Synthesis and evaluation of novel NQO2 inhibitors

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LIST OF ABBREVIATIONS

Å: Angstrom 10^{-10} m
Abs.: Absolute
Asn: Asparagine
Asp: Aspartic acid
APCI: Atmospheric-pressure chemical ionization spectrometry
CDCl$_3$: Deuterated chloroform
DCM: Dichloromethane
DMSO: Dimethyl sulfoxide
DMSO-d$_6$: Deuterated dimethyl sulfoxide
ESIMS: Electrospray ionization mass spectrometry
EtOAc: Ethyl acetate
FAD: Flavin adenine dinucleotide
Gly: Glycine
IR: Infra-red spectroscopy
Ile: Isoleucine
Leu: Leucine
Met: Methionine
MT3: Melatonin-binding site 3
NADH: Reduced adenine dinucleotide
NADPH: Reduced adenine phosphate dinucleotide
NCI: National Cancer Institute
NF-kB: Nuclear factor-kappa B
NRH: N-ribosyl dihydronicotinamide
Ppm: Parts per million
Phe: Phenylalanine
rt: Room temperature
R$_f$: Retardation factor
Cys: Cysteine
THF: Tetrahydrofuran
Thr: Threonine
TNF: Tumor necrosis factor
Trp: Tryptophan
Tyr: Tyrosine
Abstract

The NRH: quinone oxidoreductase 2 enzyme (NQO2) is a potential therapeutic target in cancer, malaria and neurodegenerative diseases. The inhibition of NQO2 enzyme activity may have a role in cancer chemoprevention and chemotherapy.

The objective of this research is the design, synthesis and evaluation of novel selective NQO2 inhibitors with no off-target effects, for example binding to DNA. From previous virtual screening studies of the NCI database, symmetric and asymmetric furan-amidines were identified as lead inhibitors of the NQO2 enzyme, with IC
\(_{50}\) values of 630 nM for 4,4'-(furan-2,5-diyl)dibenzamidine, 50 nM for 4,4'-(3,4-dimethylfuran-2,5-diyl)dibenzamidine and 140 nM for 4-(5-phenylfuran-2-yl)benzamidine.

A synthetic pathway for the synthesis of the asymmetric furan-amidines was established, which involved the cyclisation of the 1,4-diketone intermediates to give the furan ring. Several furan analogues with a range of substituents on the aromatic ring (e.g. fluoro, bromo, nitro, methyl, ethyl, isopropyl, tert-butyl, methoxy) were prepared. In addition, isosteres of the amidine group were made, including imidate, N-aryl amidine (reversed amidine), N-aryl amide and N-hydroxyamidine (amidoxime). The furan ring was replaced with other 5-membered heterocycles, including pyrrole, N-methylpyrrole, thiophene, imidazole, N-methylimidazole and oxazole. All compounds were fully characterized by \(^1\)H and \(^13\)C NMR spectroscopy, IR spectroscopy and mass spectrometry.

The synthesized asymmetric furan-amidines and their analogues showed potent NQO2 inhibition activity with IC
\(_{50}\) values in the nano-molar range. The most active compounds were asymmetric furan-amidines with meta- and para-nitro substitution on the aromatic ring, with IC
\(_{50}\) values of 15 nM.

In contrast to the symmetric furan-amidines, which showed potent intercalation in the minor grooves of DNA, the synthesized asymmetric furan-amidines and N-methylpyrrole-amidine showed no affinity towards DNA, as shown by DNA melting temperature experiment.

The high NQO2 inhibition activity of some analogues together with their high toxicity against several breast cancer cell lines, make these lead compounds worthy of further development and optimization as potential drugs.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning

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I dedicate my thesis to my lovely family
Chapter I. Introduction

1. Cancer

Cancer is a life-threatening illness that accounts for high mortality in the world.\(^1\) Cancer is a disease of uncontrolled cell proliferation\(^2\) that initiates as a result of the modification or damage of the genetic material that controls and regulates cell cycle.\(^3\) The process by which the normal cell transforms into cancerous cell is called carcinogenesis, which occurs through three stages: initiation, promotion and progression. The uncontrolled division and replication of cancer cells will result in the formation of a group of cells known as a tumour.\(^4\) Malignant tumour cells can travel from their initial site and invade other parts of the body leading to cancer metastasis.\(^5\)

Tumours are classified into solid and liquid tumours. Solid tumours are defined as a solid mass of cancer cells that grow in organs and can occur anywhere in the human body. Two types of solid tumours can occur: epithelial tumours (also called carcinomas) and connective tissue tumours (also called sarcomas). On the other hand, liquid tumours occur in the blood (lymphoma, which starts in the immune system as a liquid tumour and transforms to a solid tumour), bone marrow (leukaemia) and lymph nodes (cancer of plasma cells).\(^6\) The treatment of tumours involves surgery, radiotherapy or systemic administration of chemotherapeutic drugs known as antineoplastic agents. It is chemotherapy that is the focus of this research.

1.1. Cancer chemotherapy

Cancer chemotherapy is the use of drugs to selectively inhibit or kill proliferating cancer cells without affecting normal cells. Cancer chemotherapy started in the 1940s with the use of nitrogen mustards and antifolate drugs to cure cancer.\(^7\)

Chemotherapeutic drugs can be classified depending on their mechanism of action:

1- Antimetabolite drugs: Folate antagonists or antifolate drugs and nucleoside analogues
2- Alkylating agents
3- DNA topoisomerase inhibitors
4- DNA intercalators
5- Microtubule-targeting anticancer drugs
6- L-Asparaginase
7- Drugs that target the cell cycle

A detailed discussion about each group is given in the following sections
Chapter I. Introduction

1.1.1. Antimetabolites

Antimetabolite drugs were amongst the first effective chemotherapeutic agents discovered and are folic acid, pyrimidine or purine analogues. They have similar structures as naturally occurring molecules used in nucleic acid (DNA and RNA) synthesis. Antimetabolites are similar to chemicals needed for normal biochemical activity, but differ sufficiently so that they interfere with normal cell function. Generally, antimetabolites induce cell death during the S phase of cell growth when incorporated into RNA and DNA or inhibit enzymes needed for nucleic acid production. These agents are used for a variety of cancer therapies including leukaemia, breast, ovarian and gastro-intestinal cancers.8

1.1.1.1. Folate antagonists

Methotrexate (amethopterin) 1 (Figure 1) was first used as a chemotherapeutic drug in 1948 to treat acute leukaemia in children.9 Methotrexate was shown to have antitumour activity in a range of epithelial malignancies, including breast, ovarian, bladder, head and neck cancers. Also, methotrexate was the first drug administered systemically to cure solid tumour such as choriocarcinoma.7

![Figure 1. The structure of methotrexate (1).](image)

Methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase. Dihydrofolate reductase is responsible for the reduction of dihydrofolate into tetrahydrofolate, which is the one-carbon carrier in purine and thymidine base biosynthesis. The depletion of tetrahydrofolate leads to inhibition of thymidylate (TMP) synthesis and subsequently inhibition of DNA synthesis. Also, methotrexate inhibits purine biosynthesis both directly and by inhibiting tetrahydrofolate formation.10
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Trimetrexate 2 and edatrexate 3 (Figure 2) are antifolate drugs that are used as alternatives to methotrexate in case of the resistance of cancerous cells to methotrexate.\textsuperscript{11}

![Structures of methotrexate analogues, trimetrexate (2) and edatrexate (3).](image)

Figure 2. Structures of methotrexate analogues, trimetrexate (2) and edatrexate (3).

1.1.1.2. Nucleoside analogues

Natural nucleosides are nitrogen base compounds with purine (adenine and guanine) or pyrimidine (cytosine, uracil and thymine) scaffolds bound to a ribose or deoxyribose sugar (Table 1). The natural nucleosides are important in the molecular mechanisms of conservation, replication and transcription of the genetic information.\textsuperscript{12}

Nucleoside analogues are cytotoxic agents, which exert their cytotoxic activity by interfering with the nucleic acids, DNA and RNA in different ways. These drugs incorporate into and alter nucleic acids, interfere with various enzymes involved in nucleic acid biosynthesis and modify metabolism of physiological nucleosides.\textsuperscript{13}
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Table 1. The structures of nitrogenous bases and sugars constituting the natural nucleoside

<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purine</strong></td>
<td><strong>Guanine</strong></td>
</tr>
<tr>
<td>Adenine</td>
<td>Guanine</td>
</tr>
<tr>
<td>Pyrimidine</td>
<td>Pyrimidine</td>
</tr>
<tr>
<td>Cytosine</td>
<td>Uracil</td>
</tr>
<tr>
<td>Thymine</td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>Sugar</td>
</tr>
<tr>
<td>Ribose</td>
<td>Deoxyribose</td>
</tr>
</tbody>
</table>

1.1.1.2.1. 5-Fluorouracil and its nucleoside metabolites

5-Fluorouracil (5-FU) 4 is structurally similar to the pyrimidine base uracil in which the hydrogen on C-5 of uracil is isosterically replaced by a fluoro-atom in 5-FU (Figure 3).

![Figure 3. The structure of 5-fluorouracil (4).](image)

5-FU is metabolized inside the cell into different active nucleoside and nucleotide metabolites that exert their action by disrupting RNA synthesis and inhibiting the enzyme thymidylate synthase (TS). Thymidylate synthase catalyses the reductive methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) using 5,10-methylenetetrahydrofolate (CH₂THF) as the methyl
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donor (Scheme 1). This reaction provides the thymidylate, which is important in DNA synthesis and repair.\textsuperscript{14}

\textbf{Scheme 1}. The site of action of 5-fluorouracil.

1.1.1.2.2. Deoxycytidine derivatives

Cytarabine is a deoxycytidine analogue used in the treatment of haematological malignant disease, namely acute myeloid leukaemia. Cytarabine directly inhibits DNA polymerase and its arabinosyl CTP derivative is incorporated into DNA leading to inhibition of DNA synthesis.\textsuperscript{13}

1.1.1.2.3. 6-Thioguanine and 6-mercaptopurine nucleosides

6-Thioguanine 5 and 6-mercaptopurine 6 are thio-analogues of the naturally occurring 6-ketopurine bases, guanine and hypoxanthine, respectively (Figure 4). 6-Mercaptopurine was initially evaluated for the treatment of leukaemia in the early 1950s.\textsuperscript{15}

\textbf{Figure 4}. Structures of 6-thioguanine (5) and 6-mercaptopurine (6).

1.1.2. Alkylating agents

The use of alkylating agents in cancer chemotherapy was started in the early 1940s. Alkylating agents are used to treat a wide variety of cancers, most effectively on solid tumours and leukaemia. They are cytotoxic agents known to act during all phases of the cell cycle. They exert their action directly on DNA leading to the crosslinking
between DNA strands and causing the formation of DNA strand breaks. Consequently, cell division will be inhibited and cell apoptosis will be induced.\textsuperscript{16}

In general, the alkylating agents exert their action inside the cell by the formation of highly reactive cation species. For example, the nitrogen mustard mechlorethamine, undergoes $S_N2$ cyclization step followed by nucleophilic attack of N-7 guanine residue of DNA forming a covalent bond with DNA, followed by crosslinking between the DNA strands (Scheme 2).\textsuperscript{17}

\begin{center}
\textbf{Scheme 2.} The mechanism of the alkylating agent mechlorethamine inside the cell.
\end{center}

Alkylating agents are classified into six groups, as shown with examples, in Table 2.
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<table>
<thead>
<tr>
<th>Alkylating agent group</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl sulfonates</td>
<td><img src="image" alt="Busulfan" /></td>
</tr>
<tr>
<td>Methylmelamine</td>
<td><img src="image" alt="Hexamethylmelamine" /></td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td><img src="image" alt="Cyclophosphamide" /> <img src="image" alt="Chlorambucil" /> <img src="image" alt="Melphalan" /></td>
</tr>
<tr>
<td>Triazenes</td>
<td><img src="image" alt="Temozolomide" /> <img src="image" alt="Dacarbazine" /></td>
</tr>
<tr>
<td>Nitrosoureas</td>
<td><img src="image" alt="Carmustine" /></td>
</tr>
<tr>
<td>Platinum drugs</td>
<td><img src="image" alt="Cisplatin" /></td>
</tr>
</tbody>
</table>
1.1.3. Topoisomerase enzymes inhibitors

Topoisomerase enzymes control the topology of DNA function at several steps in the replication of cells. These enzymes remove the negative and positive supercoils of DNA, which result from DNA duplex-unwinding. The enzymes are classified into topoisomerase I and II, which are different in their mechanism of action. Topoisomerase I cuts a single strand of the DNA double helix while topoisomerase II cuts both strands of DNA, using adenosine triphosphate (ATP) for fuel. The rest of the process by which the two enzymes work is very similar. The process entails the relaxation of the coil of the two DNA strands, and then after the cuts are made and replication or repair is complete, the strands are ligated back together and reform a coil.\textsuperscript{19}

Topoisomerase enzymes are potential targets in the treatment of cancer because their inhibition results in cell death. Therefore inhibitors of the topoisomerase enzymes have the ability to kill all cells undergoing DNA replication, reading of the DNA for protein production or undergoing repair of DNA damage. Since cancer cells divide much more rapidly than normal cells, the cancer cells will be killed by the topoisomerase inhibitors, though some normal cells with topoisomerase activity will also be killed.

Camptothecin \textsuperscript{7} and its derivatives topotecan \textsuperscript{8} and irinotecan \textsuperscript{9} (Figure 5) are alkaloids with anticancer activity. They exert their action through the inhibition of topoisomerase I.\textsuperscript{20}

\textbf{Figure 5.} Structures of the topoisomerase I enzyme inhibitors, camptothecin (7), topotecan (8) and irinotecan (9).
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On the other hand, topoisomerase II is considered a potential target for widely used anticancer drugs currently in clinical use. These drugs include anthracyclines (discussed in section 1.1.1.4.) and epipodophyllotoxins. Teniposide 10 and etoposide 11 are anticancer drugs that share the podophyllotoxin scaffold 12 (Figure 6).\(^{21}\)

![Figure 6](image)

**Figure 6.** Structures of epipodophyllotoxin anticancer drugs, teniposide (10), etoposide (11) and podophyllotoxin (12).

Several compounds were approved as novel topoisomerase II enzyme inhibitors (Figure 7)\(^ {21}\)

1- Merbarone (NSC 336628) 13: a conjugate of thiobarbituric acid and aniline joined by an amide linkage.
2- Suramin 14: is a polyanionic sulphonate compound.
3- Fostriecin 15: is a polyene lactone phosphate ester.
4- Quinolone derivatives: Quinolones are widely used antibiotics, which exert their action through the inhibition of the bacterial topoisomerase II enzyme (DNA gyrase). Quinolone derivatives, namely quinobenzoxazines showed good cytotoxic activity toward tumour cell lines such as HT-29 human colon carcinoma and A546 human breast carcinoma. Examples of these cytotoxic agents are A-62176 16 and A-74932 17.
1.1.4. DNA intercalators

DNA intercalation is the insertion of a planar molecule in the space between two base pairs of DNA without breaking the H-bonding between DNA strands. This process induces local structural changes to the DNA including unwinding of the double helix and lengthening of the DNA strand. As a consequence, the transcription and replication of DNA will be retarded or inhibited.22
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The drugs known to exert their cytotoxic activity through DNA intercalation are acridines and anthracyclines.

1.1.4.1. Acridines

Acridines intercalate DNA via π-π stacking interactions with DNA base pairs. In 1970s, nitracrine 18 and amsacrine 19 (Figure 8) were the first acridines developed for the treatment of cancer. Nitracrine is 1-nitroacridine derivative and amsacrine is a 9-anilinoacridine derivative.

![Figure 8. Structures of acridine DNA intercalators, nitracrine (18) and amsacrine (19).](image)

1.1.4.2. Anthracyclines

Anthracyclines are planar polycyclic compounds that intercalate DNA. The cytotoxic activity of anthracyclines is believed to occur through different mechanisms of action beside their ability to intercalate DNA:

1. Generation of free radicals through the one-electron reduction of the quinone part. The generated free radicals lead to DNA damage and lipid peroxidation.
2. DNA binding and alkylation
3. DNA cross-linking
4. Interference with DNA unwinding or DNA strand separation
5. The inhibition of topoisomerase II enzyme causing induction of cell apoptosis.

The first anthracyclines were doxorubicin 20 and daunorubicin 21 (Figure 9), which were isolated from Streptomyces peucetius in the 1960s. Doxorubicin is used for the treatment of breast cancer, childhood solid tumours, soft tissue sarcomas and aggressive lymphomas. On the other hand, daunorubicin is used to cure acute lymphoblastic or myeloblastic leukaemias.
1.1.5. Microtubule-targeting anticancer drugs

Microtubules are long, filamentous, tube-shaped proteins present in all eukaryotic cells. They are important in the development and maintenance of cell shape, in cell signalling and in cell division and mitosis.

Microtubules are potential target in cancer because of their high importance in the process of cell mitosis: the drugs that target microtubules are named as antimitotic drugs. Microtubules are mainly targeted by naturally occurring alkaloids derived from plants and animals. Table 3 summarizes the anti-microtubules drugs and the cancer type for their activity.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cancer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinblastine</td>
<td>Hodgkin’s disease, testicular germ-cell cancer</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Leukaemia, lymphomas</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Ovarian, breast and lung tumours</td>
</tr>
<tr>
<td>Combretastatins</td>
<td>Clinical studies on anaplastic thyroid cancer</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>Clinical studies on breast cancer</td>
</tr>
<tr>
<td>Estramustine</td>
<td>Prostate</td>
</tr>
</tbody>
</table>

1.1.6. L-Asparaginase

The enzyme L-asparaginase is a chemotherapeutic agent used in the treatment of acute lymphoblastic leukaemia and other lymphoid malignancies. It was first used
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clinically in 1966. L-Asparaginase catalyzes the hydrolysis of L-asparagine (L-Asn) into L-aspartic acid (L-Asp) and ammonia (Scheme 3).

\[
\begin{array}{c}
\text{H}_2\text{N} & \text{O} & \text{O} & \text{NH}_2 & \text{OH} \\
\text{O} & \text{NH}_2 & \text{OH} & \text{O} & \text{NH}_2 & \text{OH}
\end{array}
\xrightarrow{\text{L-Asparaginase}}
\begin{array}{c}
\text{HO} & \text{O} & \text{NH}_2 \\
\text{O} & \text{NH}_2 & \text{OH} & \text{+} & \text{NH}_3
\end{array}
\]

**Scheme 3.** The reaction catalysed by L-asparaginase enzyme.

The effectiveness of L-asparaginase as a chemotherapeutic agent rose from the fact that lymphoblasts and certain other tumour cells do not synthesize L-Asn de novo. The inability of the tumour cells to synthesize L-Asn causes them to rely on L-Asn supplied from serum for survival. As a consequence, the depletion in the supply of L-Asn to the tumour cells will lead to cell death.

1.1.7. Drugs that target the cell cycle

The cell cycle is a complex process which is divided into four distinct phases:
1- G1 phase (first gap phase): cell is preparing for DNA synthesis.
2- S phase (Synthesis phase): cell is synthesizing DNA.
3- G2 phase (second gap phase): cell is preparing for mitosis.
4- M phase (mitosis phase): cell divides into two daughter cells.

The cell is considered in G0 phase (quiescent phase) when the cell is not in the cycle, but it has the potential to divide.\(^{27}\)

The process of the cell cycle is usually regulated by cyclin proteins and by cyclin-dependent kinases.\(^{27}\) The inhibition of cyclin-dependent kinases leads to cell cycle arrest and induction of apoptosis. Flavopiridol 22 and UCN-01 23 are examples of drugs that inhibit cyclin-dependent kinases (Figure 10).\(^{28}\)
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![Chemical structures of anticancer drugs](image)

**Figure 10.** Structures of anticancer drugs that target the cell cycle: flavopiridol (22) and UCN-01 (23).

1.2. Cancer resistance to chemotherapy- the need for new targets and approaches

The major problem facing the use of chemotherapeutic drugs for the treatment of cancer is the resistance of cancerous cells to these drugs. The resistance develops because of the rapid metabolic changes occurring in cancer cells, and the hypoxic conditions of the tumour environment, specifically in solid tumours. The hypoxic conditions affect the action of most chemotherapeutic agents, which need molecular oxygen to generate free radicals that induce cell toxicity.

The understanding of signal-transduction pathways involved in carcinogenesis pathways led to the discovery of new targets. This aids in the rational drug design of compounds that can modify the action of these targets leading to the discovery of new chemotherapeutic drugs.

1.3. Cancer chemoprevention- first line of defence

The high toxicity of conventional anticancer drugs and the failure to reduce the mortality rate for some cancers, means that chemoprevention of cancer is of great importance. Cancer chemoprevention is the use of a synthetic or natural product which has the ability to modulate the progress of normal cells into tumour cells. Table 4 summarizes the chemoprevention agents that have been approved for use in the clinic by the Food and Drug Administration.
Table 4. Cancer chemo-preventive agents

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Structure</th>
<th>Cancer type</th>
<th>Year first approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen&lt;sup&gt;33&lt;/sup&gt;</td>
<td><img src="image1" alt="" /></td>
<td>Breast</td>
<td>1998</td>
</tr>
<tr>
<td>Raloxifene&lt;sup&gt;34&lt;/sup&gt;</td>
<td><img src="image2" alt="" /></td>
<td>Breast</td>
<td>2007</td>
</tr>
<tr>
<td>HPV vaccine&lt;sup&gt;35&lt;/sup&gt;</td>
<td>---</td>
<td>Cervix, vagina, anus</td>
<td>2006</td>
</tr>
<tr>
<td>5-Fluorouracil&lt;sup&gt;36&lt;/sup&gt;</td>
<td><img src="image3" alt="" /></td>
<td>Skin</td>
<td>1970</td>
</tr>
<tr>
<td>Diclofenac sodium&lt;sup&gt;36&lt;/sup&gt;</td>
<td><img src="image4" alt="" /></td>
<td>Skin</td>
<td>2000</td>
</tr>
<tr>
<td>5-Aminolevulinic acid&lt;sup&gt;36&lt;/sup&gt;</td>
<td><img src="image5" alt="" /></td>
<td>Skin</td>
<td>1999</td>
</tr>
<tr>
<td>Imiquimod&lt;sup&gt;36&lt;/sup&gt;</td>
<td><img src="image6" alt="" /></td>
<td>Skin</td>
<td>2004</td>
</tr>
</tbody>
</table>
2. Quinones as carcinogenic compounds

Quinones 24 are organic cyclic compounds present as endogenous biochemical in pro- and eukaryotic cells and they are abundant in the environment. Quinones are cytotoxic compounds as they are highly electrophilic compounds that can react with the nucleophilic sites of DNA, nucleophilic residues of proteins and glutathione (GSH) leading to protein alkylation and GSH depletion. In mammalian cells, quinones are reduced into either more stable derivatives known as hydroquinones 25 or to reactive species known as semiquinones 26. Hydroquinones are produced from two-electron transfer, but semiquinones from one-electron transfer (Scheme 4).

![Scheme 4. The one-electron and two-electron reduction of quinone (24).](image)

The one-electron transfer is catalysed by flavin- or metal-dependent catalytic enzymes, such as NADH-cytochrome b5 reductase, NADPH-cytochrome P450 reductase and chloroplast ferredoxin-NADP⁺ reductase. The semiquinone species 26, which result from the one-electron reduction of quinones have a role in the oxidative stress in cells. This can be explained by their high toxicity, which forces the cells to detoxify them through the reaction with oxygen molecules. Reaction of the semiquinone
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species with oxygen molecules leads to the formation of superoxide radicals, which can damage DNA. The increase in the production of these superoxide radicals can lead to cancer.\textsuperscript{38,40}

The two-electron reduction of quinones into hydroquinones, which is catalysed by flavo-quinone reductase enzymes (QRs) competes with the one-electron reduction of quinones. The resulting hydroquinones \textsuperscript{25}, which are relatively stable compounds, are removed from cells by conjugation with glutathione and UDP-glucuronic acid.\textsuperscript{41} The quinone reductase enzymes utilize either flavin mononucleotide (FMN) \textsuperscript{27} or flavin adenine dinucleotide (FAD) \textsuperscript{28} as a cofactor (Figure 11). The role of the cofactor in the reduction process is the transfer of the hydride ion from an electron donor, such as NAD(P)H to the quinone substrate.\textsuperscript{38}

![Figure 11. Structures of FMN (27) and FAD (28) cofactors.](image)

2.1. Quinone reductases (QRs)

NAD(P)H: quinone oxidoreductase 1 (NQO1) and NRH: quinone oxidoreductase 2 (NQO2) are examples of mammalian quinone reductase enzymes. Both enzymes are homodimeric with two FAD molecules bound non-covalently to the enzymes. The isoalloxazine ring of the FAD molecule forms the floor of both enzyme active sites. The FAD molecules are involved in the reduction processes catalysed by the two enzymes, each of which will be discussed further.
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2.1.1. NAD(P)H: quinone oxidoreductase 1 (NQO1) enzyme

NQO1 (EC 1.6.99.2) is a cytosolic flavoprotein first isolated from rat liver by Ernster and co-workers in 1958.\textsuperscript{32} NQO1 is also known as DT-diaphorase as the enzyme which non-specifically catalyses the oxidation of di- and tri-phosphopyridine nucleotide NADH and NADPH, respectively.\textsuperscript{42} NQO1 is highly expressed in liver, brain, kidney, heart, lungs and testis in human.\textsuperscript{43} Besides that, NQO1 is highly expressed in most human solid tumours,\textsuperscript{44} particularly in non-small cell lung, prostate, pancreatic and breast tumours.\textsuperscript{45}

NQO1 is a homodimeric protein with a molecular weight of around 55,000 Da,\textsuperscript{46} which is composed from two monomers. Each 273-amino acid monomer is divided into two domains: a large catalytic domain contains the amino acid residues 1-220 and a small C-terminal domain contains the amino acid residues 221-273. The NQO1 enzyme has two FAD molecules bound to the catalytic domain of each monomer.\textsuperscript{47} The FAD molecules are anchored through hydrogen bonding with the amino acid residues Trp\textsuperscript{105}, Phe\textsuperscript{106}, Tyr\textsuperscript{155}, Gly\textsuperscript{150}, Gly\textsuperscript{149}, Leu\textsuperscript{103}, Thr\textsuperscript{147}, Asp\textsuperscript{18}, Arg\textsuperscript{200}, Gln\textsuperscript{66}, His\textsuperscript{11} and Tyr\textsuperscript{104}.\textsuperscript{48}

More than 90\% of the enzyme’s catalytic activity was found to be cytosolic and minor portions are associated with mitochondria and microsomes.\textsuperscript{43}a NQO1 catalyses the two-electron reduction of quinones and converts them into hydroquinones. The reduction process proceeds through a ping-pong mechanism (Scheme 5). This mechanism involves the complete transfer of two electrons and protons from NQO1 co-substrate, NAD(P)H to its cofactor flavin adenine dinucleotide (FAD\textsuperscript{+}) molecule forming reduced flavin adenine dinucleotide (FADH\textsubscript{2}) (Scheme 5).\textsuperscript{49} Subsequently, a hydride ion will transfer from FADH\textsubscript{2} to the quinone substrate.\textsuperscript{50}
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Scheme 5. Ping-pong mechanism of quinone reduction by the NQO1 enzyme.

NQO1 is considered as a detoxifying enzyme as its role in the reduction of quinones aids in the protection of cells from harmful effects of quinones.41

2.1.2. N-Ribosyl dihydronicotinamide (NRH): quinone oxidoreductase 2 (NQO2) enzyme

NRH: quinone oxidoreductase 2 (NQO2) enzyme is a cytosolic flavoprotein enzyme discovered by Liao and Williams-Ashman in 1961.51 It is widely distributed in human tissue, mainly in heart, brain, lung, liver and skeletal muscle.52 NQO2 is a homodimeric protein53 consisting of 231-amino acids.54 Each monomer is divided into two domains: the N-terminal domain (1-220 amino acid residues) and the C-terminal domain (221-231 amino acid residues) (Figure 12).55
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Figure 12. The 3D structure of NQO2 enzyme (PDB code 1QR2; Resolution at 2.10 Å).\textsuperscript{54}

The NQO2 enzyme active site is a narrow deep hydrophobic cavity\textsuperscript{55} which is surrounded by the side chains of Tyr\textsuperscript{132’}, Phe\textsuperscript{178’}, Phe\textsuperscript{126’}, Met\textsuperscript{154’} and Cys\textsuperscript{121’} amino acid residues from one monomer and Tyr\textsuperscript{155} and Phe\textsuperscript{106} amino acid residues from the other monomer.\textsuperscript{56} The isoalloxazine ring of the FAD molecule\textsuperscript{54} and the side chain of Trp\textsuperscript{105} amino acid form the floor of the cavity.\textsuperscript{56} The side chain of Asn\textsuperscript{161} and the hydroxyl groups of Tyr\textsuperscript{155} and Tyr\textsuperscript{132} amino acid residues form a hydrophilic surface at one end of the cavity.\textsuperscript{56} The hydroxyl group of Thr\textsuperscript{71’} and the main chain of Gly\textsuperscript{68} and Asp\textsuperscript{117} amino acid residues point towards the other end of the cavity.\textsuperscript{56} The positions of these amino acid residues indicate that both ends have numerous functional groups accessible for hydrogen bonding. The fourth side is accessible to solvent (Figure 13).\textsuperscript{56}
Figure 13. NQO2 enzyme active site (PDB code 1QR2; Resolution at 2.10 Å).\textsuperscript{54}

NQO2 catalyzes the two-electron reduction of quinones \textit{via} a ping-pong mechanism similar to the NQO1 enzyme.\textsuperscript{57} NQO2 utilizes non-naturally occurring \textit{N}-ribosyl dihydronicotinamide (NRH) co-substrate 29 as an electron donor in the reduction processes.\textsuperscript{58} The \textit{N}-methyl 30, \textit{N}-\,(n-propyl) 31, and \textit{N}-benzyl 32 analogues of NRH (Figure 14) were studied as alternative co-substrates of NQO2.\textsuperscript{59} It was found that NQO2 has a high affinity for the \textit{N}-benzynicotinamide analogue 32.\textsuperscript{60}
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Figure 14. Structures of NQO2 enzyme co-substrates, N-ribosyl (29), N-methyl (30), N-(n-propyl) (31) and N-benzyl (32) dihydronicotinamide.

The substrates for the NQO2 enzyme are either para-quinones, such as menadione (vitamin K3, menadione) 33, coenzyme Q0 34, or ortho-quinones (catechol quinones), such as the estrogen ortho-quinone metabolites, estrone 35 and estradiol 36 (Figure 15). Menadione (2-methyl-1,4-napthaquinone, Vitamin K3, 33) is activated by NQO2 leading to hepatic toxicity.

Figure 15. Structures of the NQO2 substrates, menadione (33), coenzyme Q0 (34), estrone (35) and estradiol (36).

The role of NQO2 in the reduction of estrogen ortho-quinones is considered a detoxification reaction. The estrogen quinones are electrophilic compounds that react with DNA and they are related to carcinogenesis of breast cancer.62
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On the other hand, NQO2 is considered as an activating enzyme as its role in the reduction of benzo[a]pyrene-3,6-quinone \(37\) \(^{41}\) is related to cell toxicity. The reduction of benzo[a]pyrene-3,6-quinone \(37\) yields benzo[a]pyrenediol product \(38\). Benzo[a]pyrenediol product can re-oxidize back to benzo[a]pyrenediones generating hydrogen peroxide, hydroxyl and semiquinone radicals (Scheme 6) that can attack cell macromolecules \(^{64}\) leading to mutagenicity.\(^{65}\)

Scheme 6. Reduction of benzo(a)pyrenediones (37) into benzo(a)pyrenediol (38).

2.1.3. NQO1 and NQO2: similarities and differences

The overall topology of NQO2 is highly similar to its homologous enzyme NQO1, with 54% and 49% similarity between human NQO2 and NQO1 cDNA and protein, respectively.\(^{53}\) Both enzymes are homodimeric binding two FAD cofactos \(^{66}\) and they share the same active site.\(^{54}\) The active sites are hydrophobic, favouring the binding of hydrophobic substrates and inhibitors.\(^{57}\) The NQO2 active site is slightly larger and more hydrophobic than the NQO1 active site because Tyr\(^{126}\), Tyr\(^{128}\) and Met\(^{131}\) residues in NQO1 are replaced by Phe\(^{126}\), Ile\(^{128}\) and Phe\(^{131}\) in NQO2 (Figure 16).\(^{54}\)
The difference in the binding sites’ nature and size between NQO1 and NQO2 enzymes has an impact on the inhibitor specificity for each enzyme. The potent polycyclic aromatic hydrocarbon inhibitors of NQO2, such as benzo(a)pyrene 39 and 7-methylbenzo(a)anthracene 40 (Figure 17), do not inhibit the NQO1 enzyme.\textsuperscript{57, 59, 67} Also, NQO2 is not be inhibited by the potent NQO1 inhibitor dicoumarol 41 (Figure 17).

Both NQO1 and NQO2 catalyse the two-electron reduction of quinone and four-electron reduction of nitro-compounds,\textsuperscript{66b} with differences in the co-substrate that each
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enzyme uses as electron donor. NQO2 uses reduced non-phosphorylated derivatives of nicotinamides such as NRH 29 and its analogues 30-32, but it is completely inert toward the phosphorylated derivatives of nicotinamide such as NADH 42 or NADPH 43 (Figure 18) 59 that are used by NQO1.

![Figure 18. Structures of NADH (42) and NADPH (43).](image)

The difference in co-substrate affinity between NQO1 and NQO2 enzymes can be explained by the lack of 43 amino acids in the C-terminal domain in NQO2. 57 This C-terminal domain in NQO1 provides the site for binding of the pyrophosphate-ribose-adenine moiety of phosphorylated nicotinamides 42 and 43. 57 For the same reason, NQO2 cannot be inhibited by dicoumarol 41, a known potent inhibitor of NQO1, because it exerts its action by competing with the NAD(P)H binding site. 57

2.1.4. The catalytic consequences of NQO1 and NQO2 on cells

The impact of the catalytic function of NQO1 and NQO2 in cells is different between the two enzymes. NQO1 is considered as a protective detoxifying enzyme, but NQO2 is an activating enzyme with some detoxification activity against estrogen ortho-quinones. NQO2-null mice exhibited myeloid hyperplasia and hyperactivity of bone marrow. 63 The reduction of menadione by NQO1 and NQO2 has a different impact on the cells. The reduction of menadione into menadiol by NQO1 leads to the decrease of its toxicity on cells as menadiol will be removed by conjugation with glutathione and UDP-glucuronic acid. 37 On the other hand, the reduction of menadione by NQO2 leads to hepatic toxicity. 63

NQO1 and NQO2 enzymes act as chaperone proteins by binding to the tumour suppressor factor p53 leading to its stabilization against 20S proteasomal degradation. 68 Tumour suppressor factor p53 ‘the guardian of the genome’ regulates and controls cell
growth and protects cells against adverse effects of radiation and chemicals by the induction of cell growth arrest or programmed cell death (apoptosis). 69

2.1.5. Importance of NQO1 and NQO2 enzymes in cancer-targeted therapy

The targeted anti-cancer prodrug therapy is an approach used to increase the local delivery of the parent cytotoxic drugs to the cancer cells in humans. This targeting depends on the activation of the prodrugs by a certain enzyme, which is over-expressed in the cancer cells. This has an advantage of limiting the side effects of the cytotoxic drugs on normal cells of the human. 70 Prodrugs are compounds that must be transformed in vivo to exert their pharmacological activity.

The high expression of NQO1 71 and NQO2 enzymes in tumour cells 72 and their ability to reduce quinone and nitro compounds was utilized for the targeting of prodrugs such CB1954 44 and nitro-cryptolepine derivatives 45 (Figure 19) to the cancer cells. 72-73

![Chemical structures of CB1954 and nitro-cryptolepine derivatives](image)

**Figure 19.** Structures of anti-tumour prodrugs, CB1954 (44) and nitro-cryptolepine derivatives (45).

The bio-activation of cytotoxic prodrugs into cancer cells produces highly toxic derivatives able to covalently bind macromolecules, especially DNA, leading to cell toxicity and death. The catalytic efficiency of the two enzymes is different: NQO2 is 3000 times more efficient than NQO1 66b in the four-electron reduction such as CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide, 44] into DNA alkylating 4-hydroxylamine derivative 46 (Scheme 7). 66b, 72
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Scheme 7. Reduction of the cytotoxic compound CB1954 (44) by NQO2 enzyme.

Nitro-cryptolepine derivatives are activated by NQO1 and not NQO2 with the exception of the compound 2-fluoro-7,9-dinitrocryptolepine 47 (Figure 20). Compound 47 was found to exhibit higher toxicity in the RT112 cell line (high in NQO2) in the presence of N-ribosyl dihydronicotinamide 29 (NRH).73

Figure 20. The structure of 2-fluoro-7,9-dinitrocryptolepine (47).

The anticancer prodrug mitomycin C 48 (Figure 21) is activated by the two enzymes through two-electron transfer forming a highly electrophilic hydroquinone metabolite, which has the ability to cross-link DNA.74 However, NQO1 has poor affinity for mitomycin C 75 and the product of this reduction can irreversibly bind NQO1 leading to its inhibition.76 Mitomycin C analogues namely, EO9 49 can be activated by NQO1 77 and NQO2 41, and BMY25067 50 (Figure 21) can be activated by NQO2 only.41
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Figure 21. Structures of the anticancer drugs: mitomycin C (48), EO9 (49) and BMY25067 (50).

3. NQO2 Enzyme and its role in human diseases

The NQO2 enzyme is considered a potential therapeutic target in cancer and this importance has arisen for a number of reasons.

NQO2 is highly expressed in tumour cells, and its role in the reduction of quinones is related to cell toxicity and mutagenicity. NQO2 is required for the tumour necrosis factor (TNF)-induced activation of NF-kB. NF-kB is a transcription factor, which controls the genes responsible for cell proliferation and survival. The activation of NF-kB by the cytokine TNF results in the suppression of cell apoptosis and the protection of tumour cells from chemotherapeutic drugs and ionizing radiation.

NQO2 binds some anti-tumour drugs as their off-target effect. It was found that Imatinib (2-phenylaminopyrimidine compound, 51) and nilotinib (4-pyridyl-2-phenylaminopyrimidine compound, 52) (Figure 22) bind to the active site of NQO2 enzyme leading to its inhibition. Imatinib and nilotinib are tyrosine kinase inhibitors used to treat leukaemia.
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Figure 22. Structures of tyrosine kinase inhibitors, Imatinib (51) and nilotinib (52).

The inhibition of the NQO2 enzyme activity has a role in the chemoprevention and chemotherapy of cancer. The inhibition of the NQO2 enzyme activity leads to the modulation of NF-kB signalling. The modulation of NF-kB signalling has an effect on tumour cell proliferation and the potentiation of the activity of chemotherapeutic drugs and ionizing radiation leading to induction of tumour cell apoptosis.

In addition, the high expression of NQO2 enzymes in tumour cells and its ability to reduce quinone and nitro compounds was utilized for the targeting of bioreducible cytotoxic agents such CB1954 and nitro-cryptolepine analogues to the tumour cells.

Also, the NQO2 enzyme has a role in other human diseases neurodegenerative diseases, for example Parkinson’s disease, schizophrenia and Alzheimer’s disease. NQO2 gene polymorphism is correlated with Parkinson’s disease and schizophrenia. On the other hand, the levels of the NQO2 enzyme in the hippocampus of Alzheimer’s disease patients was found to be high, but still the relation between Alzheimer's disease and NQO2 has not yet been determined.

The NQO2 enzyme in human red blood cells has been identified as a potential target of the anti-malarial aminoquinoline compounds primaquine and chloroquine, which can selectively inhibit this enzyme in the low micro-molar range.

3.1. NQO2 inhibitors

The first compounds studied as inhibitors for NQO2, with the aim to determine the enzyme properties, were quinacrine (Atabrine®, 53) and chlorpromazine 54 (Figure
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23) which can inhibit NQO2 at concentration of 20.0 μM.\textsuperscript{51} Polycyclic aromatic hydrocarbons such as benzo[a]pyrene \textsuperscript{39} (Figure 17) and benzo[a]anthracene \textsuperscript{55} (Figure 23), can also potently inhibit the NQO2 enzyme with an IC\textsubscript{50} value less than 10 nM.\textsuperscript{51}

![Figure 23. Structures of first reported NQO2 inhibitors, quinacrine (53), Chloropromazine (54) and benzo(a)anthracene (55).](image)

3.1.1. Flavones

Flavones are polyphenol compounds that can inhibit NQO2 competitively with respect to NRH.\textsuperscript{66b} In the literature many of these compounds were reported as potent inhibitor of NQO2 through exerting their inhibition action in the 100 nM range such as Epigenin \textsuperscript{56}, Genistein \textsuperscript{57}, and Kaempferol \textsuperscript{58}.\textsuperscript{60} The most potent flavone inhibitor known is Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, \textsuperscript{59}) (Figure 24) that can inhibit NQO2 with an IC\textsubscript{50} value of 80 nM.\textsuperscript{66b}

![Figure 24. Structures of flavonoid inhibitors of NQO2, Epigenin (56), Genistein (57), Kaempferol (58) and Quercetin (59).](image)
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3.1.2. Resveratrol

Resveratrol (trans-3,4',5-trihydroxystilbene, 60), a constituent of red grapes (Figure 25), is a chemopreventive compound that can affect the three stages in carcinogenesis, namely, tumour initiation, promotion and progression. Its action as a chemopreventive agent was first reported with its inhibition of cyclooxygenase at a concentration of ~15 μM. The chemopreventive action of resveratrol is also linked to the inhibition of the NQO2 enzyme through binding to the enzyme active site competing with the substrate.

![Figure 25. The structure of resveratrol (60).](image)

3.1.3. Aminoquinolines

The antimalarial aminoquinoline compounds namely, quinacrine 53 (Figure 23), primaquine 61 and chloroquine 62 (Figure 26) showed high affinity for inhibition of NQO2 enzyme in the 1 μM range.

![Figure 26. Structures of aminoquinoline inhibitors of NQO2, Primaquine (61) and Chloroquine (62).](image)

3.1.4. MT3-ligands

Melatonin (N-acetyl-5-methoxytryptamine, 63) (Figure 27) is a neurohormone secreted from the pineal glands. It exerts its action in the body by binding two G protein coupled receptors, namely MT1 and MT2, and a non G-protein coupled receptor with enzymatic properties identified and characterized as NQO2 (MT3). Melatonin 63 and its analogue iodomelatonin 64 can bind to the active site of the NQO2 enzyme leading to its inhibition with IC\(_{50}\) values of 11.3 and 1.1 μM, respectively. Several MT3
receptor ligands are reported in the literature as potent NQO2 inhibitors. These ligands (Figure 27) can specifically inhibit the NQO2 enzyme in the low nanomolar range. The most potent ligand is compound S29434 67 that can inhibit NQO2 with an IC\textsubscript{50} value of 2.4 nM.\cite{93,94}

![Figure 27. Structures of MT3 receptor ligands.](image)

### 3.1.5. Imatinib (Glivec®)

The anti-leukaemic drug Imatinib 51 (Figure 22) is a tyrosine kinase inhibitor that can inhibit the NQO2 enzyme with an IC\textsubscript{50} value of 80 nM. It competes with the substrate for the active site which is considered as an off-target effect for this drug.\cite{81}

![Image of Imatinib structure](image)
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3.1.6. Dabigatran and its ethyl ester prodrug⁹⁵

Dabigatran 71 and dabigartan ethyl ester 72 (Figure 28) are anti-coagulant drugs, which exert their action through the inhibition of thrombin. Michaelis and co-workers reported that these drugs have similar NQO2 inhibitory potency as Imatinib 51.

![Figure 28. Structures of the anticoagulants, dabigatran (71) and its ethyl ester (72).](image)

3.1.7. Casimiroin and its analogues⁶⁶

Casimiroin 73 (Figure 29) is a natural product derived from the fruit of the *Casimiroa edulis* Rutaceae plant. It is a lead quinolinone compound investigated for its potential chemopreventive and chemotherapeutic activity, which can be explained by its ability to inhibit the NQO2 enzyme with an IC₅₀ of 54.1 µM. To optimize its NQO2 inhibition activity many analogues were synthesized (Table 5) and the most potent, compound 78 exerted its activity with an IC₅₀ of 1.9 µM.

![Figure 29. The structure of casimiroin (73).](image)
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Table 5. NQO2 inhibition data for casimiroin and its analogues.\textsuperscript{56}

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>R\textsubscript{3}</th>
<th>R\textsubscript{4}</th>
<th>R\textsubscript{5}</th>
<th>IC\textsubscript{50} (\textmu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casimiroin 73</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>54.1 ± 6.7</td>
</tr>
<tr>
<td>74</td>
<td>-OCH\textsubscript{2}O-</td>
<td>H</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>6.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>5.8 ± 0.9</td>
</tr>
<tr>
<td>76</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>9.3 ± 2.3</td>
</tr>
<tr>
<td>77</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>8.8 ± 1.1</td>
</tr>
<tr>
<td>78</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>79</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>10.8 ± 1.5</td>
</tr>
<tr>
<td>80</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>OCH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>81</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>82</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>83</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>84</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>OCH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>

Casimiroin and its analogues bind deeply in the NQO2 enzyme active site through hydrophobic interactions between the quinoline ring and Trp\textsuperscript{105}, Gly\textsuperscript{68}, Phe\textsuperscript{126}, Phe\textsuperscript{178} amino acids residues and the isoalloxazine ring of FAD.

These analogues are more potent than casimiroin in their NQO2 inhibition: replacement of methoxy group at position 4 by a methyl group increases the potency of analogues, e.g. compound 74 is 8 times more potent than casimiroin. N-Methylated analogues are more potent than non N-methylated analogues, e.g. compounds 78 and 80 are more active than compounds 77 and 79. Di- and tri-methoxy analogues are more potent than casimiroin, but tetramethoxy analogues are inactive because of an increase in the steric bulk of these groups, which affects the co-planarity with the FAD isoalloxazine ring dis favouring \pi-\pi stacking with the benzene ring.
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3.1.8. Triazoloacridin-6-ones

NSC645827 85 (Figure 30) is a triazoloacridin-6-one compound that was identified by Stratford and co-workers using virtual screening of the NCI databases to be a lead NQO1 inhibitor in the low micro-molar potency range.

Figure 30. The structure of triazoloacridin-6-one NSC645827 (85).

Analogues of the lead 85 were synthesized and evaluated for their NQO1 and also NQO2 inhibition activity. This resulted in introducing three N-oxide derivatives (Table 6) that can specifically and potently inhibit NQO2 in the low 100 nM range.

Table 6. NQO2 inhibition data for N-oxide derivatives of triazoloacridin-6-one

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
<td>H</td>
<td>167 ± 42</td>
</tr>
<tr>
<td>87</td>
<td>Br</td>
<td>117 ± 29</td>
</tr>
<tr>
<td>88</td>
<td>OCH₃</td>
<td>98 ± 10</td>
</tr>
</tbody>
</table>

3.1.9. Imidazoloacridin-6-ones

Series of the National Cancer Institute (NCI) compounds with imidazoloacridin-6-one scaffold were tested for their ability to inhibit the NQO2 enzyme by Stratford and co-workers. The imidazoloacridin-6-one NSC660841 89 (Figure 31) was identified as a lead NQO2 inhibitor with an IC₅₀ of 6 nM.
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![Figure 31. The structure of imidazoloacridin-6-one NSC660841 (89).](image)

In order to develop potentially potent NQO2 inhibitors, analogues containing the imidazoloacridin-6-one scaffold were synthesized. An N-oxide moiety was introduced to many imidazoloacridin-6-ones to ensure their selectivity as NQO2 inhibitors without DNA binding effects (Table 7). The imidazoloacridin-6-ones with the N-oxide moiety proved to be potent inhibitors of the NQO2 enzyme in cells at non-toxic concentrations.98

**Table 7. NQO2 inhibition data for N-oxide derivatives of imidazoloacridin-6-one**98

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R₁</th>
<th>R₂</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>OCH₃</td>
<td>H</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>91</td>
<td>OH</td>
<td>H</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>92</td>
<td>Br</td>
<td>CH₃</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>93</td>
<td>H</td>
<td>CH₃</td>
<td>56 ± 10</td>
</tr>
</tbody>
</table>

**3.1.10. Indolequinones**99

The compounds with the indolequinone scaffold 94 (Figure 32) can inhibit the NQO2 enzyme selectively. The observed selective inhibition of NQO2 is mechanism-based (suicide substrate) involving irreversible modification of the protein.
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![Figure 32. Indolequinone scaffold (94).](image)

3.1.11. Quinoline and pyrroloquinoline ammosamide analogues

Ammosamide B (Figure 33) is a natural product isolated from marine *Streptomyces* strain CNR-698. Ammosamide B potently inhibits NQO2 with an IC$_{50}$ value of 61.0 nM.

![Figure 33. The structure of ammosamide B (95).](image)

Several bicyclic 96 and tricyclic 97 analogues of ammosamide B (Figure 34) were synthesized to explore the structural requirements for NQO2 inhibitory activity.

![Figure 34. Bicyclic (96) and tricyclic (97) analogues of ammosamide B.](image)

The IC$_{50}$ values for the synthesized compounds ranged from 4.1-25.2 µM. The compounds that were structurally distinct from the scaffold of ammosamide B were not active as NQO2 inhibitors. The most potent compound was the N-methyl derivative at C-8 of ammosamide B 98 (Figure 35), with an IC$_{50}$ value of 4.1 nM.
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![Figure 35](image1.png)

**Figure 35.** The structure of the most potent analogue of ammosamide B (98).

### 3.1.12. 9-Aminoacridine

NSC13000 99 (Figure 36) is a 9-aminoacridine compound that was identified by Stratford and co-workers using virtual screening of the NCI database to be a lead NQO2 inhibitor with an IC\(_{50}\) of 420 nM.

![Figure 36](image2.png)

**Figure 36.** The structure of 9-aminoacridine (99).

### 3.1.13. Ellipticine

Virtual screening of the NCI database by Stratford and co-workers, led to the discovery of ellipticine compounds as NQO2 inhibitors with an IC\(_{50}\) range of 20-160 nM (Table 8).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC71795 (100)</td>
<td>H</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>NSC164016 (101)</td>
<td>(CH(_2))(_2)NHCH(_2)C(_6)H(_5)</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>NSC322087 (102)</td>
<td>CH(_3)C(_6)H(_5)</td>
<td>30 ± 0.0</td>
</tr>
<tr>
<td>NSC12547 (103)</td>
<td><img src="image3.png" alt="Compound" /></td>
<td>160 ± 50</td>
</tr>
</tbody>
</table>
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3.1.14. Quinolines

Series of quinolines were found to inhibit NQO2 in the low nanomolar range. The virtual screening of the NCI database by Stratford and co-workers, led to the discovery of several quinoline compounds as NQO2 inhibitors with IC\textsubscript{50} values of 40-640 nM (Table 9).

Table 9. NQO2 inhibition data for quinoline compounds

<table>
<thead>
<tr>
<th>NSC No.</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>R\textsubscript{3}</th>
<th>IC\textsubscript{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>76750 (104)</td>
<td>H</td>
<td>H</td>
<td>500 ± 60</td>
<td></td>
</tr>
<tr>
<td>13484 (105)</td>
<td>Ph</td>
<td>OCH\textsubscript{3}</td>
<td>550 ± 50</td>
<td></td>
</tr>
<tr>
<td>101984 (106)</td>
<td>H</td>
<td>H</td>
<td>640 ± 50</td>
<td></td>
</tr>
<tr>
<td>617933 (107)</td>
<td></td>
<td></td>
<td>40 ± 10</td>
<td></td>
</tr>
<tr>
<td>273829 (108)</td>
<td>n = 7</td>
<td></td>
<td>250 ± 50</td>
<td></td>
</tr>
<tr>
<td>270904 (109)</td>
<td>n = 8</td>
<td></td>
<td>170 ± 90</td>
<td></td>
</tr>
</tbody>
</table>
3.1.15. Furan-amidines

The virtual screening of the NCI database and NQO2 enzyme studies performed by Nolan and co-workers identified three compounds with furan-amidine scaffold as lead NQO2 inhibitors (Table 10). The scaffold of furan-amidines can be classified into symmetric (compounds 111 and 112) or asymmetric (compound 110).

Table 10. Furan-amidine lead compounds as inhibitors of NQO2

<table>
<thead>
<tr>
<th>NSC</th>
<th>R₁</th>
<th>R₂</th>
<th>NQO2 Inhibition IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17602</td>
<td>H</td>
<td>H</td>
<td>140 ± 40</td>
</tr>
<tr>
<td>305831</td>
<td>C(=NH)NH₂</td>
<td>H</td>
<td>630 ± 70</td>
</tr>
<tr>
<td>305836</td>
<td>C(=NH)NH₂</td>
<td>CH₃</td>
<td>50 ± 10</td>
</tr>
</tbody>
</table>

3.1.16. Mode of binding of the NQO2 inhibitors in the active site

The binding of the inhibitors in the active site of the NQO2 protein was studied. The inhibitors of the NQO2 enzyme exhibit a flat conformation in order to fit inside the narrow cavity of the active site. They bind deeply inside the active site cavity co-planar with the isoalloxazine ring of the FAD molecule. FAD is involved in the enzyme reduction activity. Any structural modification which disfavours this position will lead to inactive compounds, e.g. tetramethoxy analogues of casimiroin are inactive compared to mono-, di-, and tri-methoxy analogues because of the steric bulk of the fourth methoxy groups that disturbs the co-planarity.

The occupancy of the active site area is different among the known NQO2 inhibitors. Small inhibitors such as, casimiroin 73 (Figure 37) and its analogues occupy two thirds of the active site area. Larger inhibitors such as flavones, resveratrol 60 (Figure 38), triazoloacridin-6-ones, and imidazoloacridin-6-ones occupy the whole...
area of the active site. All the inhibitors maintain their co-planar situation with the FAD molecule through hydrophobic interactions with the isoalloxazine ring of the FAD molecule and the side chains of the amino acid residues lining the inner surface of the cavity. The hydrophobic interaction between the enzyme and the inhibitors is very important because of the highly hydrophobic nature of the active site cavity. In addition, the inhibitors form hydrogen bonds with hydrophilic amino acids at the sides of the cavity either directly or through water bridges.

Figure 37. Casimiroin (73) occupies two thirds of the active site area of NQO2 (PDB code 3GAM; Resolution at 1.98 Å).
Figure 38. Resveratrol (60) occupies the whole area of the active site area of NQO2 (PDB code 1SG0; Resolution at 1.50 Å).\textsuperscript{55}

4. Amidine functional group

Amidine is a binitrogen analogue of the carboxylic acid and ester groups (Figure 39). The amino nitrogen free electrons are in conjugation with the \( \pi \)-electrons of the imine (C=N) double bond. The bonds between the central carbon and the two nitrogen atoms have partial double bond properties because of resonance. Amidine combines the properties of azomethine-like C=N double bond and amide-like C-N single bond.\textsuperscript{101}

\[
\begin{align*}
&\text{Amidine} & \text{Carboxylic acid} & \text{Ester} \\
&\text{NR'} \quad \text{NR''} & \text{R' OH} & \text{R' O- R''}
\end{align*}
\]

Figure 39. Amidine and its carbonyl analogues.

Amidine is an organo-superbase with pK\(_a\) range 5.0-12.0. The basicity of the amidine depends on the extent and type of substitution at the imino and amino nitrogen atoms and the central carbon atom. The protonation occurs at the imino nitrogen atom, so the substitution at this nitrogen atom has the largest influence on the pK\(_a\) value of amidines followed by substitution at the functional carbon atom.\textsuperscript{102}

Amidine is more basic than the amine functional group and less basic than guanidine. The pK\(_a\) values of methylamine, acetamide and guanidine are 10.6, 12.4
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and 13.6, respectively. The high basicity of amidine and guanidine is due to the formation of a highly effective conjugation system after the protonation (Figure 40).\textsuperscript{102}

\[
\begin{align*}
\text{Amidine} & : \quad \begin{array}{c}
\text{R} - \text{NH} - \text{R} \\
\text{NH} - \text{R} \\
\end{array} \quad \text{H}^+ \quad \begin{array}{c}
\text{R} - \text{NH} - \text{R} \\
\text{NH}^+ - \text{R} \\
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{Guanidine} & : \quad \begin{array}{c}
\text{RHN} - \text{NH} - \text{RHN} \\
\text{NH} - \text{RHN} \\
\end{array} \quad \text{H}^+ \quad \begin{array}{c}
\text{RHN} - \text{NH} - \text{RHN} \\
\text{NH}^+ - \text{RHN} \\
\end{array}
\end{align*}
\]

Figure 40. The protonation states of amidine and guanidine.

Amidines can be classified into five general types depending on the number and the distribution of the substituents on the amino- and imino-nitrogen atoms.\textsuperscript{103} These types are:

1. Unsubstituted

2. Mono-substituted

3. Symmetric di-substituted

4. Asymmetric di-substituted

5. Tri-substituted

4.1. Amidine-containing pharmacologically active molecules

The amidine group is present in different pharmacologically active molecules. Some examples are given in the following sections.
Chapter I. Introduction

4.1.1. Fibrinogen antagonists

The peptidomimetic low molecular weight fibrinogen antagonists named, as Ro 43-5054 113 and Ro 44-9883 114 are amidine compounds (Figure 41). These two amidine compounds inhibit the binding of fibrinogen to glycoprotein IIb-IIIa leading to the prevention of platelet aggregation. p-Amidino-compounds 113 and 114 are potent fibrinogen antagonists with IC$_{50}$ values of 60 and 30 nM, respectively.

![Figure 41](image.png)

**Figure 41.** Structures of amidine fibrinogen antagonists, Ro 43-5054 (113) and Ro 44-9883 (114).

4.1.2. Anticoagulant drugs

Dibasic (amidinoaryl)propionic acid derivatives are identified as lead anticoagulant compounds. These low molecular weight, non-peptidic and orally active inhibitors are potent and selective inhibitors of factor Xa. The best inhibitor is DX-90659 115 (Figure 42), which can inhibit factor Xa with an IC$_{50}$ value of 70 nM.

![Figure 42](image.png)

**Figure 42.** The structure of factor Xa inhibitor, DX-90659 (115).

5. Aim and objectives of the research

The overall objective of this research is the optimization of the furan-amidine leads (see section 3.1.15 and Table 10) as novel selective NQO2 inhibitors with no off-target effect, for example DNA intercalation. The research involves the design and synthesis of a series of asymmetric furan-amidine and analogues with potential NQO2 inhibition and good drug-like properties.
Chapter I. Introduction

To ensure selectivity of the designed compounds for NQO2, the focus will be on the design of asymmetric furan-amidines and their analogues. To ensure desirable pharmacokinetic properties of the designed inhibitors, the amidine group will be replaced with isosterically similar groups.

Symmetric and asymmetric furan-amidines possess different distribution properties inside the cell, which depends on the number of the positive charges on the compounds. The high basicity of the amidine group leads to its total protonation at physiological pH.\textsuperscript{101} The symmetric furan-amidines with two amidine groups, distribute inside the nucleus and bind to DNA.\textsuperscript{106} In contrast, the asymmetric furan-amidines with one amidine group distributes in the cytoplasm, into mitochondria, without nuclear accumulation.\textsuperscript{106a}
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1. Introduction

The synthesis of the lead furan-amidines 110 and 111, identified as inhibitors of NQO2 from the published virtual screening of the NCI database by Nolan and coworkers,82 was the first objective in this research. The synthesis of these known compounds was required to complete their further biological evaluation; however it also allowed method development of a new synthetic pathway to prepare a range of symmetric and asymmetric furan-amidines and their analogues.

The effect of the isosteric replacements of the furan ring in 110 on NQO2 inhibition activity and water solubility was studied. The furan ring was replaced with a wide range of 5-membered heterocycles such as pyrrole, N-methylpyrrole, thiophene, imidazole, N-methylimidazole and oxazole.

All of the synthesized furan-amidines and their analogues have the highly basic amidine groups. These aryl amide groups are known to give a low oral bioavailability, which can be explained by their high ionization at physiological pH. The reported pKa of benzamidine is 11.89 ± 0.50,101 which makes this compound totally ionized at pH 7.4. The isosteric replacements of the amidine group by less basic imidate (iminoether) (pK_a = 6.2)107, N-aryl amide (neutral), N-aryl amidine (reversed amidine, pK_a = 9.64 ± 0.50) and amidoxime (N-hydroxy amidine, pK_a = 6.53 ± 0.69) groups were completed to study the effect of these changes firstly on NQO2 inhibition ability. These less basic analogues, having more of the neutral form at physiological pH, may enhance the oral bioavailability of the NQO2 inhibitors.

The details of the synthetic routes of all of the targeted compounds are discussed in the following sections.

2. Synthesis of the furan-amidine scaffold

The literature synthetic pathway for the preparation of the furan-amidines, specifically the symmetric furan-amidines, requires the initial preparation of the symmetric 1,4-diketone 4-bromo substituents 116 (Scheme 8).108 The bromine atoms in the 1,4-diketone 116 are substituted later by nitrile groups using a cyanide-containing reagent, such as copper (I) cyanide. This substitution step is usually done after the cyclization of the 1,4-diketones 116 into furan 117. The nitrile groups in compound 118 are the precursors for amidine groups. The conversion of the nitrile groups 118 into amidines 111 is typically completed through the preparation of an imidate hydrochloride (iminoether) intermediate 119. The reaction between imidate
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hydrochloride intermediate with ammonia gas results in the formation of amidine group 111.

Scheme 8. Synthetic pathway for symmetric furan-amidine 111; Reagents and conditions: i- \( \text{H}_2\text{SO}_4, \text{Ac}_2\text{O}, \text{reflux} \); ii- \( \text{CuCN, quinolone, reflux} \); iii- \( \text{EtOH, HCl (g), CHCl}_3, 0^\circ\text{C} - \text{rt} \); iv- \( \text{NH}_3(g), \text{CHCl}_3, \text{rt} \).

The limitations for the use of this synthetic pathway for the synthesis of the symmetric furan-amidines generally and asymmetric furan-amidines specifically are:

1. The use of a highly toxic cyanide reagent to substitute the bromine in step ii. Upon the acidic work-up of this step, the highly toxic hydrogen cyanide gas is formed, therefore, this reagent will not be used unless absolutely necessary.

2. The use of a large volume of dry hydrogen chloride gas to saturate the reaction in step iii. The traditional method of generating hydrogen chloride gas is done by the reaction between concentrated sulfuric acid and sodium chloride salt.\(^{109}\) The disposal of large quantities of concentrated sulfuric acid is not preferable. The alternative use of hydrogen chloride gas cylinders may be an issue because of the highly corrosive properties of hydrogen chloride gas.

3. The use of ammonia gas.

These limitations led to the search for a safer and simpler synthetic pathway for the synthesis of the furan-amidines. An alternative multi-step synthetic pathway for the
synthesis of the furan-amidines was proposed. This pathway depends on the approach described by Suthiwangcharoen and Stephens\textsuperscript{110} (Scheme 9), which starts from reagents already possessing the aryl nitrile groups. The aryl nitrile groups in the starting materials serve as precursors for the aryl amidine groups later.

Scheme 9. Proposed synthetic pathway for symmetric furan-amidine \textsuperscript{111}; Reagents and conditions: i- Paraformaldehyde, HN(CH\textsubscript{3})\textsubscript{2}.HCl, EtOH, reflux; ii- 5-(2-Hydroxyethyl)-3,4-dimethyl-1,3-thiazolium iodide \textsuperscript{128}, NEt\textsubscript{3}, dry THF, reflux,; iii- H\textsubscript{2}SO\textsubscript{4}, Ac\textsubscript{2}O, reflux.

The proposed alternative synthetic pathway was applied for the synthesis of the asymmetric furan-amidine \textsuperscript{110}. The multi-step synthetic pathway was optimized and used later to synthesize the symmetric furan-amidine \textsuperscript{111}. 

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3. Synthesis of asymmetric furan-amidine scaffold: Synthetic pathway optimization

The asymmetric furan-amidine 110 was synthesized using a modified approach to the one described by Suthiwangcharoen and Stephens. The 1,4-diketone 126 was first synthesised through the coupling between Mannich base 125 and 4-formylbenzonitrile 122 under Stetter reaction conditions (Scheme 10). Mannich base 125 was firstly prepared from the reaction between acetophenone 124, paraformaldehyde and dimethylamine hydrochloride under anhydrous acidic conditions. Each step in the synthesis of the asymmetric furan-amidine 110 was discussed in detail in the following sections.

Scheme 10. Proposed synthetic pathway for the asymmetric furan-amidine 110; Reagents and conditions: i- Paraformaldehyde, NH(CH₃)₂.HCl, EtOH, reflux; ii- 5-(2-Hydroxyethyl)-3,4-dimethyl-1,3-thiazolium iodide 128, NEt₃, dry THF , reflux; iii- H₂SO₄, Ac₂O, reflux.


3.1. Mannich base reaction

The Mannich base reaction is an equilibrium reaction, which starts by the formation of Schiff base from the reaction of paraformaldehyde and dimethylamine hydrochloride under acidic conditions. The resulting imine reacts as an electrophile, which is attacked by the enolate form of the methyl aryl ketone (Scheme 11). The best molar ratio to be used for the Mannich base reaction is 1 methyl aryl ketone: 1.5 paraformaldehyde: 1.5 dimethylamine hydrochloride.

\[
\begin{align*}
\text{H}_2\text{C}=\text{O} & \xrightarrow{\text{H}^+} \text{H}_2\text{C}=\text{O}^+ \\
\text{C}_{\text{H}_3}\text{N}^+\text{H}^+ & \xrightarrow{\text{H}^+} \text{C}_{\text{H}_3}\text{N}^+\text{H}^+ \text{H}^+ \text{N}^+\text{H}_2\text{C}=\text{O}^+ \\
\text{H}_2\text{O} & \xrightarrow{\text{H}^+} \text{H}_2\text{O}^+ \\
\end{align*}
\]

Scheme 11. The mechanism of the preparation of Mannich base 125.

3.2. Aryl 1,4-diketones synthesis

Aryl 1,4-diketones or γ-diketones are useful intermediates used in the preparation of 5-membered heterocyclic rings such as furan, pyrrole and thiophene. Aryl 1,4-diketones are synthesized by carbon-carbon coupling between two carbonyl compounds. Aryl 1,4-diketones can be classified as symmetric and asymmetric depending on the starting carbonyl compounds used in their synthesis.

The synthesis of the asymmetric 1,4-diketones is challenging when compared to the synthesis of the symmetric 1,4-diketones. This is attributed to the cross reactions between the two different starting carbonyl compounds, which can lead to the formation of different products.
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Two synthetic pathways were used to synthesize the 1,4-diketone 126, the key intermediate in the synthesis of the targeted asymmetric furan-amidine 110. The first synthetic pathway used was the Stetter reaction, which implies the coupling between Mannich base 125 and 4-formylbenzonitrile 122. The Stetter Reaction is a 1,4-addition (conjugate addition) of an aldehyde to an α,β-unsaturated compound, catalyzed by cyanide or a thiazolium salt. This reaction competes with the corresponding 1,2-addition, which is known as the benzoin condensation. The benzoin-condensation is a reversible reaction, but the Stetter reaction leads to more stable products.\textsuperscript{111} The main product derived from this reaction is the 1,4-diketone 126.

The key step in the reaction mechanism is the conversion of the aldehyde carbonyl group from an electrophile to a nucleophile in an umpolung process (Scheme 1) through the use of 5-(2-hydroxyethyl)-3,4-dimethyl-1,3-thiazolium iodide 128 as a catalyst. Catalyst 128 for the Stetter reaction needed to be synthesized which is detailed in section 3.2.1.

The reaction is initiated by the activation of the quaternary thiazolium iodide 128 into ylide 129 through the deprotonation of the aryl hydrogen by triethylamine. Then, the nucleophilic ylide 129 attacks the carbonyl group in 4-cyanobenzylaldehyde 122 forming the tetrahedral intermediate 130. The 1,2-rearrangement of the methylene proton in 130 results in the formation of the carbanion 131. The carbanion 131 reacts with the enone derived from the Mannich bases 125 forming the adduct 132. Finally, the thiazolium compound 128 is expelled generating the 1,4-diketone 126 and completing the catalytic cycle (Scheme 12).\textsuperscript{111}

The Stetter reaction must be carried out in an aprotic solvent and under anhydrous conditions. The best solvent to get a good yield from this heterogeneous reaction is 1,2-dimethoxyethane. Dry DMF, toluene and THF were tried as alternatives to this solvent because of the high toxicity of 1,2-dimethoxyethane on fertility. Dry THF was the best solvent used to prepare the 1,4-diketone 126.
Scheme 12. The mechanism of the Stetter reaction in the synthesis of the 1,4-diketone 126.

The nitrile groups in the 1,4-diketone 126 serve as precursors for the amidine groups. In this synthetic pathway the nitrile group has not been affected by the reaction conditions. In the infrared spectrum, the stretching frequency of the nitrile group in 126 appeared as a strong peak at $2229.3 \text{ cm}^{-1}$ (Figure 43).
The use of the Stetter reaction as the main synthetic pathway in the preparation of the 1,4-diketones 126 had some limitations:

1. The long reaction time of the Stetter reaction under reflux, which is 24-72 hr.
2. The difficult purification of the 1,4-diketones from the Stetter heterogeneous reaction mixture.
3. The poor yield of the 1,4-diketone 126, which was 36%.

The 1,4-diketone 126 was used as a model compound to optimize the conditions needed to synthesize the targeted 1,4-diketone intermediates. The ease in the synthesis of the 1,4-diketones together with a good yield are essential issues to be considered in choosing general synthetic pathway to be applied for the preparation of different 1,4-diketones with different substituents. Also, the symmetrical 1,4-diketone 123 was synthesized using the Stetter reaction in a yield of only 20%.

The new synthetic pathway to synthesize the 1,4-diketone 126 was proposed adapting the method described by Nevar and co-workers. This synthetic pathway requires the coupling between methyl aryl ketone and α-bromomethyl aryl ketones using zinc chloride, triethylamine and t-butanol as a condensation reagent (Scheme 13).
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Ethanol was used as an alternative to tert-butanol because of the lower boiling point of ethanol compared to tert-butanol and its easier evaporation during the work-up step. The 1,4-diketone 126 was prepared using this synthetic pathway by the coupling between the methyl aryl ketone 120 and the α-bromomethyl aryl ketone 133 (Scheme 13).

Scheme 13. Synthesis of 1-(4-cyanophenyl)-4-phenyl-1,4-butadiene 126 through the coupling between methyl aryl ketone 120 and α-bromomethyl aryl ketone 133;
Reagents and conditions: i- ZnCl₂, NEt₃, EtOH, dry toluene, rt.

In general, the methyl aryl ketone reacts as an nucleophile and α-bromomethyl aryl ketone reacts as an electrophile (Scheme 14). The aldol condensation between the enolate form of the methyl aryl ketone 120 and the α-bromomethyl aryl ketone 133 leads to the formation of the 4-bromo-3-hydroxyketone intermediate 134. The intermediate 134 subsequently rearranges to a 1,4-diketone through the formation of activated cyclopropane intermediate 135.\textsuperscript{112}
Scheme 14. The mechanism of the synthesis of 1-(4-cyanophenyl)-4-phenyl-1,4-butadione by the coupling between methyl aryl ketone 120 and \( \alpha \)-bromomethyl aryl ketone 133.

The formation of unsymmetrical 1-(4-cyanophenyl)-4-phenyl-1,4-butadiene 126 using the two synthetic pathways detailed above was confirmed by the use of \( ^1 \)H NMR spectroscopy. In the \( ^1 \)H NMR spectrum, the chemical shift of the \( \text{COCH}_2\text{CH}_2\text{CO} \) protons (H-2 and H-3 protons) were observed as two triplets merged to form a multiplet in the region between 3.42 ppm to 3.52 ppm with an integration of 4 protons (Figure 44).
Figure 44. $^1$H NMR (CDCl$_3$) spectrum of 1-(4-cyanophenyl)-4-phenyl-1,4-butadione 126.

Also, the symmetric 1,4-diketone 123 was synthesized through the coupling between the methyl aryl ketone 120 and the $\alpha$-bromomethyl aryl ketone 136 (Scheme 15).

Scheme 15. The synthetic pathway for the symmetric 1,4-diketone 123 through the coupling between methyl aryl ketone 120 and $\alpha$-bromomethyl aryl ketone 136;

Reagents and conditions: i- ZnCl$_2$, NEt$_3$, EtOH, dry toluene, rt.

Table 11 shows the differences between the two synthetic pathways used in the preparation of 1,4-diketones 123 and 126.
Table 11. The differences between the two synthetic pathways used for the preparation of the 1,4-diketones 123 and 126

<table>
<thead>
<tr>
<th></th>
<th>Stetter reaction</th>
<th>Methyl aryl ketone and the α-bromomethyl aryl ketones coupling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction conditions</td>
<td>Reflux</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Reaction time</td>
<td>2-3 days</td>
<td>3-4 days</td>
</tr>
<tr>
<td>1,4-diketones purification</td>
<td>Column chromatography</td>
<td>Crystallization</td>
</tr>
<tr>
<td>% Yield of 123</td>
<td>20</td>
<td>61</td>
</tr>
<tr>
<td>% Yield of 126</td>
<td>36</td>
<td>39</td>
</tr>
</tbody>
</table>

Considering these results, the coupling between the methyl aryl ketones and α-bromomethyl aryl ketones was used as the main route for the synthesis of other 1,4-diketones intermediates, required to prepare the target heterocyclic compounds.

3.2.1. Synthesis of 5-(2-hydroxyethyl)-3,4-dimethyl-1,3-thiazolium iodide catalyst 128

Thiazolium catalyst 128 was synthesized by heating the compound 137 at reflux with iodomethane under anhydrous conditions (Scheme 16). The structure of the thiazolium catalyst 128 was confirmed by $^1$H (Figure 45), $^{13}$C and DEPT135 NMR and IR spectroscopy. The purity of the catalyst 128 was confirmed using thin layer chromatography (TLC). The catalyst was used in the Stetter reaction step (as detailed in section 3.2.) without any further purification.

Scheme 16. Route for the synthesis of the thiazolium catalyst 128; Reagents and conditions: i- CH$_3$I, dry CH$_3$CN, reflux.
3.3. 2,5-Diarylfuran synthesis

Substituted 2,5-diarylfurans are aromatic compounds synthesized starting from 1,4-dicarboxyls compounds, such as 1,4-diketones. The 1,4-diketones are cyclized into a furan ring in the presence of acid. The acid-catalyzed cyclization of 1,4-diketones into furans is known as the Paal-Knorr furan synthesis (Scheme 17). The first step in the reaction is the rapid and reversible protonation of one of the carbonyls forming the positively charged intermediate. Intermediate undergoes a concerted enolization and ring closure to give, which is the slowest step in the reaction. The dehydration of leads to the formation of 2,5-diarylfuran. The dehydration step is rapid and irreversible due to the aromatization.
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Scheme 17. Mechanism of Paal-Knorr furan synthesis.

The acid-catalyzed cyclization of 1,4-diketone 126 to give the 2,5-diaryl furan 127 was accomplished using two synthetic pathways. The first synthetic pathway requires the heating of the 1,4-diketone 126 in acetic anhydride in the presence of few drops of sulfuric acid (Scheme 18).\(^{113}\)

Scheme 18. Synthetic pathway to give 4,4’-(furan-2,5-diyl)benzonitrile 127; Reagents and conditions: i- Ac\(_2\)O, H\(_2\)SO\(_4\), reflux.

Using this synthetic pathway 4-(5-phenylfuran-2-yl)benzonitrile 127 was obtained in a high yield of 78.0%. The formation of 127 was confirmed by \(^1\)H NMR spectroscopy. The formation of the furan ring was confirmed by \(^1\)H NMR spectroscopy as the furan protons H-3’ and H-4’ were observed as doublets at 6.81 ppm (\(J\) 3.6 Hz) and 6.93 ppm (\(J\) 3.3 Hz). The phenyl protons H-2”, H-3” and H-4” were observed as a doublet at 7.78 ppm (\(J\) 7.2 Hz), a triplet at 7.45 ppm (\(J\) 7.8 Hz) and a triplet at 7.34 ppm (\(J\) 7.5 Hz), respectively. The phenyl protons H-2 and H-3 were observed as doublets at 7.70 ppm (\(J\) 8.4 Hz) and at 7.83 ppm (\(J\) 8.4 Hz) (Figure 46).
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The harsh acidic conditions of the reaction did not affect the nitrile group. The stretching frequency of the nitrile group 127 was present at 2225 cm\(^{-1}\) in the IR spectrum and at 119.0 ppm in the \(^{13}\)C-NMR spectrum.

A symmetric 2,5-diarylfuran 118 was also synthesized by heating the 1,4-diketone 123 in acetic anhydride in the presence of concentrated sulfuric acid (Scheme 19).

**Figure 46.** \(^1\)H NMR (CDCl\(_3\)) spectrum of 4-(5-phenylfuran-2-yl)benzonitrile 127.

**Scheme 19.** Synthetic pathway for 4,4’-(furan-2,5-diyl)benzonitrile 118; Reagents and conditions: i- Ac\(_2\)O, H\(_2\)SO\(_4\), reflux.

The structure of 118 was characterized by \(^1\)H NMR spectroscopy. Because of the plane of symmetry in the compounds, the protons H-3’ and H-4’ of the furan ring
were observed as a singlet at 7.44 ppm. The protons H-2 and H-3 of the phenyl rings were observed as doublets at 7.92 ppm (J 8.1 Hz) and 8.06 ppm (J 8.4 Hz) (Figure 47).

![Chemical Structure](image)

**Figure 47.** $^1$H NMR (DMSO-$d_6$) spectrum of 4,4’-(furan-2,5-diyl)benzonitrile 118.

The second synthetic pathway used to synthesize 127 depends on the use of hydrogen chloride gas generated *in situ* from the reaction between ethanol and acetyl chloride (Scheme 20). The molar ratio that was used from acetyl chloride and ethanol was 8:12 for each 1.0 mole of the 1,4-diketone compound.
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Scheme 20. Synthetic pathway for 4,4’-(furan-2,5-diyl)benzonitrile 127 using *in situ* generated dry hydrogen chloride gas.

This *in situ* hydrogen chloride gas generation method was used as an alternative to the typical method, which uses concentrated sulfuric acid. This was an attempt to avoid handling and disposal of concentrated sulfuric acid.

### 3.4. Carboximidate synthesis

Carboximidates are organic compounds with the general formula RC(=NR’)OR’. They are also known as imino ethers as an imine group (C=N), together with an oxygen atom are connected to the carbon atom. Carboximidates are prepared as intermediates for the preparation of unsubstituted amidines from nitriles. Carboximidates intermediates can be prepared by either base-catalyzed reaction of nitriles with alcohols (Scheme 21a)¹¹⁴ or the Pinner synthesis, which consists of condensing a nitrile and an alcohol under anhydrous conditions in the presence of hydrogen chloride or hydrogen bromide gas (Scheme 21b).¹¹⁵ In the Pinner synthesis the hydrogen chloride gas activates the nitrile group making it liable to be attacked by alcohol leading to the formation of carboximidate hydrochlorides (Scheme 22).

\[
\text{Scheme 21. Pathways for the synthesis of carboximidates.}
\]
According to the research reported by Yadav and Babu for the synthesis of an imidate hydrochloride salt from a nitrile group using dry hydrogen chloride gas, the best molar ratio to use was 1 (nitrile): 8 (acetyl chloride): 12 (alcohol). Utilizing these conditions, it was expected that the reaction between hydrogen chloride gas, ethanol and the 1,4-diketones 126 will form the ethyl imidate hydrochloride compound 142 (Figure 48), however the furan 127 was formed in this reaction (see Scheme 23).

This can be explained by the higher reactivity of the ketone groups towards protonation compared to the nitrile group. The effect of the number of hydrogen chloride gas moles on the product formed from the reaction with 1,4-diketones compounds bearing the nitrile group was studied further.

According to the results reported by Yadav and Babu, the nitrile group reacted with 8 moles of hydrogen chloride gas (generated \textit{in situ} from the reaction between 8 moles of acetyl chloride and 12 moles of alcohol), giving 4 moles of alcohol
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to provide the nucleophile to attack the nitrile group to form the imidate hydrochloride. In this reaction, as the ketone groups were more reactive towards hydrogen chloride gas than the nitrile, the moles needed from acetyl chloride and alcohol were doubled. Using 16 moles of acetyl chloride and 24 moles of ethanol with each mole of the 1,4-diketone 126 resulted in both the cyclization to the furan and the conversion of the nitrile group into the ethyl imidate hydrochloride 143 (Scheme 23). Monitoring of the reaction using $^1$H NMR spectroscopy, showed that the furan ring formed first, followed by the conversion of the nitrile group to ethyl imidate hydrochloride.

![Scheme 23. Synthesis of ethyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 143.](image)

The structure of 143 was confirmed by $^1$H NMR and IR spectroscopy. The furan protons H-3’ and H-4’ were observed as doublets at 7.22 ppm ($J$ 3.3 Hz) and 7.45 ppm ($J$ 3.6 Hz). The phenyl protons H-2”, H-3” and H-4” were observed as a doublet at 7.90 ppm ($J$ 7.8 Hz), triplet at 7.49 ppm ($J$ 7.5 Hz) and triplet at 7.37 ppm ($J$ 7.5 Hz), respectively. The phenyl protons H-2 and H-3 were observed as doublets at 8.08 ppm ($J$ 8.7 Hz) and at 8.17 ppm ($J$ 8.4 Hz). IR spectroscopy confirmed that the nitrile group had been converted to the imidate group, with a new carbon-nitrogen double bond stretch at 1606 cm$^{-1}$. 
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This imidate Pinner synthesis was also used to synthesize the symmetrical diethyl 4,4’-(furan-2,5-diyl)dibenzimidate hydrochloride 119. Compound 119 was synthesized starting from the 1,4-diketone 123 (Scheme 24).

Scheme 24. Synthetic pathway for diethyl 4,4’-(furan-2,5-diyl)dibenzimidate dihydrochloride 119; Reagents and conditions: i- HCl(g), EtOH, CHCl₃, 0 °C- rt.

In order to ensure the synthesis of diethyl 4,4’-(furan-2,5-diyl)dibenzimidate 119 starting from the 1,4-diketones 123, 24 equivalents of hydrogen chloride gas were used to affect the transformation of the two-nitrile groups into ethyl imidate hydrochloride. The symmetrical structure of 119 was confirmed by ¹H NMR spectroscopy. The protons for the ethyl groups were observed as a triplet at 1.52 ppm (J 6.9 Hz) and a quartet at 4.66 ppm (J 6.9 Hz). The furan protons H-3’ were observed as a singlet at 7.54 ppm. The aryl protons H-2 and H-3 were observed as two doublets at 8.15 ppm (J 8.1 Hz) and 8.25 ppm (J 8.4 Hz).

3.5. Amidine functional group synthesis

The most common route reported in the literature for the synthesis of unsubstituted amidines is the Pinner method. As described in the previous section, the Pinner method transforms the nitrile group into the carboximidate by reacting it with hydrogen chloride gas and alcohol. The carboximidate intermediate can then be transformed into the amidine group by the action of ammonia (Scheme 25).¹⁰³

\[
\text{R-CN + HCl(g) + R'OH} \rightarrow \text{R-OR' + NH₃(g) \rightarrow R-NH₂ + R'OH}
\]

Scheme 25. Pinner synthesis of unsubstituted amidine.

An alternative synthesis of the amidine group involves transformation of the nitrile group into the thioamide using hydrogen sulfide gas. The thioimidate can then be
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converted into the unsubstituted amidines using methyl iodide followed by ammonium acetate (Scheme 26).

\[
\text{R-CN} \xrightarrow{\text{H}_2\text{S(aq)}} \text{R-} \text{S} \text{NH}_2 \xrightarrow{\text{CH}_3\text{I}} \text{R-} \text{S} \text{NH} \xrightarrow{\text{NH}_4\text{OAc}} \text{R-} \text{NH}_2
\]

**Scheme 26.** Synthesis of an unsubstituted amidine from nitrile via the thioimidate.

In addition, unsubstituted amidines can be synthesized directly from nitrile by heating the nitrile-containing compound with an ammonium salt in liquid ammonia at a high temperature (150-200 °C) (Scheme 27).

\[
\text{NH}_4\text{X} \xrightarrow{\text{NH}_3 + \text{HX}} \text{R-CN} \xrightarrow{\text{NH}_4^+} \text{R}^+\text{C}^-\text{NH}_2 \xrightarrow{\text{NH}_2^-} \text{R}^-\text{C}^+\text{NH}_2 + \text{NH}_3
\]

**Scheme 27.** Synthesis of an unsubstituted amidine from nitrile using ammonium salts in liquid ammonia.

The nucleophilic addition of amine to a nitrile group using methyl chloro aluminum amide can also afford amidine under mild conditions (Scheme 28).

\[
\text{Cl} \xrightarrow{\text{Al-CH}_3} \text{R-CN} + \text{CH}_3\text{Al(Cl)NH}_2 \xrightarrow{\text{H}_2\text{O}} \text{R}^-\text{C}^-\text{NH}_2 \rightarrow \text{R}^+\text{C}^+\text{NH}_2
\]

**Scheme 28.** Unsubstituted amidine synthesis using aluminum amide reagents.

In another approach, substituted amidine can be synthesized from the reaction of a nitrile with substituted amines. The reaction is catalyzed by copper (I) chloride (Scheme 29).

\[
\text{R-CN} + \text{HN}^-\text{R'} \xrightarrow{\text{CuCl}} \text{R}^-\text{N}^-\text{R'} \xrightarrow{\text{HN}^-\text{R}''} \text{R}^-\text{N}^+\text{R''}
\]

**Scheme 29.** Substituted amidine synthesis using copper (I) chloride.

In this research the Pinner synthesis was used to transform the ethyl benzimidate intermediates 143 and 119 into unsubstituted amidines 4-(5-phenylfuran-2-yl)benzamidine acetate 110 and 4,4''-(furan-2,5-diyl) dibenzamidine acetate 111,
respectively. The ethyl benzimidates 143 and 119 reacted with ammonium acetate (the source of ammonia) in ethanol at room temperature (Scheme 30).\textsuperscript{121}

\textbf{Scheme 30}. Synthetic pathway to give 4-(5-phenylfuran-2-yl)benzamidine acetate 110 and 4,4’-(furan-2,5-diyl)dibenzamidine acetate 111; Reagents and conditions: i-CH\textsubscript{3}COO\textsuperscript{−}NH\textsubscript{4}, EtOH, rt.

The formation of the amidine groups in 110 and 111 was confirmed by the disappearance of the ethyl peaks for ethyl benzimidates 143 and 119 in the \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra and the appearance of the amidine carbons in the \textsuperscript{13}C NMR spectrum at 164.9 and 164.8 ppm, respectively (Figure 49).
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Figure 49. NMR (DMSO-d$_6$) spectra of 4-(5-phenylfuran-2-yl)benzamidine acetate

110: A- $^1$H NMR; B- $^{13}$C NMR.
3.6. Conclusion

A general multi-step synthetic pathway for the synthesis of asymmetric furan-amidines with different substituents (R group) was developed (Scheme 31). The synthetic pathway depends on the use of commercially available methyl aryl ketones and α–bromomethyl aryl ketones as starting materials in the synthesis of the 1,4-diketone intermediates. One of the starting materials has a nitrile group that serves as the precursor for the target amidine group. The 1,4-diketone intermediates are then cyclized to give the furan ring and the nitrile group is transformed into ethyl benzimidate hydrochloride. The imidate group is an intermediate functional group between the nitrile group and the final amidine group. Finally the ethyl imidate hydrochloride intermediates are converted into amidine by the use of ammonium acetate.

Scheme 31. General synthetic pathway for the preparation of asymmetric furan-amidines; A- starting from methyl aryl ketone having a nitrile group; B- starting from α–bromomethyl aryl ketone having a nitrile group; Reagents and conditions: i- ZnCl₂, NEt₃, EtOH, dry toluene or THF, rt; ii- HCl(g), EtOH, CHCl₃, 0 °C- rt; iii- CH₃COO⁺\text{NH₄}, EtOH, rt.
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4. Applications of the optimized multi-step synthetic pathway

The multi-step synthetic pathway developed in section 3, was used to synthesise a range of asymmetric furan-amidines with different substituents on the phenyl ring. This was completed to study the effect of different substituents (electronics and size) on the NQO2 inhibition activity.

The asymmetric furan amidines synthesized are summarized in Table 12. Different substituents were introduced, which can be classified as electron-withdrawing or electron-donating groups. The effect of the electron-withdrawing groups, namely, fluoro 144 and 145, bromo 146 and nitro groups 147 and 148 on NQO2 inhibition activity was studied. The regio-effect for the fluoro- and nitro-groups on NQO2 inhibition activity was also considered through the preparation of different regio-isomers, with meta 144 and 147 and para 145 and 148 analogues. In addition, the effect of the electron-donating groups, namely, methoxy 149, methyl 150, ethyl 151, isopropyl 152 and t-butyl 153 groups on NQO2 inhibition ability was also studied. The length and size of the alkyl chains was evaluated through the preparation of a series of methyl, ethyl, isopropyl and t-butyl substituents.

In addition, one compound with a meta-amidine group 154 was prepared to study the effect of the amidine position on the NQO2 inhibition activity.
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Table 12. The synthesized targeted furan-amidines.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>3-F</td>
</tr>
<tr>
<td>145</td>
<td>4-F</td>
</tr>
<tr>
<td>146</td>
<td>4-Br</td>
</tr>
<tr>
<td>147</td>
<td>3-NO₂</td>
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<tr>
<td>148</td>
<td>4-NO₂</td>
</tr>
<tr>
<td>149</td>
<td>4-CH₃</td>
</tr>
<tr>
<td>150</td>
<td>4-OCH₃</td>
</tr>
<tr>
<td>151</td>
<td>4-CH₂CH₃</td>
</tr>
<tr>
<td>152</td>
<td>4-CH(CH₃)₂</td>
</tr>
<tr>
<td>153</td>
<td>4-C(CH₃)₃</td>
</tr>
<tr>
<td>154</td>
<td>----</td>
</tr>
</tbody>
</table>

The detailed synthesis of the target asymmetric furan-amidines and their precursors is discussed in the following sections.

4.1. Asymmetric aryl 1,4-diketones synthesis

The 1,4-diketones 165-174 with different substituents on the aromatic ring were synthesized from the coupling reaction between the methyl aryl ketones 155-164 and the α-bromomethyl aryl ketone 136 (Scheme 32).
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Scheme 32. Pathway for the synthesis 1,4-diketones 165-174; Reagents and conditions:
i- ZnCl₂, NEt₃, EtOH, dry toluene or THF, rt.

The preparation of the 1,4-diketone intermediates 165-174 is the key step in the synthetic pathway of the target amidine compounds; their preparation in good yields is desirable. Variable yields of the 1,4-diketone intermediates 165-174 were obtained depending on the substituent on the phenyl group of the precursor methyl aryl ketones 155-164 (Table 13).

Table 13. The reaction times and % yields for the synthesis of the 1,4-diketones 165-174.

<table>
<thead>
<tr>
<th>1,4-diketone ID</th>
<th>Reaction time</th>
<th>%Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>165</td>
<td>4 days</td>
<td>16</td>
</tr>
<tr>
<td>166</td>
<td>3 days</td>
<td>26</td>
</tr>
<tr>
<td>167</td>
<td>3 days</td>
<td>56</td>
</tr>
<tr>
<td>168</td>
<td>3 days</td>
<td>47</td>
</tr>
<tr>
<td>169</td>
<td>3 days</td>
<td>51</td>
</tr>
<tr>
<td>170</td>
<td>7 days</td>
<td>27</td>
</tr>
<tr>
<td>171</td>
<td>7 days</td>
<td>9</td>
</tr>
<tr>
<td>172</td>
<td>7 days</td>
<td>15</td>
</tr>
<tr>
<td>173</td>
<td>7 days</td>
<td>11</td>
</tr>
<tr>
<td>174</td>
<td>7 days</td>
<td>9</td>
</tr>
</tbody>
</table>
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The first step in this synthetic pathway is the formation of the enolate anion (conjugate base) of the methyl aryl ketones (see Scheme 14). This slow step is base-catalysed and it was accomplished using triethylamine. Shorter reaction times were observed in the case of starting with the methyl aryl ketones 157-159 giving the 1,4-diketones 167-169 in the best yields. This is attributed to the starting methyl aryl ketones 157-159 having large electron-withdrawing groups on the aryl rings increasing the acidity of the α-hydrogens adjacent to the carbonyl group of the ketone, leading to the stabilization of the negative charge on the enolate anion. In contrast, low yields of 1,4-diketones 171-174 were obtained and attributed to the methyl aryl ketones precursors bearing electron-donating groups 161-164. This can also be explained by the inductive effect of the electron-donating alkyl groups on de-stabilizing the enolate intermediate formed during the reaction.

An attempt to increase the yields of the 1,4-diketones, which were synthesized from the methyl aryl ketones with electron-donating groups, was investigated. The synthesis of the 1,4-diketone 172 was attempted through the coupling between methyl 4-cyanophenyl ketone 120 and the α-bromomethyl 4-ethylphenyl ketone 175 (Scheme 33). In this synthetic pathway, the starting ketone 120 had the electron-withdrawing nitrile group, whereas in the earlier synthesis methyl 4-ethylphenyl ketone 162 had an ethyl electron-donating group.

![Scheme 33. Alternative pathway for the synthesis 1,4-diketone 172; Reagents and conditions: i- ZnCl₂, NEt₃, EtOH, dry toluene, rt.](image)

The percentage yield of the 1,4-diketone 172 obtained from this reaction was 18.0 % compared to 15.0 % obtained from the first synthetic pathway. No improvement can be seen either starting from the methyl aryl ketone 162 or 120. The α-bromomethyl aryl ketone 175 was synthesized as discussed in section 4.1.3.

The methyl aryl ketones 163 and 164 were required to be synthesized, as described in section 4.1.2.
4.1.1. Spectroscopic characterization of the 1,4-diketones 165-174

The structures of the 1,4-diketones 165-174 were characterized by $^1$H, $^{13}$C NMR IR spectroscopy and mass spectrometry. The formation of the 1,4-diketones 165-174 was confirmed by the appearance of two triplet peaks merged as a multiplet or a singlet peak in the aliphatic region (3.42 ppm to 3.52 ppm) of the $^1$H NMR spectra for the COCH$_2$CH$_2$CO protons integrating to 4H.

The presence of the aryl nitrile group (CN) in the 1,4-diketones 165-174 was confirmed by $^{13}$C NMR and IR spectroscopy. The aryl nitrile group is usually observed in the 118-119 ppm region in the $^{13}$C NMR spectrum and the peak is absent from the $^{13}$C-DEPT135 spectrum. In the IR spectrum the nitrile group has a distinct sharp peak with a frequency in the region 2220-2230 cm$^{-1}$.

The 1,4-diketones 165 and 166 with meta-fluoro and para-fluoro atoms, respectively, showed coupling between the fluoro-atom and the protons in the $^1$H NMR spectrum and carbons in the $^{13}$C NMR spectrum.

The $^1$H NMR spectrum of 1-(4-cyanophenyl)-4-(3-fluorophenyl)-1,4-butadione 165 showed the coupling between the fluorine atom and the ortho-protons H-2” and H-4” and the meta-proton H-5” (Figure 50A). The H-2”, H-4” and H-5” were observed as doublet at 7.71 ppm ($^3$J$_{HF}$ = 9.6 Hz), triplet (formally dd) at 7.33 ppm ($^3$J$_{HF}$ = $J_{HH}$ = 8.4 Hz) and quartet (formally ddd) at 7.50 ppm ($^4$J$_{HF}$ = $J_{HH}$ = 6.0 Hz), respectively. $^{13}$C-$^{19}$F Coupling was also observed in the $^{13}$C NMR spectrum of 170 (Figure 50B). The coupling between the fluorine atom and C-1”, C-2”, C-3”, C-4”, C-5” and C-6” was observed as doublets with different coupling constants depending on the proximity of the fluorine to the carbon. The carbons C-1”, C-2”, C-3”, C-4”, C-5” and C-6” were observed at 138.5 ppm ($^3$J$_{CF}$ 6.0 Hz), 120.4 ppm ($^5$J$_{CF}$ 21.0 Hz), 162.9 ppm ($^4$J$_{CF}$ 247.0 Hz), 114.9 ppm ($^3$J$_{CF}$ 22.0 Hz), 130.4 ppm ($^3$J$_{CF}$ 8.0 Hz) and 123.9 ppm ($^4$J$_{CF}$ 3.0 Hz), respectively.
Figure 50. NMR (CDCl₃) spectra of 1-(4-cyanophenyl)-4-(3-fluorophenyl)-1,4-butadione 165: A-¹H NMR; B-¹³C NMR.
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The $^1$H-NMR spectrum of 1-(4-cyanophenyl)-4-(4-fluorophenyl)-1,4-butadione 166 showed the coupling between the fluorine atom and the ortho-protons H-3" and meta-protons H-2" (Figure 51A). The H-3" protons were observed as a triplet at 7.09 ($^3J_{HF} = J_{HH} = 8.4$ Hz) and the protons H-2" were observed as a doublet of doublets at 7.99 ppm ($^4J_{HF} = J_{HH} = 7.8$ Hz). The $^{13}$C-NMR spectrum of 1-(4-cyanophenyl)-4-(3-fluorophenyl)-1,4-butadione 166 showed the coupling between the fluorine atom and the carbon atoms through 1, 2, 3 and 4 bonds. The C-1", C-2", C-3" and C-4" carbons were observed as doublets at 132.9 ppm ($^4J_{CF} 3.0$ Hz), 130.8 ppm ($^3J_{CF} 9.0$ Hz), 115.8 ppm ($^2J_{CF} 22.0$ Hz) and 165.9 ppm ($^1J_{CF} 253.0$ Hz), respectively (Figure 51B). $^{19}$F-NMR spectroscopy showed the presence of one aryl-fluoro atom in the 1,4-diketones 165 and 166 at -111.7 and -104.7 ppm.
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Figure 51. NMR (CDCl₃) spectra of 1-(4-cyanophenyl)-4-(4-fluorophenyl)-1,4-butadione 166: A -¹H NMR; B -¹³C NMR.

4.1.2. Synthesis of the methyl aryl ketones 163 and 164

The methyl aryl ketones 163 and 164 were synthesized through the Friedel-Craft acylation of isopropylbenzene (cumene) 176 and t-butylbenzene 177 with acetyl chloride (Scheme 34). These methyl aryl ketones were used as the building units in the synthesis of the 1,4-diketones 173 and 174 as described in section 4.1.

Scheme 34. Pathway for the synthesis of 163 and 164 using a Friedel-Craft acylation; Reagents and conditions: i- AcCl, AlCl₃, dry DCM, 0 °C - rt.

The Friedel-Craft acylation is an electrophilic aromatic substitution reaction in which the aromatic ring reacts as a nucleophile and attacks the electrophilic acylium ion. The acylium ion is a highly reactive electrophile resulting from the reaction between an acyl chloride and Lewis acid, such as aluminium chloride (Scheme 35). ¹²²
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The substituents on the aromatic ring will direct the reaction position. Alkyl groups are known to be ortho- and para-directing groups because of the stabilization of the positive charge by inductive effect. Because of the bulkiness of the isopropyl and tert-butyl groups, by t.l.c the acylation gave only one product, forming only the methyl aryl ketones 163 and 164, respectively. The structures of 4-isopropylacetophenone 163 and 4-tert-butylacetophenone 164 were confirmed by ¹H-NMR spectroscopy.

4.1.3. Synthesis of α-bromomethyl aryl ketone 175

α-Bromomethyl aryl ketone 175 was synthesized from the reaction of 4-ethylacetophenone 162 with N-bromosuccinimide (NBS) in the presence of p-toluenesulfonic acid (PTSA) as a catalyst (Scheme 36).

Scheme 35. Friedel-Craft acylation mechanism.

Scheme 36. Pathway for the synthesis of 2-bromo-1-(4-ethylphenyl)ethanone 175.

p-Toluenesulfonic acid was used in large amount to aid in the formation of the bromonium ion (Br⁺), which reacted with the enol of 162 (Scheme 37).
Scheme 37. Mechanism of bromination of ketone 162 using NBS in the presence of PTSA.

The structure of 2-bromo-1-(4-ethylphenyl)ethanone 175 was confirmed by $^1$H NMR spectroscopy.

4.2. Synthesis of the aryl 1,4-diketone with a meta-nitrile group 179

Aryl 1,4-diketone 179 was synthesized by the coupling between 3-acetylbenzonitrile 178 and 2-bromoacetophenone 133 using zinc chloride, triethylamine and ethanol as condensation agent (Scheme 38) in a yield of 15.2%. 1-(3-Cyanophenyl)-4-phenyl-1,4-butadione 179 is an asymmetric 1,4-diketone with a meta-nitrile group. This compound was synthesized as an intermediate to prepare the asymmetric furanamidine 154 described in section 4.4. The meta-aryl amidine group is reported to have lower binding affinity towards DNA compared to the para-aryl amidine. If the furanamidine 154 shows good NQO2 inhibition activity, this compound could be a selective NQO2 inhibitor.
Scheme 38. Synthesis of the 1,4-diketone 179; Reagents and conditions: i- ZnCl$_2$, NEt$_3$, EtOH, dry toluene, rt.

4.3. Synthesis of the ethyl imidate intermediates

The ethyl imidate hydrochloride intermediates 180-190 were synthesized by reacting the 1,4-diketones 165-174 and 179 with dry hydrogen chloride and ethanol (Scheme 39). Sixteen equivalents of hydrogen chloride gas were used to ensure firstly the cyclization of the 1,4-diketones and secondly the transformation of the nitrile group into carboximidate, to give the furan-ethyl imidate intermediates.

Scheme 39. Pathway for the synthesis of the ethyl imidate hydrochloride intermediates; 180-190; Reagents and conditions: i- HCl(g), EtOH, CHCl$_3$, 0 °C - rt.

4.4. Synthesis of the targeted amidines

The target amidines 144-154 were synthesized by treating the ethyl imidate hydrochloride intermediates 180-190 with ammonium acetate in ethanol (Scheme 40).
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Good to excellent yields of the amidines 144-154, except for 146 (18.2%) were obtained.

Scheme 40. Synthetic pathway of the targeted amidines; 144-154; Reagents and conditions: i- CH₃COO⁺NH₄, EtOH, rt.

4.5. Physical properties of the amidines 144-154

The synthesized asymmetric furan-amidines 110 and 144-154 showed different solubilities in water (hydrophilicity). A drug candidate must have optimal hydrophilicity properties to be tested in a cell system. The calculated log $P$ value for a drug candidate can be used as an indication for its hydrophilicity. The Log $P$ value of a drug is the logarithm of its (n-octanol-water) partition coefficient $P_{(o/w)}$. The partition coefficient for a substance X can be given by the following equation:

$$P_{(o/w)} = [X]_{o} / [X]_{w}$$

In general, the synthesized amidines 110 and 144-154 showed poor water solubility and the least soluble were the furan-amidines 147 and 148. The study of the predicted log $P$ values (Table 14) for the base-form of the synthesized asymmetric furan-amidines 110 and 144-154 aided in addressing the problem of low water solubility of the synthesized amidines.
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Table 14. The calculated log $P$ values$^{126}$ of the synthesized asymmetric furan-amidines 110 and 144-154.

<table>
<thead>
<tr>
<th>ID</th>
<th>R</th>
<th>Calculated log $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>H</td>
<td>2.8</td>
</tr>
<tr>
<td>144</td>
<td>3-F</td>
<td>2.9</td>
</tr>
<tr>
<td>145</td>
<td>4-F</td>
<td>2.9</td>
</tr>
<tr>
<td>146</td>
<td>4-Br</td>
<td>3.5</td>
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<tr>
<td>147</td>
<td>3-NO$_2$</td>
<td>2.7</td>
</tr>
<tr>
<td>148</td>
<td>4-NO$_2$</td>
<td>2.7</td>
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<tr>
<td>149</td>
<td>4-OCH$_3$</td>
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<td>4-CH$_3$</td>
<td>3.2</td>
</tr>
<tr>
<td>151</td>
<td>4-CH$_2$CH$_3$</td>
<td>3.5</td>
</tr>
<tr>
<td>152</td>
<td>4-CH(CH$_3$)$_2$</td>
<td>3.9</td>
</tr>
<tr>
<td>153</td>
<td>4-C(CH$_3$)$_3$</td>
<td>4.4</td>
</tr>
<tr>
<td>154</td>
<td>----</td>
<td>2.8</td>
</tr>
</tbody>
</table>

One of the ways that was used to increase the hydrophilicity of the asymmetric furan-amidines was to exchange the furan ring into more water-soluble heterocycles. Pyrrole, N-methylpyrrole, imidazole, N-methylimidazole and oxazole were the heterocycles that were chosen. The calculated log $P$ values for the proposed heterocycle-isosteres of the asymmetric furan-amidine 110 are listed in Table 15, all of which are lower than the furan analogue.
Table 15. Calculated log $P$ values$^{126}$ of the proposed asymmetric furan-amidine isosteric analogues of the asymmetric furan-amidine 110

<table>
<thead>
<tr>
<th>ID</th>
<th>Compound structure</th>
<th>Calculated log $P$</th>
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</thead>
<tbody>
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<td>2.8</td>
</tr>
<tr>
<td>191</td>
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<td>2.5</td>
</tr>
<tr>
<td>192</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>2.4</td>
</tr>
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<td><img src="image4" alt="Chemical Structure" /></td>
<td>1.8</td>
</tr>
<tr>
<td>194</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>1.7</td>
</tr>
<tr>
<td>195</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>2.2</td>
</tr>
</tbody>
</table>

5. Asymmetric furan-amidines isosteres

5.1. Hydrophilicity optimization

The isosteres 191-195 of the asymmetric furan-amidine 110 were synthesized with the aim to optimize the water solubility of the potential NQO2 inhibitors. The details of the synthesis of the heterocyclic amidines 191-195 are discussed in the following sections.
5.1.1. Synthesis of substituted 2,5-diarylpyrrole-amidines 191 and 192

The pyrrole ring is a very abundant heterocycle in nature and in commercially available drugs. In general, pyroles are synthesized from the condensation reaction between a 1,4-dicarbonyl compound and a primary amine, which is known as the Paal-Knorr pyrrole synthesis.\textsuperscript{127}

The first step in the synthesis of the pyrrole- 191 and N-methylpyrrole-amidines 192 was the cyclization of the 1,4-diketones 126 into pyrrole 198 and N-methylpyrrole 199. The condensation between the aryl 1,4-diketone 126 and an amine results in the formation of the hemiaminal 196 intermediate. The elimination of water molecule from the hemiaminal 196 gives the imine 197. Finally, the ring closure and elimination of a water molecule yields 2,5-diarylpyrrole compounds 198 (Scheme 41A) and 199 (Scheme 41B). The amines, which were used to synthesize 198 and 199, were ammonium acetate and methylamine, respectively.
Scheme 41. Pathways for the synthesis of: A - 4-(5-phenyl-1H-pyrrol-2-yl)benzonitrile 198; B - 4-(1-methyl-5-phenyl-1H-pyrrol-2-yl)benzonitrile 199.

The structure of 4-(5-phenyl-1H-pyrrol-2-yl)benzonitrile 198 was confirmed by $^1$H NMR spectroscopy. The $N$-H proton was observed as a broad singlet at 8.65 ppm. H-2 and H-3 protons were observed as doublets at 7.68 ppm ($J$ 8.7 Hz) and in the region of 7.56-7.62 ppm merged with H-2” proton of the second aryl ring. H-3” and H-4” were observed as triplets at 7.44 ppm ($J$ 7.8 Hz) and 7.30 ppm ($J$ 7.5 Hz), respectively (Figure 52).
Figure 52. $^1$H NMR (CDCl$_3$) spectrum of 4-(5-phenyl-\textit{H}-pyrrol-2-yl)benzonitrile 198.

The H-3’ and H-4’ protons were observed as triplets at 6.64 ppm and 6.75 ppm. A long-range coupling through four bonds ($J = 2.4\text{-}3.0$ Hz) was observed between $N$-H and the H-3’/ H-4’ protons, possible as they adopt a W arrangement (Figure 53).

Figure 53. The W arrangement of $N$-H, H-3’ and H-4’ protons in 4-(5-phenyl-$H$-pyrrol-2-yl)benzonitrile 198.

To confirm the long-range coupling between $N$-H, H-3’ and H-4’ protons, a D$_2$O exchange experiment was completed. Four drops of D$_2$O were added to the NMR tube and the tube was shaken. The deuterium from D$_2$O replaces the exchangeable $N$-H proton, which could no longer be observed. Due to the lack of $N$-H coupling, H-3’ and...
H-4’ were each observed as a doublet at 6.65 ppm and 6.81 ppm with $J$ values of 3.9 Hz and 3.6 Hz (Figure 54B).

![NMR spectra](image)

**Figure 54.** $^1$H NMR spectrum of N-H, H-3’ and H-4’ protons of 4-(5-phenyl-$H$-pyrrol-2-yl)benzonitrile 198; A-CDCl$_3$; B- 4 drops of D$_2$O + CDCl$_3$.

$^{13}$C NMR spectroscopy also confirmed the structure of 4-(5-phenyl-$H$-pyrrol-2-yl)benzonitrile 198. The peaks of C-3’ and C-4’ of the pyrrole ring were observed at 107.0 ppm and 108.4 ppm and the aryl nitrile peak was observed at 119.3 ppm. Also,
the nitrile peak was observed at 2214 cm\(^{-1}\) in the infrared spectrum, which means that the nitrile group remained intact during the reaction conditions.

The structure of 4-(1-methyl-5-phenyl-1H-pyrrol-2-yl)benzonitrile 199 was confirmed by \(^{1}\)H-NMR spectroscopy. The protons of the N-methyl group were observed as singlet at 3.63 ppm and the H-3\(^{\prime}\) and H-4\(^{\prime}\) protons were observed as doublets at 6.35 ppm (J 3.6 Hz) and 6.51 ppm (J 3.6 Hz). H-2 and H-3 protons were observed as doublets at 7.72 ppm (J 8.1 Hz) and 7.89 ppm (J 8.4 Hz). On the other hand, H-2\(^{\prime}\) and H-3\(^{\prime}\) were observed as a multiplet at 7.45-7.54 ppm and H-4\(^{\prime}\) as a triplet at 7.36 ppm (J 7.2 Hz) (Figure 55).

\[\text{Figure 55.} \ ^{1}\text{H NMR (DMSO-d\(_6\)) spectrum of 4-(1-methyl-5-phenyl-1H-pyrrol-2-yl)benzonitrile 199.}\]

\(^{13}\)C-NMR spectroscopy confirmed the structure of the 4-(1-methyl-5-phenyl-1H-pyrrol-2-yl)benzonitrile 199. The peaks of C-3\(^{\prime}\) and C-4\(^{\prime}\) of the pyrrole ring were observed at 108.2 ppm and 109.2 ppm (Figure 56).
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Figure 56. $^{13}$C NMR (DMSO-d$_6$) spectrum of 4-(1-methyl-5-phenyl-H-pyrrol-2-yl)benzonitrile 199.

The aryl nitrile groups in 198 and 199 were converted into the amidine derivatives 191 and 192 using the Pinner synthesis through the preparation of the ethyl imidate hydrochloride intermediates 200 and 201, respectively (Scheme 42). The amidine was observed at 165.2 ppm in the $^{13}$C NMR spectrum of 4-(1-methyl-5-phenyl-1H-pyrrol-2-yl)benzamidine acetate 192.

Scheme 42. Pathway for the synthesis of 4-(5-phenyl-H-pyrrol-2-yl)benzamidine acetate 191 and 4-(1-methyl-5-phenyl-H-pyrrol-2-yl)benzamidine acetate 192; Reagents and conditions: i- HCl$_2$g, EtOH, CHCl$_3$, 0 °C - rt; ii- CH$_3$COO$^+$NH$_4^+$, EtOH, rt.
5.1.2. Synthesis of substituted imidazole-amidines 193 and 194

Two imidazole-amidines, 4-(4-phenyl-1H-imidazol-2-yl)benzamidine acetate 193 and N-methylimidazole-amidine 194 were synthesized. The first step in the preparation of 193 and 194 was the synthesis of the diarylimidazole 203. The imidazole 203 was synthesized from the reaction between 4-cyanobenzaldehyde 122 and phenylglyoxal monohydrate 202 in the presence of ammonium acetate (Scheme 43).128

\[
\begin{align*}
\text{NC} & \quad \text{122} \\
\text{O} & \quad + \\
\text{H} & \quad \text{H}_2\text{O} \cdot \text{H} \\
\text{202} & \quad \text{i} \\
\text{NC} & \quad \text{203}
\end{align*}
\]

Scheme 43. Synthetic pathway of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203;
Reagents and conditions: i- CH$_3$COO$^+$NH$_4^+$, MeOH, rt.

The structure of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203 was confirmed by $^1$H NMR spectroscopy. The H-3” and H-4” were observed as triplets at 7.41 ppm ($J$ 7.5 Hz) and 7.25 ppm ($J$ 6.9 Hz), respectively. The aryl protons; H-2, H-3, H-4’ and H-2” were present in the range 7.86-8.20 ppm. The N-H proton was observed as a broad singlet at 12.98 ppm (Figure 57). The addition of 2 drops of deuterium dioxide led to the disappearance of the N-H proton from the $^1$H NMR spectrum.
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Figure 57. $^1$H NMR (DMSO-d$_6$) spectrum of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203.

The conversion of the aryl nitrile group in 203 to the amidine using the Pinner synthesis did not work. Attempts to synthesize the ethyl imidate hydrochloride intermediate failed because of the basicity of the nitrogen atom of the imidazole ring ($pK_a$ of the conjugate acid = 6.9). Compound 203 precipitated as hydrochloride salt at the beginning of the reaction in a large range of organic solvents such as chloroform, ethanol and tetrahydrofuran.

The imidazole-amidine 193 was prepared through the conversion of the aryl nitrile in 203 into an amidoxime ($N$-hydroxyamidine) intermediate 204.$^{129}$ The intermediate 204 was then reduced to afford the imidazole-amidine 193 (Scheme 44).$^{130}$
Scheme 44. Pathway for the synthesis of 4-(4-phenyl-1H-imidazol-2-yl)benzamidine acetate 193; Reagents and conditions: i- NH₂OH.HCl, t-BuOK, dry DMSO, 0 °C - rt; ii- HCOO⁻⁺NH₄⁺, Pd/C, AcOH, reflux.

The structure of N-hydroxy-4-(4-phenyl-1H-imidazol-2-yl)benzamidine 204 was confirmed by ¹H NMR spectroscopy. The N-OH and NH₂ protons were observed at 9.75 and 5.85 ppm, respectively (Figure 58), which exchanged with deuterium oxide in the NMR tube.
Figure 58. $^1$H NMR (DMSO-d$_6$) spectrum of N-hydroxy-4-(4-phenyl-1H-imidazol-2-yl)benzamidine 204.

The reaction of the nitrile group 203 with hydroxylamine to form the amidoxime 204 was confirmed by the absence of the nitrile peak in the IR spectrum (Figure 59). The nitrile stretching frequency was observed at 2228.3 cm$^{-1}$ in the IR spectrum of 203.
The conversion of amidoxime 204 into amidine 193 was accomplished by catalytic hydrogen-transfer using ammonium formate as the source of hydrogen. The catalytic hydrogen-transfer is the use of an organic molecule as a donor for hydrogen in the presence of a metal catalyst.\textsuperscript{131} The structure of 4-(4-phenyl-1H-imidazol-2-yl)benzamidine acetate 193 was confirmed by observing the amidine carbon in the $^{13}$C-NMR spectrum at 165.4 ppm. The carbon of the amidoxime group in 204 was observed at 150.3 ppm in the $^{13}$C-NMR spectrum.

$N$-Methylimidazole 205 was synthesized from the reaction of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203 with iodomethane (Scheme 45). There is a possibility to get two regioisomers 205A or 205B from the reaction of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203 with iodomethane.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure59.png}
\caption{IR spectrum of $N$-hydroxy-4-(4-phenyl-1H-imidazol-2-yl)benzamidine 204.}
\end{figure}
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Scheme 45. Pathway for the synthesis of the N-methylimidazole regioisomers 205A and 205B; Reagents and conditions: i- CH$_3$I, KOH, acetone, rt.

Depending on the $^1$H NMR spectrum of the imidazole 205, the methyl group was added to the acidic nitrogen of the imidazole tautomer 203. $^1$H NMR spectrum of 205 (Figure 60) showed the disappearance of the N-H proton that was observed in the $^1$H-NMR spectrum of 203 at 12.98 ppm (see Figure 57). The protons H$_a$ and H$_b$ were observed as doublets at 7.97 ppm ($J$ 8.7 Hz) and 8.00 ppm ($J$ 8.7 Hz). The protons H$_c$, H$_d$, H$_e$ and H$_f$ were observed as a singlet at 7.86 ppm, a doublet at 7.81 ppm ($J$ 7.2 Hz), a triplet at 7.39 ppm ($J$ 7.8 Hz) and a triplet at 7.23 ppm ($J$ 7.2 Hz), respectively.

Figure 60. $^1$H NMR (DMSO-d$_6$) spectrum of N-methylimidazole regioisomers 205A and 205B.
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The regioisomer 205A was formed from the reaction of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203 with iodomethane as confirmed by the NOESY spectrum (Figure 61). A long-range interaction between the N-methyl protons and H-5' can be observed from the NOESY spectrum. The formation of the regioisomer 205A can be explained by an easier attack of N-1' by iodomethane, as N-3' is more sterically hindered as it is adjacent to two aryl rings.

Figure 61. The NOESY (DMSO-d6) spectrum of 4-(1-methyl-4-phenyl-1H-imidazol-2-yl)benzonitrile 205A.
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4-(1-Methyl-4-phenyl-1H-imidazol-2-yl)benzamidine 194 was synthesized from 4-(1-methyl-4-phenyl-1H-imidazol-2-yl)benzonitrile 205A through the formation of the \( N \)-hydroxyamidine intermediate 206 (Scheme 46).

Scheme 46. Synthetic pathway for 4-(1-methyl-4-phenyl-1H-imidazol-2-yl)benzamidine acetate 194; Reagents and conditions: i- NH\(_2\)OH.HCl, t-BuOK, dry DMSO, 0 °C - rt; ii- HCOO\(^+\)NH\(_4\), Pd/C, AcOH, reflux.

5.1.3. Synthesis of oxazole-amidine 195

The oxazole-amidine 195 was synthesized from the key precursor 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209. First the benzamide 209 was prepared from the coupling between 4-cyanobenzoyl chloride 207 and 2-amino-1-phenylethanone hydrochloride 208 in the presence of sodium bicarbonate as the base (Scheme 47).\(^{132}\)

Scheme 47. Reaction for the synthesis of 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209; Reagents and conditions: i- NaHCO\(_3\), DCM, 0 °C - rt.
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The identification of 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209 was confirmed by $^1$H NMR spectroscopy. The protons N-H, H-1’, H-3”, H-4” were observed as a triplet at 9.16 ppm ($J$ 5.7 Hz), a doublet at 4.83 ppm ($J$ 5.7 Hz), a triplet at 7.58 ppm ($J$ 7.8 Hz) and a triplet at 7.70 ppm ($J$ 7.2 Hz), respectively. The protons H-2, H-3 and H-2” were observed as a multiplet at 7.99-8.08 ppm (Figure 62).

![Figure 62](image_url)

Figure 62. $^1$H NMR (DMSO-d$_6$) spectrum of 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209.

It was then anticipated that hydrogen chloride gas would both cyclize 209 to the oxazole and convert the aryl nitrile to the imidate, however only ethyl 4-((2-oxo-2-phenylethyl)carbamoyl)benzimidate hydrochloride 210 was formed without the cyclization of the oxazole heterocycle (Scheme 48). The structure of 210 was confirmed by $^1$H NMR spectroscopy and IR spectroscopy. The formation of ethyl imidate 210 was confirmed by the appearance of methyl and methylene proton peaks as a triplet at 1.51 ppm ($J$ 6.9 Hz) and a quartet at 4.66 ppm ($J$ 6.9 Hz), respectively, in $^1$H NMR spectrum. IR spectroscopy confirmed the disappearance of the nitrile peak, but also the presence of two carbonyl groups (ketone stretching was observed at 1694 cm$^{-1}$ and secondary amide stretching was observed at 1647 cm$^{-1}$ and 1533 cm$^{-1}$) for the non-cyclized
compound. The absence of cyclization was also consistent with a doublet peak in the $^1\text{H}$ NMR spectrum at 4.84 ppm ($J\ 5.7$ Hz) integrating to 2H for H-1”.

Scheme 48. Synthetic pathway for 4-((2-oxo-2-phenylethyl)carbamoyl)benzimidate hydrochloride 210; Reagents and conditions: i- HCl$_{(g)}$, EtOH, CHCl$_3$, 0 °C - rt.

The cyclization to the oxazole was accomplished under alternative conditions: the reaction of 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209 in acetic anhydride with a few drops of concentrated sulfuric acid at room temperature led to the formation of the 2,5-diphenyloxazole ring 211 within minutes (Scheme 49).

Scheme 49. Pathway for the synthesis of 4-(5-phenyloxazol-2-yl)benzonitrile 211; Reagents and conditions: i- Ac$_2$O, conc. H$_2$SO$_4$, rt.

The formation of an oxazole from the dehydration of 2-acylaminoketone is known as Robinson-Gabriel synthesis.$^{133}$ The study of the mechanism of the reaction showed that the oxygen of the amide is involved in the oxazole ring formation and the oxygen of the ketone group is expelled during the reaction (Scheme 50).$^{134}$
Scheme 50. The mechanism for the synthesis of 2,5-diaryloxazole 211.

The product 4-(5-phenyloxazol-2-yl)benzonitrile 211 was characterized by $^1$H NMR spectroscopy. The H-4’ proton of the oxazole ring was observed as a singlet at 7.97 ppm (Figure 63). The nitrile had not reacted, as confirmed by the IR spectrum.
Figure 63. $^1$H NMR (DMSO-d$_6$) spectrum of 4-(5-phenyloxazol-2-yl)benzonitrile 211.

4-(5-Phenyloxazol-2-yl)benzonitrile 211 was converted to the oxazole-amidine 195 through the formation of the amidoxime intermediate 212. The reduction of the amidoxime 212 resulted in the formation of amidine 195 (Scheme 51). The structure of the amidine 195 was confirmed by $^1$H NMR spectroscopy.

Scheme 51. Pathway for the synthesis of 4-(5-phenyloxazol-2-yl)benzamidine acetate 195; Reagents and conditions: i- NH$_2$OH.HCl, t-BuOK, dry DMSO, 0 °C - rt; ii- HCOO$^+$NH$_4$, Pd/C, AcOH, reflux.
5.2. Hypothesized correlation between compound structure and NQO2 inhibitory activity

The overall objective of the research is the synthesis of novel, potent and selective inhibitors of the NQO2 enzyme. Many isosteric replacements and structural modifications of the lead asymmetric furan-amidine 110 have been completed in the search for a potent NQO2 inhibitor with optimal drug-like properties.

5.2.1. Synthesis of an asymmetric 3,4-disubstituted furan-amidine

The best lead furan-amidine with the highest inhibition activity against the NQO2 enzyme was the symmetric 3,4-dimethyl-substituted furan-amidine 112 (Figure 64 and Table 10) with an IC$_{50}$ of 50 nM.\textsuperscript{82} As the symmetric furan-amidines are known to be DNA intercalators, an asymmetric 3,4-dimethylfuran-amidine 213 (Figure 64) may prove to be a potent and selective NQO2 inhibitor without off-target effects, e.g. DNA intercalation.

![Figure 64. Structures of the symmetric furan-amidine 112 and the proposed asymmetric 3,4-dimethylfuran-amidine 213.](image)

The synthesis of furan-amidine 213 utilises 2,5-diphenylfuran 215 as the starting material. Heating 2,5-diphenylfuran 215 at reflux with paraformaldehyde in 33% (wt) HBr in acetic acid solution gave 3,4-di(bromomethyl)furan 216. The bromomethyl substituents on C-3 and C-4 of the furan ring 216 were reduced into methyl groups 217 using lithium aluminum hydride (LiAlH$_4$) to give 217.\textsuperscript{108} The methylation of C-3 and C-4 of the furan ring is not possible if the nitrile group is present in the compound as the reaction conditions are too harsh for the nitrile group. Consequently, the starting 1,4-diketone 214 in this synthetic pathway has a \textit{para}-bromine atom, which is substituted by copper cyanide to give 218 (Scheme 52).
Scheme 52. Synthetic pathway for the preparation of 4-((3,4-dimethyl-5-phenylfuran-2-yl)benzamidine 213; Reagents and conditions: i- Ac₂O, H₂SO₄, reflux; ii- paraformaldehyde, 33% wt HBr in AcOH, reflux; iii- LiAlH₄, dry THF, rt; iv- CuCN, quinoline, reflux.

To avoid the use of the toxic copper cyanide, an alternative synthesis of the asymmetric furan-amidine 213 was proposed starting from the preparation of the key aryl-substituted 1,4-diketones intermediate 219, which has a nitrile group as the amidine precursor (Scheme 53).
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Scheme 53. Proposed alternative synthetic pathway for the preparation of 4-(3,4-dimethyl-5-phenylfuran-2-yl)benzamidine 213; Reagents and conditions: i- Ac₂O, H₂SO₄, reflux.

The synthesis of 1,4-diketone 219 was proposed from the coupling between ethyl aryl ketone 220 and the α-bromoethyl aryl ketone 221 using diethylamido magnesium bromide as condensation agent (Scheme 54). Diethylamido magnesium bromide is prepared in situ from the reaction between ethyl magnesium bromide and diethylamine.¹³⁵

Scheme 54. Synthetic pathway of the 1,4-diketone 219; Reagents and conditions: i- EtMgBr, Et₃NH, dry THF, 0 °C - rt.

The ethyl aryl ketone 220 is commercially available on a kilogram scale. The difficulty of purchasing smaller quantities of this reagent led to the proposal of the synthesis of aryl mono-substituted 1,4-diketone 222 to serve as an intermediate in the synthesis of the 4-methylfuran-amidine 223 (Scheme 55). The 1,4-diketone 222 was synthesized through the coupling between the methyl aryl ketone 220 and α-bromomethyl aryl ketone 221 using in situ prepared diethylamido magnesium bromide, followed by triethylamine.
Scheme 55. Pathway for the synthesis of 4-(4-methyl-5-phenylfuran-2-yl)benzamidine 223; Reagents and conditions: i- EtMgBr, Et$_3$NH, dry THF, 0 °C - rt.

Unexpectedly, the condensation of the methyl aryl ketone 120 and α-bromomethyl aryl ketone 221 led to the formation of the furan 4-(4-methyl-5-phenylfuran-2-yl)benzonitrile 224 instead of the expected 1,4-diketone 222. The structure of compound 224 was confirmed by $^1$H (Figure 65) and $^{13}$C NMR spectroscopy.

Figure 65. $^1$H NMR (DMSO-d$_6$) spectrum for the furan compound 4-(4-methyl-5-phenylfuran-2-yl)benzonitrile 224.
The nitrile group of 224 was observed at 2224 cm$^{-1}$ in the IR spectrum and no peaks were observed for ketone groups, which confirmed that cyclization to give the furan ring had occurred (Figure 66).

![IR spectrum of 4-(4-methyl-5-phenylfuran-2-yl)benzonitrile 224.](image)

**Figure 66.** IR spectrum of 4-(4-methyl-5-phenylfuran-2-yl)benzonitrile 224.

In the mechanism of the reaction to prepare the 1,4-diketone 222 the enolate anion of 120 attacks the carbonyl group of the α-bromoethyl aryl ketone 221 forming the 4-bromo-3-hydroxyketone intermediate A. The 4-bromo-3-hydroxyketone intermediate A converts to the 1,4-diketone 222 through the formation of the unstable cyclopropane intermediate B. Under the basic conditions the presence of the methyl group in 222 caused the diketone to cyclise to give the furan 224 (Scheme 56).
Scheme 5.6. Mechanism for the synthesis of 4-(4-methyl-5-phenylfuran-2-y1)benzonitrile 224.

The nitrile group of 224 was converted into amidine 223 through the formation of the amidoxime 225 (Scheme 5.7). The use of amidoxime as an intermediate in the synthesis of amidine is fully discussed in section 5.1.2.
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Scheme 57. Pathway for the synthesis of 4-(4-methyl-5-phenylfuran-2-yl)benzamidine 223 via amidoxime intermediate 225; Reagents and conditions: i- Ac$_2$O, conc. H$_2$SO$_4$, reflux; ii- NH$_2$OH. HCl, t-BuOK, dry DMSO, 0 °C - rt.

5.2.2. Synthesis of the asymmetric 2,5-diarylthiophene-amidine 226

The thiophene-amidine 226 (Figure 67), an analogue of the lead asymmetric furan-amidine 110, is expected to be a potential inhibitor of NQO2 as the active site is highly hydrophobic in nature, leading to the preference of the hydrophobic inhibitors. Thiophene is a 5-membered ring heterocycle analogue of furan, which is more hydrophobic than the furan itself.

Figure 67. The structure of thiophene-amidine 226.

The synthesis of the thiophene-amidine 226 first required the preparation of 2,5-diarylthiophene 228 from the reaction between the 1,4-diketone 126 and Lawesson’s reagent 227 (Scheme 58).
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Scheme 58. Synthetic pathway for the preparation of the asymmetric 4-(5-phenylthiophen-2-yl)benzamidine 226.

In general, 2,5-diarylthiophenes, such as 228 are synthesized starting from 1,4-diketones using different thionation agents. Thionation agents can be either inorganic phosphorus compound such as sulfur pentasulfide (P₄S₁₀) or organophosphorus compounds, such as Lawesson’s reagent (2,4-bis-(p-methoxyphenyl)-1,2,3,4-dithiaphosphetane disulfide) 227. The use of phosphorus pentasulfide as a thionation agent for the cyclization of 1,4-diketones into 2,5-diarylthiophene has many limitations. These limitations include the need for a large excess of phosphorus pentasulfide, long reaction times, the formation of tarry by-products and variable low yields of 2,5-diarylthiophene are usually obtained.¹³⁶

The use of Lawesson’s reagent is preferred because of the mild reaction conditions together with higher yields of pure 2,5-diarylthiophenes. In general, the heating of Lawesson’s reagent 227 results in the formation of a highly reactive dithiophosphine ylide 229, which is in equilibrium with 227. The dithiophosphine ylide 229 reacts with the 1,4-diketones 126 forming a thiaoxaphosphetane intermediate 230, which decomposes to the corresponding thiokeitone 231 (Scheme 59).¹³⁶b Finally, spontaneous dehydration of the thiokeitone 231 yields the 2,5-diarylthiophene 228.¹³⁷
Scheme 59. Mechanism for the synthesis of 2,5-diaryltiophene 228 using Lawesson’s reagent 227.

The structure of 4-(5-phenylthiophen-2-yl)benzonitrile 228 was confirmed by $^1$H NMR and IR spectroscopy. The H-3’ and H-4’ protons of the thiophene ring were each observed as doublets downfield at 7.64 ppm ($J = 3.9$ Hz) and 7.79 ppm ($J = 3.9$ Hz). Surprisingly, the aryl protons H-2 and H-3 were observed as a singlet integrating to 4H at 7.90 ppm. On the other hand, the aryl protons H-2”, H-3” and H-4” were observed as a doublet at 7.74 ppm ($J = 7.5$ Hz), a triplet at 7.47 ppm ($J = 7.5$ Hz) and a triplet at 7.37 ppm ($J = 7.5$ Hz), respectively.
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The IR spectrum of 228 showed the presence of the nitrile group at 2227 cm\(^{-1}\), which means that the nitrile was not affected by the reaction conditions (Figure 68).

Figure 68. IR spectrum of 4-(5-phenylthiophen-2-yl)benzonitrile 228.

To synthesize the thiophene-amidine 226, the nitrile group in 228 must be transformed into the amidine. The use of Pinner synthetic method to prepare the amidine from nitrile through the formation of an imidate intermediate failed. The conversion of the nitrile group in 228 into ethyl imidate 232 using in situ generated hydrogen chloride gas and ethanol also did not work (Scheme 60).

Scheme 60. Attempted synthesis of ethyl 4-(5-phenylthiophen-2-yl)benzimidate hydrochloride 232; Reagents and conditions: i- HCl\(_{(g)}\), EtOH, CHCl\(_3\), 0 °C - rt.

The alternative synthetic pathway to prepare the thiophene-amidine 226 was through the preparation of amidoxime intermediate 233, followed by reduction of the amidoxime intermediate into amidine (Scheme 61).
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Scheme 61. The alternative synthetic pathway for 4-(5-phenylthiophen-2-yl)benzamidine 226; Reagents and conditions: i- NH₂OH.HCl, t-BuOK, dry DMSO, 0 °C - rt; ii- HCOO⁻⁺NH₄, Pd/C, AcOH, reflux.

The structure of N-hydroxy 4-(5-phenylthiophen-2-yl)benzamidine 233 was confirmed by ¹H NMR spectroscopy. The reduction of the amidoxime group 233 into amidine 226 using ammonium formate as organic hydrogen donor did not work. The starting material 233 was recovered after heating at reflux in acetic acid in the presence of ammonium formate and palladium. This can be explained by poisoning of the palladium catalyst by thiophene. The reduction of 233 into 226 was achieved using triethylsilane as hydrogen donor and palladium (II) chloride as a catalyst (PdCl₂) (Scheme 62).¹³⁸

Scheme 62. The reduction of N-hydroxy 4-(5-phenylthiophen-2-yl)benzamidine 233 using catalytic transfer hydrogenation.

The reduction of N-hydroxy 4-(5-phenylthiophen-2-yl)benzamidine 233 into 4-(5-phenylthiophen-2-yl)benzamidine acetate 226 was confirmed by low resolution
(ESIMS m/z 279.3 for the parent amidine compound) and high resolution mass spectrometry (Mass measured for the parent amidine compound was 279.0952).

5.3. Amidine isosteres: Bioavailability optimization

Aryl amidine compounds are highly basic. The pKₐ of the conjugate acid of benzamidine is 11.8, giving complete ionisation at pH 2.0 (pH of stomach) (Equation 1).

\[ \text{pH} = \text{pK}_a + \log_{10} \left( \frac{[\text{PhC}(=\text{NH})\text{NH}_2]}{[\text{PhC}(=\text{NH}_2^+)\text{NH}_2]} \right) \]  

Drugs with the amidine group showed low oral bioavailability because of the high basicity of the amidine group. The amidine group is protonated at a wide range of pH, which decreases its permeation through the phospholipid layers of the stomach. Several analogues of the asymmetric furan-amidine were synthesized in which the amidine group was isosterically replaced with imidate, N-aryl amidine (reversed amidine) and N-aryl amide and amidoxime (Table 16). The amidine isosteres have lower pKₐ values, which would be expected to enhance oral bioavailability of the compounds.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td><img src="image1.png" alt="Structure 110" /></td>
</tr>
<tr>
<td>234</td>
<td><img src="image2.png" alt="Structure 234" /></td>
</tr>
<tr>
<td>235</td>
<td><img src="image3.png" alt="Structure 235" /></td>
</tr>
</tbody>
</table>
5.3.1. Amidines isosteres: Synthesis of 1,4-diketone substrates

The first step in the synthesis of the amidine analogues 234-240 was the preparation of the key 1,4-diketone intermediates 126 (synthesis reported in section 2.2), 241 and 242. The 1,4-diketones 241 and 242 were synthesized through the coupling between the methyl aryl ketones 158 and 159 and the α-bromomethyl ketone 133 using zinc chloride, triethylamine and ethanol as the condensation agent (Scheme 63). The 1,4-diketones 241 and 242 were obtained in modest yields of 44% and 31%, respectively. The structures of the 1,4-diketones 241 and 242 were confirmed by $^1$H NMR spectroscopy.

Scheme 63. Pathway for the synthesis of 1,4-diketones 241 and 242; Reagents and conditions: i- ZnCl$_2$, NEt$_3$, EtOH, dry toluene, rt.
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The imidate 234 and amidoxime 240 were synthesized starting from the 1,4-diketones 126 as discussed in detail in sections 5.3.7 and 5.3.8, respectively.

5.3.2. Amidines isosteres: 2,5-Diarylfurans syntheses

2-(3-Nitrophenyl)-5-phenylfuran 243 and 2-(4-nitrophenyl)-5-phenylfuran 244 were prepared from the reaction of the 1,4-diketones 241 and 242, respectively, using dry hydrogen chloride gas to catalyse the cyclization to the furan ring (Scheme 64).

\[ \begin{align*} \text{O}_2\text{N} & \quad \text{O} \\ \text{3-NO}_2 & \quad \text{4-NO}_2 \\ 241 & \quad 242 \end{align*} \]

\[ \begin{align*} \text{O}_2\text{N} & \quad \text{O} \\ \text{3-NO}_2 & \quad \text{4-NO}_2 \\ 243 & \quad 244 \end{align*} \]

Scheme 64. Pathway for the synthesis of 2-(3-nitrophenyl)-5-phenylfuran 243 and 2-(4-nitrophenyl)-5-phenylfuran 244; Reagents and conditions: i- HCl(g), CHCl\(_3\), 0 °C - rt.

5.3.3. Amidines isosteres: 2,5-diaryltiopenes syntheses

The thiophenes 2-(3-nitrophenyl)-5-phenylthiophene 245 and 2-(4-nitrophenyl)-5-phenylthiophene 246 were preparation from the reactions of the 1,4-diketones 241 and 242, respectively, with Lawesson’s reagent 227 (Scheme 65).

\[ \begin{align*} \text{O}_2\text{N} & \quad \text{O} \\ \text{3-NO}_2 & \quad \text{4-NO}_2 \\ 241 & \quad 242 \end{align*} \]

\[ \begin{align*} \text{O}_2\text{N} & \quad \text{O} \\ \text{3-NO}_2 & \quad \text{4-NO}_2 \\ 245 & \quad 246 \end{align*} \]

Scheme 65. Pathway for the synthesis of 2-(3-nitrophenyl)-5-phenylthiophene 245 and 2-(4-nitrophenyl)-5-phenylthiophene 246; Reagents: i- Lawesson’s reagent 227, THF, 55 °C.

The structures of the thiophenes 245 and 246 were characterized by \(^1\)H NMR spectroscopy, here discussed for 246. The H-3 and H-4 protons of the thiophene ring in 246 were each observed as doublets at 7.66 ppm (J 3.9 Hz) and 7.84 ppm (J 3.6 Hz). The aryl H-2”, H-3” and H-4” protons were observed as a doublet at 7.75 ppm (J 7.5 Hz), a triplet at 7.47 ppm (J 7.2 Hz) and a triplet at 7.34 ppm (J 7.2 Hz). The aryl H-
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2’and H-3’ protons were each observed as doublets at 7.97 ppm (J 8.4 Hz) and 8.27 ppm (J 8.4 Hz).

5.3.4. Amidines isosteres: Reduction of the nitro-groups into aromatic amines

The nitro-groups in the compounds 243-246 were reduced to amines 247-250 using sodium borohydride in the presence of a catalytic amount of copper sulfate (Scheme 66).140

![Scheme 66. Reduction of the nitro-group in compounds 243-246 to give amines 247-250; Reagents and conditions: i- NaBH₄, CuSO₄, EtOH, 0 °C - rt.](image)

The reduction of the nitro-groups into amines was confirmed by ¹H NMR spectroscopy. The key differences between the ¹H NMR spectra of 3-(5-phenylfuran-2-yl)aniline 247 (Figure 69A) and 2-(3-nitrophenyl)-5-phenylfuran 243 (Figure 69B) are discussed.
Figure 69. A- $^1$H NMR (CDCl$_3$) spectrum of A- 2-(3-nitrophenyl)-5-phenylfuran 243; B- $^1$H NMR (DMSO-$d_6$) spectrum of 3-(5-phenylfuran-2-yl)aniline 247.
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The aryl protons of the aromatic ring A in 243 were shifted up-field upon the reduction of the nitro-group into amine. The peaks of the H-2’, H-4’, H-5’ and H-6’ protons of 243 were shifted up-field from 8.57, 8.12, 7.59 and 8.05 ppm to 6.87, 6.52, 7.08 and 6.94 ppm in 247, respectively. The up-field shift is attributed to the difference in the nature of the nitro-group when compared to the amino-group. The nitro-group is an electron-withdrawing group by resonance, which leads to a decrease in the electron density on the aryl ring A. In contrast, the amino-group is an electron-donating group through resonance, which leads to an increase in the electron density in the aryl ring A (Figure 70).

![Figure 70. Resonance states of the aryl ring A in 3-(5-phenylfuran-2-yl)aniline 247.](image)

5.3.5. Amidines isosteres: N-Aryl amidines “reversed amidine” syntheses

Two N-aryl amidines, namely N-(4-(5-phenylfuran-2-yl)phenyl)acetamidine hydrobromide 235 and N-(4-(5-phenylthiophen-2-yl)phenyl)acetamidine hydrobromide 236 were synthesized from the reaction of the amines 248 and 250, respectively, with S-2-naphthylmethyl thioacetimidate hydrobromide 251 (Scheme 67).\(^{141}\) The synthesis of reagent 251 is discussed in the next section 5.3.5.1.
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Scheme 67. Routes for the synthesis of \( N-(4-(5\text{-phenylfuran-2-yl})\text{phenyl})\text{acetamidine hydrobromide} \) 235, \( X = O \) and \( N-(4-(5\text{-phenylthiophen-2-yl})\text{phenyl})\text{acetamidine hydrobromide} \) 236, \( X = S \).

The \( N\)-aryl amide products 235 and 236 were characterized by \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectroscopy and the spectra for 235 are discussed here. In the \(^1\text{H}\) NMR spectrum, the protons H-2, H-3, H-3’, H-4’, H-2”, H-3” and H-4” were observed as a doublet at 7.34 ppm (\( J = 8.4 \) Hz), a doublet at 7.91 ppm (\( J = 8.7 \) Hz), a doublet at 7.08 ppm (\( J = 3.3 \) Hz), a doublet at 7.14 ppm (\( J = 3.6 \) Hz), a doublet at 7.78 ppm (\( J = 7.2 \) Hz), a triplet at 7.41 ppm (\( J = 7.5 \) Hz) and a triplet at 7.28 ppm (\( J = 7.2 \) Hz), respectively. The methyl protons were observed as a singlet at 2.28 ppm. The three non-equivalent \( N\)-H protons were observed as three singlet peaks at 8.58 ppm, 9.44 ppm and 11.14 ppm. The quaternary amidine carbon was observed at 164.3 in the \(^{13}\text{C}\) NMR spectrum, which was appropriately absent in the DEPT135 spectrum.

5.3.5.1. Synthesis of S-2-naphthylmethyl thioacetimidate hydrobromide 251

S-2-Naphthylmethyl thioacetimidate hydrobromide 251 was required for use as a reagent in the synthesis of \( N\)-aryl amidines (reversed amidines) 235 and 236. S-2-Naphthylmethyl thioacetimidate hydrobromide 251 was synthesized by the alkylation of thioacetamide 252 with 2-bromomethylnaphthalene 253 with heating at reflux in chloroform (Scheme 68). \(^{141c}\)
Scheme 68. Pathway for the synthesis of S-2-naphthylmethyl thioacetimidate hydrobromide 251.

The structure of 251 was characterized by $^1$H NMR spectroscopy. The methyl and methylene protons were observed as singlets at 2.64 ppm and 4.75 ppm, respectively. The aryl protons of the naphthalene ring were observed as multiplets at 7.54-7.56 ppm and 7.91-8.00 ppm.

5.3.6. Amidines isosteres: N-Aryl amide syntheses

Three N-aryl amides, namely N-(3-(5-phenylfuran-2-yl)phenyl)acetamide 237, N-(4-(5-phenylfuran-2-yl)phenyl)acetamide 238 and N-(3-(5-phenylthiophen-2-yl)phenyl)acetamide 239 were synthesized from the reaction of the amines 247, 248 and 249 with acetyl chloride, respectively (Scheme 69).

Scheme 69. Synthetic pathway of N-aryl amides 237-239; Reagents and conditions: i- AcCl, dry CH$_3$CN, rt.

The structures of the N-aryl amides 237-239 were characterized by $^1$H and $^{13}$C NMR spectroscopy. The $^1$H and $^{13}$C NMR spectra for N-(4-(5-phenylfuran-2-yl)phenyl)acetamide 238 is discussed as an example. In the $^1$H NMR spectrum of 238 the methyl group and N-H proton were observed as singlets at 2.06 ppm and 10.06 ppm,
respectively. The aryl protons, H-2, H-3, H-3’, H-4’, H-2”, H-3” and H-4” were observed as a doublet at 7.66 ppm (J 8.1 Hz), a doublet at 7.74 ppm (J 8.4 Hz), a singlet at 6.95 ppm, a singlet at 7.05 ppm, a doublet at 7.79 ppm (J 7.5 Hz), a triplet at 7.44 ppm (J 7.8 Hz) and a triplet at 7.29 ppm (J 7.2 ppm), respectively (Figure 71). In the $^{13}$C-NMR spectrum the amide carbon was observed at 168.3 ppm.

![Figure 71](image)

**Figure 71.** $^1$H NMR (DMSO-d$_6$) spectrum of N-(4-(5-phenylfuran-2-yl)phenyl)acetamide 238.

5.3.7. Amidines isosteres: Carboximidate synthesis

It was anticipated that the heating of ethyl benzimidate hydrochloride 143 at reflux with ammonium chloride salt into a mixture of methanol and water would give the furan-amidine 110, however the isolated product was the methyl imidate 234 (Scheme 70).
Scheme 70. Reaction of 143 gave methyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 234 and not 110; Reagents and conditions: i- NH₄Cl, MeOH/ H₂O, reflux.

Methanol attacked the carbon atom of benzimidate group in compound 143 to give methyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 234. This is an analogue of the asymmetric furan-amidine 110 and was therefore tested for inhibition of NQO2. The imidate group is considered as a much less basic isostere (pKa of conjugate acid 6.2)¹⁰⁷ than the highly basic amidine group, which has a pKa of 11.8.¹⁰¹

The structure of compound 234 was confirmed by ¹H NMR spectroscopy, with the methyl group present as a singlet at 3.94 ppm (Figure 72A). In the ¹³C NMR spectrum, the methyl group was observed at 52.1 ppm and the imidate carbon was observed at 166.8 ppm (Figure 72B).
5.3.8. Amidines isosteres: Amidoxime synthesis

One compound with the amidoxime (N-hydroxyamidine) group 240 was synthesized as an isostere for the asymmetric furan-amidine 110. The amidoxime is considered as a less basic isostere than the amidine as the pKₐ value of the conjugate
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acid is approximately 5-6\textsuperscript{143} compared with 5-12 for the amidine.\textsuperscript{101} In addition, amidoximes are considered as potential prodrugs for amidines and used as isosteres to optimize the oral bio-availability of amidine-containing drugs.\textsuperscript{129, 139} The amidoxime prodrugs are reduced into the parent amidine drugs in human liver microsomes.\textsuperscript{144}

\(N\)-Hydroxy-4-(5-phenylfuran-2-yl)benzamidine 240 was synthesized starting from the 1,4-diketone 126. The 1,4-diketone 126 was cyclized into furan 127, which reacted with hydroxylamine to give 240 (Scheme 71).

\[
\begin{align*}
\text{126} & \xrightarrow{\text{Ac}_2\text{O, conc. } \text{H}_2\text{SO}_4, \text{reflux}} \text{127} \\
\text{127} & \xrightarrow{\text{NH}_2\text{OH, } \text{HCl, } \text{t-BuOK, dry DMSO, } 0 \degree\text{C - rt.}} \text{240}
\end{align*}
\]

**Scheme 71.** Pathway for the synthesis of \(N\)-hydroxy-4-(5-phenylfuran-2-yl)benzamidine 240; Reagents and conditions: i- Ac\(_2\)O, conc. H\(_2\)SO\(_4\), reflux; ii- \(\text{NH}_2\text{OH, HCl, } \text{t-BuOK, dry DMSO, } 0 \degree\text{C - rt.}\)

The characterization of 4-(5-phenylfuran-2-yl)benzonitrile 127 was discussed in Section 3.3. The structure of \(N\)-hydroxy-4-(5-phenylfuran-2-yl)benzamidine 240 was characterized by \(^1\text{H}\) NMR and IR-spectroscopies. The NH\(_2\) and N-OH protons were observed as singlets at 5.85 ppm and 9.69 ppm, respectively (Figure 73). These exchangeable protons disappeared from the \(^1\text{H}\) NMR spectrum after the addition of D\(_2\)O.
6. Furan analogue of resveratrol

Resveratrol 60 is a known inhibitor for NQO2 with an IC\textsubscript{50} of 450 nM\textsuperscript{82}. Resveratrol has a planar conformation allowing its accommodation inside the deep cleft of the NQO2 active site\textsuperscript{55}. Resveratrol has a \textit{trans}-stilbene nucleus with hydroxyl substituents on both aromatic rings. The furan-analogue of resveratrol 250 (Figure 74) with a \textit{cis}-geometry was proposed as a potential inhibitor of NQO2.

The synthetic pathway to prepare 5-((5-(4-hydroxyphenyl)furan-2-yl)benzene-1,3-diol 250 (Scheme 72) first requires the preparation of the starting 1,4-diketone 251.

\textbf{Figure 73.} \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) spectrum of \textit{N}-hydroxy-4-(5-phenylfuran-2-yl)benzamidine 240.

\textbf{Figure 74.} The structures of resveratrol (60) and the furan-analogue of resveratrol (250).
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As the hydroxyl groups can affect the synthetic pathway, they were protected with acetyl groups at the start of the synthesis.

\[
\text{Scheme 72. Pathway for the synthesis of 5(5-(4-hydroxyphenyl)furan-2-yl)benzene-1,3-diol 250; Reagents and conditions: i- HCl(g), CHCl}_3, 0 \, ^\circ\text{C} - rt.}
\]

Two synthetic pathways were proposed to prepare the 1,4-diketone 251 (Scheme 73) and both required the coupling between a methyl aryl ketone and an \(\alpha\)-bromomethyl aryl ketone.

\[
\text{Scheme 73. Pathways for the synthesis of 1-(3,5-di(acetyloxy)phenyl)-4-(acetyloxyphenyl)-1,4-butadione 251.}
\]

The choice between the synthetic pathway A or B to prepare 1-(3,5-di(acetyloxy)phenyl)-4-(acetyloxyphenyl)-1,4-butadione 251 depends on the ease of preparation of the methyl aryl ketones 253 or 254 and the \(\alpha\)-bromomethyl aryl ketones 252 or 255. As the methyl aryl ketone 254 and the \(\alpha\)-bromomethyl aryl ketone 255 were easier to prepare, synthetic pathway B was preferred for the synthesis of 1,4-diketone 251. The syntheses of the compounds 252-255 are discussed further in sections 6.1-6.4.

The coupling between 3',5'-di(acetyloxy)cetophenone 54 and 2-bromo-4'- (acetyloxy)acetophenone 255 was performed using two different coupling agents. With zinc chloride, triethylamine and ethanol as the condensation reaction conditions, no
reaction was observed between 254 and 255 by t.l.c. The use of t-butoxymagnesium bromide as a condensation agent resulted in the formation of 256 instead of the 1,4-diketone 251 without the consumption of 255 (Scheme 74). The reactivity of t-butoxymagnesium bromide led to the de-acetylation of compound 254 and no coupling occurred with compound 255.

Scheme 74. Pathways attempted for the synthesis of 1-(3,5-di(acetyloxy)phenyl)-4-(acetyloxyphenyl)-1,4-butadione 251; Reagents and conditions: i- t-BuOMgBr, dry THF, 0 °C- rt.

The structure of compound 256 was characterized by $^1$H-NMR spectroscopy. Two singlet peaks for two different methyl protons were observed at 2.39 ppm (ester) and 2.55 ppm (ketone). The OH proton was observed as a broad singlet at 7.76 ppm. The aryl protons H-2, H-4 and H-6 were observed as fine triplets (meta-coupling) at 7.29 ppm, 6.84 ppm and 7.20 ppm, respectively (Figure 75).
Figure 75. $^1$H NMR (CDCl$_3$) spectrum of 3-(acetyloxy)-5-hydroxyacetophenone 256.


The synthesis of $\alpha$-bromomethyl aryl ketone 252 was attempted by the $\alpha$-bromination of 3’-5’-dihydroxyacetophenone 257 using copper (II) bromide (Scheme 75).

The expected product was the $\alpha$-bromo-compound 252, but heating at reflux led to a mixture of products. From the $^1$H NMR spectrum, there was evidence for electrophilic aromatic substitution, aryl oxidation and the formation of an $\alpha,\alpha$-dibromo compound.

Scheme 75. Synthetic pathway for $\alpha$-bromo-compound 252; Reagents and conditions: i- CuBr$_2$, EtOAc/DCM, reflux.
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6.2. Synthesis of 4’-(acetyloxy)acetophenone 253

The acetylation of the hydroxyl group in 4’-hydroxyacetophenone 258 was performed by using acetic anhydride as the acetylator agent (Scheme 76). 4-(Dimethylamino)pyridine (DMAP) and triethylamine were used to increase the hydroxyl nucleophilicity to attack the carbonyl group of acetic anhydride.\(^{146}\)

\[
\text{HO-} \quad \text{CH}_3 \quad \xrightarrow{i} \quad \text{O-} \quad \text{H}_3\text{C} \quad \text{O} \quad \text{O-} \quad \text{CH}_3
\]

Scheme 76. Pathway for the synthesis of 4’-(acetyloxy)acetophenone 253; Reagents: i-Ac\(_2\)O, DMAP, NEt\(_3\), DCM, 0 °C - rt.

The structure of 4’-(acetyloxy)acetophenone 253 was characterized by \(^1\)H-NMR spectroscopy. The methyl protons adjacent to the ester group were observed as a singlet at 2.30 ppm and the methyl protons adjacent to the ketone group were observed as a singlet at 2.57 ppm. H-2 and H-3 protons were observed as doublets at 7.97 ppm (\(J = 8.7\) Hz) and 7.17 ppm (\(J = 8.7\) Hz), respectively.

6.3. Synthesis of 3’,5’-di(acetyloxy)acetophenone 254

The acetylation of the hydroxyl groups in 3’,5’-dihydroxyacetophenone 257 was performed by using acetic anhydride as the acetylatior agent (Scheme 77).\(^{147}\) The structure of 3’,5’-di(acetyloxy)acetophenone 254 was characterized by \(^1\)H-NMR spectroscopy.

\[
\text{HO-} \quad \text{CH}_3 \quad \text{CH}_3 \quad \xrightarrow{i} \quad \text{H}_3\text{C} \quad \text{O} \quad \text{O-} \quad \text{CH}_3 \quad \text{O} \quad \text{O-} \quad \text{CH}_3
\]

Scheme 77. Pathway for the synthesis of 3’,5’-di(acetyloxy)acetophenone 254; Reagents: i- Ac\(_2\)O, DMAP, NEt\(_3\), DCM, 0 °C - rt.
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6.4. Synthesis of 2-bromo-4’-(acetylloxy)acetophenone 255

2-Bromo-4’-(acetylloxy)acetophenone 255 was synthesized using a two-step synthetic pathway. The first step involved the preparation of 2-bromo-4’-hydroxyacetophenone using a different brominating agent. Heating 258 at reflux with copper (II) bromide led to the formation of 2-bromo-4’-hydroxyacetophenone 259 and 2,2-dibromo-4’-hydroxyacetophenone 260 in a ratio of 80%: 20%, respectively (Scheme 78). It was not easy to separate the two products 260 and 261 as they have very close Rf values (mobile phase 1.0% MeOH/CHCl3) of 0.41 and 0.49, respectively.

Scheme 78. α-Bromination of 4’-hydroxyacetophenone 258; Reagents and conditions:
i-CuBr2, EtOAc/DCM, reflux.

The 1H NMR spectrum of the reaction mixture showed the presence of the two compounds. The CH2Br protons of 259 were observed as singlet at 4.43 ppm and the CHBr2 protons of 260 was down-field giving a singlet at 6.70 ppm.

The reaction of bromine with 258 at room temperature also led to the formation of both 2-bromo-4’-hydroxyacetophenone 259 and 2,2-dibromo-4’-hydroxyacetophenone 260. The mixture of 259 and 260 was reacted with acetic anhydride to prepare 2-bromo-4’-acetylloxyacetophenone 255. The bromo-atom in 2-bromo-4’-hydroxyacetophenone 259 was replaced by the acetate group forming 2,4’-di(acetylloxy)acetophenone 261 (Scheme 79).
Scheme 79. Pathway for the synthesis of 2,4'-di(acetyloxy)acetophenone 261 and 2,2-dibromo-4'-acetylxyacetophenone 262; Reagents: i- Ac₂O, DMAP, NEt₃, DCM, 0 °C - rt.

The structure of 2,4'-di(acetyloxy)acetophenone 261 was characterized by ¹H NMR spectroscopy. The methyl protons a and b were observed as singlets at 2.25 ppm and 2.35 ppm. The CH₂ protons were observed as a singlet at 5.33 ppm and the aryl protons H-2 and H-3 were observed as doublets at 7.25 ppm (J 8.7 Hz) and 7.98 ppm (J 8.7 Hz) (Figure 76).
Figure 76. $^1$H NMR (CDCl$_3$) spectrum of 2,4'-di(acetyloxy)acetophenone 261.

In contrast, the hydroxyl group in 2,2-dibromo-4'-hydroxyacetophenone 260 was acetylated without acetate substitution of one of $\alpha$-bromo atoms. The difference in $R_f$ values between 261 and 262 was large enough to separate both compounds using column chromatography. The structure of 2,2-dibromo-4'-(acetyloxy)acetophenone 262 was characterized by $^1$H NMR spectroscopy. The CH$_3$ and CHBr$_2$ protons were observed as singlet peaks at 2.28 ppm and 6.57 ppm, respectively.

Depending on these results, 2-bromo-4'-(acetyloxy)acetophenone 255 was synthesized by firstly preparing 4'-(acetyloxy)acetophenone 253, followed by its $\alpha$-bromination using bromine (Scheme 80).

Scheme 80. Pathway for the synthesis of 2-bromo-4'-(acetyloxy)acetophenone 255; Reagents and conditions: i- Br$_2$/ AlCl$_3$, THF, 0 °C - rt.
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The reaction of 4’-(acetyloxy)acetophenone 253 with bromine in the presence of a catalytic amount of aluminium chloride yielded 2-bromo-4’-(acetyloxy)acetophenone 255.¹⁴⁸ No dibromo compound was detected from this reaction. The structure of 2-bromo-4’-(acetyloxy)acetophenone 255 was characterized by ¹H NMR spectroscopy. The CH₃ and CH₂Br protons were observed as singlet peaks at 2.35 ppm and 4.44 ppm, respectively. H-2 and H-3 protons were each observed as doublets at 7.25 ppm (J 8.7 Hz) and 8.05 ppm (J 8.7 Hz).

7. Synthesis of N-ribosyl dihydronicotinamide NRH

For the NQO2 enzyme studies, the synthesis of N-ribosyl dihydronicotinamide NRH was required. NRH was synthesized according to an enzymatic method reported by Long and coworkers⁶³ that utilizes NADH as the starting material. The hydrolysis of NADH to NRH is achieved in two steps (Scheme 81); the first step involves phosphodiesterase enzyme, which cleaves the phosphodiester bond leading to the formation of the phosphate ester of NRH. In the second step the alkaline phosphatase – which works optimally at basic pH¹⁴⁹ removes the phosphate group¹⁵⁰ leading to the formation of NRH. NRH is a stable compound⁷² that will not be affected during the purification process. NRH was purified using preparative HPLC and the sample concentrated on a freeze-dryer.
Scheme 81. Pathway for the enzymatic synthesis of the co-substrate $N$-ribosyl dihydronicotinamide NRH.
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1. Inhibition of recombinant human NQO2 enzyme activity

The ability of the synthesized compounds to inhibit the NQO2 enzyme was determined by a spectrophotometric method using the redox dye 2,6-dichlorophenolindophenol (DCPIP) \(263\) in the presence of \(N\)-ribosyl dihydronicotinamide (\(NRH\)). DCPIP \(263\) is an iminoquinone compound with a blue colour, which is reduced by NQO2 into aminophenol \(264\). The aminophenol \(264\) is a colourless compound compared to \(263\) because of the loss of conjugation with the second phenol ring (Scheme 82). In this method, the rate of DCPIP \(263\) colour change, which correlates to the rate of NQO2 activity, is monitored spectrophotometrically.

![Scheme 82. Reduction of DCPIP 263 by NQO2 in the presence of NRH (R=ribose).](image)

Resveratrol \(60\) was used as the positive control in this assay. The IC\(_{50}\) values of resveratrol \(60\) and the synthesized compounds \(110-154, 191-195, 226\) and \(234-240\) were determined. The IC\(_{50}\) value represents the concentration of a drug that is required for 50% inhibition compared with the control rate \textit{in vitro}. Table 17 summarizes the IC\(_{50}\) values of resveratrol and the synthesized compounds.
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Table 17. The IC$_{50}$ values of resveratrol and the synthesized furan-amidine compounds (compounds 60, 110, 111, 147, 148 were tested by myself and all other compounds were tested by my colleague PhD student Elham Santina).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>IC$_{50}$ (nM) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resveratrol 60</td>
<td>----</td>
<td>913.0 ± 2.3</td>
</tr>
<tr>
<td>110</td>
<td>H</td>
<td>68.0 ± 0.9</td>
</tr>
<tr>
<td>Symmetric furan-amidine 111</td>
<td>para-C(=N)NH$_2$</td>
<td>35.0 ± 0.8</td>
</tr>
<tr>
<td>144</td>
<td>meta-F</td>
<td>98.0 ± 0.5</td>
</tr>
<tr>
<td>145</td>
<td>para-F</td>
<td>505.0 ± 1.2</td>
</tr>
<tr>
<td>146</td>
<td>para-Br</td>
<td>27.0 ± 0.5</td>
</tr>
<tr>
<td>147</td>
<td>meta-NO$_2$</td>
<td>14.5 ± 2.3</td>
</tr>
<tr>
<td>148</td>
<td>para-NO$_2$</td>
<td>15.2 ± 2.2</td>
</tr>
<tr>
<td>149</td>
<td>para-OCH$_3$</td>
<td>107.0 ± 0.8</td>
</tr>
<tr>
<td>154</td>
<td>----</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

The positive control resveratrol 60 showed an IC$_{50}$ value of 913.0 ± 2.3 nM. All of the synthesized asymmetric furan-amidines and their pyrrole and N-methylpyrrole analogues with a para-amidine showed superior inhibitory activity to resveratrol, with IC$_{50}$ values in the nano-molar range. The asymmetric furan-amidines with a para-amidine group and a substituent on the other phenyl group showed very good inhibitory activity in the < 100 nM range, the exception being 145.

The best asymmetric furan-amidines inhibitors with meta- and para-NO$_2$ group 147 and 148, respectively, both showed IC$_{50}$ values of ~15 nM. The low water solubility of the most potent NQO2 inhibitors 147 and 148 limited their further evaluation to be potential drugs for cancer.

The effect of length and branching of the alkyl groups on NQO2 inhibitory ability was studied by preparing asymmetric furan-amidine with homologous alkyl
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substituents 150-153 (Table 18). Both asymmetric furan-amidines 110 (R = H) and 150 (R = Me) showed approximately similar IC_{50} values of ~70 nM. An increase in the alkyl chain length to ethyl and tert-butyl led to the increase of the IC_{50} values by 3 and 8 fold, respectively, when compared with 110. This can be explained by the increase in the lipophilicity of the synthesized compounds with the increase in the alkyl chain length. Compound 152, with the isopropyl group, could not be tested because of its low aqueous solubility.

Table 18. The IC_{50} values of the asymmetric furan-amidine 110 and its alkyl-substituted analogues (tested by my colleague PhD student Elham Santina).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>IC_{50} (nM) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>H</td>
<td>68.0 ± 0.9</td>
</tr>
<tr>
<td>150</td>
<td>Me</td>
<td>72.0 ± 0.9</td>
</tr>
<tr>
<td>151</td>
<td>Et</td>
<td>202.0 ± 0.3</td>
</tr>
<tr>
<td>152</td>
<td>iPr</td>
<td>Poor aqueous solubility</td>
</tr>
<tr>
<td>153</td>
<td>t-Bu</td>
<td>509.9 ± 0.3</td>
</tr>
</tbody>
</table>

The change of amidine from the para-position 110 to the meta-position 154 led to the loss of NQO2 inhibitory activity. The opposite effect was seen with the fluoro-substituent as the meta-fluoro compound 144 showed a five-fold enhanced inhibitory activity compared to the para-fluoro analogue 145.

The isosteric replacement of furan ring 110 into pyrrole 191, N-methylpyrrole 192, imidazole 193, N-methylimidazole 194 and thiophene 226 all led to an increase of the IC_{50} values when compared with 110 (Table 19). This can be rationalized by the loss of the planar geometry, which is an essential property for NQO2 inhibitors in order to be accommodated into the deep active site cleft.55
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Table 19. The IC$_{50}$ values of the asymmetric furan-amidine 110 and its furan isosteric analogues (tested by my colleague PhD student Elham Santina).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>IC$_{50}$ (nM) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Methylfuran-amidine 224</td>
<td>Inactive</td>
</tr>
<tr>
<td>Furan-amidine 110</td>
<td>68.0 ± 0.9</td>
</tr>
<tr>
<td>Pyrrole-amidine 191</td>
<td>332.0 ± 1.2</td>
</tr>
<tr>
<td>N-Methylpyrrole-amidine 192</td>
<td>410.0 ± 1.1</td>
</tr>
<tr>
<td>Imidazole-amidine 193</td>
<td>1111.0 ± 0.5</td>
</tr>
<tr>
<td>N-Methylimidazole-amidine 194</td>
<td>Inactive</td>
</tr>
<tr>
<td>Oxazole-amidine 195</td>
<td>Inactive</td>
</tr>
<tr>
<td>Thiophene-amidine 226</td>
<td>773.1 ± 0.4</td>
</tr>
</tbody>
</table>

The isosteric replacement of amidine group 110 by ethyl imidate 234, N-aryl amide (reversed amidine) 235 and 236, N-aryl amide 238 and N-hydroxy amidine (amidoxime) 240 all led to the loss of NQO2 inhibitory activity (Table 20). The N-aryl amides 237 and 239 showed mild NQO2 inhibitory effect with IC$_{50}$ values of approximately 1 and 2 µM, respectively. The amidine group is mainly protonated at physiological pH (the pH of the assay media) as the pKa of the conjugate acid of the amidine group is 11.8.$^{101}$ On the other hand, the protonated and neutral forms of the imidate group in compound 234 will be in equilibrium, with the preference for the neutral form as the pKa of the conjugate acid of the imidate group is 6.2.$^{107}$
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Table 20. The IC_{50} values of the asymmetric furan-amidine 110 and its amidine isosteric analogues (tested by my colleague PhD student Elham Santina).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>X</th>
<th>Y</th>
<th>IC_{50} (nM) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>O</td>
<td>H</td>
<td>68.0 ± 0.9</td>
</tr>
<tr>
<td>234</td>
<td>O</td>
<td>H</td>
<td>Inactive</td>
</tr>
<tr>
<td>235</td>
<td>O</td>
<td>H</td>
<td>Inactive</td>
</tr>
<tr>
<td>236</td>
<td>S</td>
<td>H</td>
<td>Inactive</td>
</tr>
<tr>
<td>237</td>
<td>O</td>
<td>H</td>
<td>1145 (n =1)</td>
</tr>
<tr>
<td>238</td>
<td>O</td>
<td>H</td>
<td>Inactive</td>
</tr>
<tr>
<td>239</td>
<td>S</td>
<td>H</td>
<td>2000 (n =1)</td>
</tr>
<tr>
<td>240</td>
<td>O</td>
<td>H</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

The loss of activity in the reversed amidines 235 and 236 may be explained by the steric effect of the methyl group, as the distance between the phenyl ring and methyl group in 235 and 236 is longer than the distance between the phenyl group and the amine group in 110 (Figure 77).
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The structures of the furan-amidine 110 and its furan- and thiophene-reversed amidines 235 and 236, respectively.

Figure 77. The structures of the furan-amidine 110 and its furan- and thiophene-reversed amidines 235 and 236, respectively.

The N-hydroxy amidine 240 is a potential pro-drug for the asymmetric furan-amidine 110. The pro-drug approach can provide a potential solution for the low water solubility of parent compounds, as all the isosteric modifications led to a decrease or total loss of the inhibitory activity.
1. Docking: Correlation between the predicted and experimental NQO2 inhibitory activity of the synthesized compounds

The in silico study of the binding modes of the synthesized inhibitors in the active site of NQO2 aids the design of potential highly potent inhibitors. The synthesized NQO2 inhibitors were computationally docked in the NQO2 active site using the X-ray crystal structure of human NQO2 with bound FAD (PDB code 1QR2; Resolution at 2.10 Å).

The docking calculations predicted an energetically favourable binding mode for the asymmetric furan-, pyrrole-, N-methylpyrrole-, thiophene-, imidazole-, N-methylimidazole- and oxazole-amidines. All the inhibitors are positioned in the centre of the active site co-planar with the isoalloxazine ring of the FAD molecule. The inhibitors mainly make a hydrophobic interaction with the FAD molecule and the hydrophobic amino acids Trp105, Phe178', Phe126', Met154' and Cys121' through π–π stacking interactions. The amidine group forms hydrogen bonds with the side chain of the hydrophilic amino acid Glu122 or the backbone of the simple amino acid Gly-174. Figure 78 shows that the asymmetric furan-amidine 150 (A), pyrrole-amidine 191 (B), N-methylpyrrole-amidine 192 (C) and thiophene-amidine 226 (D) adopt a flat conformation, which optimizes the hydrophobic interaction with the isoalloxazine ring of the FAD molecule.
Chapter II. Results and Discussion/ Docking

Figure 78. The most energetically favourable docking binding mode in the NQO2 active site (PDB code 1QR2; Resolution at 2.10 Å) for: A- Asymmetric furan-amidine 150; B- Asymmetric pyrrole-amidine 191; C- Asymmetric N-methylpyrrole-amidine 192; D- Asymmetric thiophene-amidine 226.

The binding affinities $\Delta G_{\text{calc}}$ of the synthesized inhibitors were calculated after their docking in the NQO2 active site (PDB code 1QR2; Resolution at 2.10 Å). The binding affinity is an indication of the strength of the non-covalent interaction between the protein receptor or enzyme active site and the ligand or inhibitor. The inhibitors’ \textit{in silico} binding affinities $\Delta G_{\text{calc}}$ (KJ/mol) were compared to the \textit{in vitro} $\Delta G_{\text{exp}}$ (KJ/mol) binding affinities derived from the experimental IC$_{50}$ values. The experimental binding affinities were calculated using the Cheng-Prusoff equation (Equation 2) from the IC$_{50}$ values:

$$\Delta G_{\text{exp}} (J) = -RT \ln (K_i)$$

\textit{Equation 2}

$R$ = ideal gas constant, which equals 8.31 J/mol.K
$T$ = Temperature at which the IC$_{50}$ was calculated, Kelvin
$K_i$ = Inhibition constant, which is equal to IC$_{50}$ (molar concentration).

Only a poor correlation (correlation coefficient $R^2 = 0.0366$) between the calculated and experimental binding affinities was observed (Figure 79).
Chapter II. Results and Discussion/ Docking

Figure 79. Experimental ($\Delta G_{\text{exp}}$; KJ/mol) and calculated ($\Delta G_{\text{cal}}$; KJ/mol) binding affinities for the synthesized inhibitors binding to NQO2.

The calculated binding affinities of the asymmetric furan-amidine 110 (R = H) and its alkyl-substituted analogues 150 (R = Me), 151 (R = Et) and 153 (R = tert-Bu) were consistent with the experimental binding affinities (Table 21). With the increase in the length and branching of the alkyl group, the possibility of the steric effect increases, which results in the overall decrease in the value of the calculated binding affinity. A very good correlation (correlation coefficient $R^2 = 0.725$) between the calculated and the experimental binding affinities was observed for these inhibitors (Figure 80).

Table 21. The experimental ($\Delta G_{\text{exp}}$; KJ/mol) and calculated ($\Delta G_{\text{cal}}$; KJ/mol) binding affinities of the asymmetric furan-amidine 110 and its alkyl-substituted analogues 150, 151 and 153.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>$\Delta G_{\text{cal}}$ (KJ/mol)</th>
<th>$\Delta G_{\text{exp}}$ (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>H</td>
<td>-40.92</td>
<td>-42.52</td>
</tr>
<tr>
<td>150</td>
<td>Me</td>
<td>-42.44</td>
<td>-42.37</td>
</tr>
<tr>
<td>151</td>
<td>Et</td>
<td>-39.69</td>
<td>-39.71</td>
</tr>
<tr>
<td>153</td>
<td>t-Bu</td>
<td>-39.31</td>
<td>-37.33</td>
</tr>
</tbody>
</table>
Chapter II. Results and Discussion/ Docking

Figure 80. Experimental (ΔG<sub>exp</sub>; KJ/mol) and calculated (ΔG<sub>calc</sub>; KJ/mol) binding affinities for the synthesized inhibitors 110, 150, 151 and 153 binding to NQO2.

Poor correlation (correlation coefficient R<sup>2</sup> = 0.0397) was observed when comparing the asymmetric furan-amidine 110 with its heterocyclic-containing analogues; pyrrole- 191, N-methylpyrrole- 192, imidazole- 193, and thiophene- 226 (Figure 81).

Figure 81. Experimental (ΔG<sub>exp</sub>; KJ/mol) and calculated (ΔG<sub>calc</sub>; KJ/mol) binding affinities for the synthesized inhibitors 110, 191, 192, 193 and 226 binding to NQO2.

In contrast, better correlation (correlation coefficient R<sup>2</sup> = 0.583) was observed when comparing the asymmetric furan-amidine 110 with its heterocyclic-containing analogues; pyrrole- 191, N-methylpyrrole- 192 and imidazole-amidines 193, except thiophene-amidine 226 (Figure 82). The calculated binding affinity for the thiophene-
Chapter II. Results and Discussion/ Docking

amidine 226 (−44.95 KJ/mol) was much higher than the experimental binding affinity (−36.25 KJ/mol). This large difference can be explained by the high lipophilicity of the thiophene heterocycle compared to the furan, pyrrole and imidazole heterocycles. As the NQO2 active site is highly hydrophobic in nature, it is expected that the thiophene-containing inhibitors form more hydrophobic interaction with the hydrophobic amino acids than the other inhibitors, leading to higher predicted binding affinity values. The same difference was observed between the calculated (−42.92 KJ/mol) and experimental (−35.24 KJ/mol) binding affinities with the thiophene-reversed amidine 239.

![Graph showing experimental (ΔGexp; KJ/mol) and calculated (ΔGcalc; KJ/mol) binding affinities for synthesized inhibitors 110, 191, 192, and 193 binding to NQO2.](image)

**Figure 82.** Experimental (ΔGexp; KJ/mol) and calculated (ΔGcalc; KJ/mol) binding affinities for the synthesized inhibitors 110, 191, 192 and 193 binding to NQO2.

Although, the computational docking gives a prediction for the affinity of a ligand to bind to a protein receptor or enzyme active site, it can only be used as a guide to aid and direct the synthesis of novel NQO2 inhibitors.
Chapter II. Results and Discussion/ DNA Melting

1. Introduction

The stability of the secondary structure of DNA in aqueous solution depends on the interaction between the complementary bases through H-bonds and the interaction between neighbouring pairs through \( \pi-\pi \) stacking of the bases.\(^{152} \) The heating of DNA over a range of 25-98 °C leads to the breaking of H-bonds and the un-stacking of the base pairs. As a result a separation of the double strands occurs, which is known as DNA thermal denaturation or melting.\(^{153} \) DNA has a characteristic UV absorption spectrum with a maximum near 260 nm and a minimum near 230 nm, and the un-stacking of DNA pairs can be readily monitored spectrophotometrically. The un-stacking of DNA pairs leads to a hyperchromic effect in which the absorbance increases by 30% as the un-stacked base pairs can absorb more light.\(^{154} \) The plot of the DNA absorbance versus the temperature gives a sigmoidal curve. At the beginning of the experiment, double-stranded DNA is present. With the increase in the temperature, the bonds between the base pairs start to break and a hyperchromic shift is observed. At the end of the experiment, the DNA has melted and only DNA single strands are present (Figure 83).

![Figure 83. Thermal denaturation sigmoidal curve for DNA.](image)

The temperature at which the DNA melts is known as the DNA melting temperature (\( T_m \)), which is defined as the temperature in degrees Celsius, at which 50% of DNA is hybridized into a double strand, and 50% is present as single strands (Figure 84).
Chapter II. Results and Discussion/ DNA Melting

The binding of a ligand to DNA usually leads to an increase in the DNA $T_m$ value as more energy is needed to overcome the interactions between the ligand and DNA. The DNA thermal denaturation method can be used as an indicator for the binding of the drugs to DNA.

2. DNA binding: Is it an off-target effect for the synthesized asymmetric furan-and $N$-methylpyrrole-amidines?

Symmetric furan-amidines are known to be DNA minor grooves binder and they bind strongly in the minor groove of the AT sequence. To ensure the selectivity of the synthesized asymmetric amidine-compounds towards the inhibition of NQO2 without DNA off-target effects, the $T_m$ values of double-stranded DNA in the presence of the synthesized asymmetric furans- 110, 144-146 and 149-150 and $N$-methylpyrrole-amidines 192 were measured. Two positive controls were used in this experiment, which were doxorubicin 20 and the symmetric furan-amidine 111, both known to intercalate into DNA. The melting of DNA in the presence of doxorubicin 20, symmetric furan-amidine 111, furan- 110, 144-146 and 149-150 and $N$-methylpyrrole-amidines 192 were monitored spectrophotometrically over a range of 25-98 °C in low salt solution. To get the DNA melting temperature in a more accurate way than the sigmoidal curve, the first derivative of the DNA sigmoidal was calculated. The first derivative curve is the plot of $-\left(\frac{dA}{dT}\right)$ versus temperature (Figure 85).
Chapter II. Results and Discussion/ DNA Melting

![DNA thermal denaturation first derivative curve, asymmetric furan-amidine](image)

**Figure 85.** DNA thermal denaturation first derivative curve, asymmetric furan-amidine 144.

The melting point temperatures of doxorubicin 20, 111, 110, 144-146, 149-150 and 192 are summarized in Table 22. Table 22 showed also the difference in DNA melting temperatures in the absence and presence of the compound ($\Delta T_m = T_m^{\text{compound-DNAComplex}} - T_m^{\text{DNA alone}}$).

**Table 22.** DNA melting temperatures of doxorubicin, symmetric and asymmetric furan-amidines and asymmetric N-methylpyrrole-amidine in low salt solution.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>$T_m$ (°C)</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>70.8 ± 1.5</td>
<td>0</td>
</tr>
<tr>
<td>Doxorubicin 20</td>
<td>91.2 ± 1.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Symmetric furan-amidine 111</td>
<td>90.8 ± 0.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 110</td>
<td>72.3 ± 0.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 144</td>
<td>74.0 ± 0.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 145</td>
<td>74.0 ± 0.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 146</td>
<td>75.2 ± 1.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 149</td>
<td>72.7 ± 0.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Asymmetric furan-amidine 150</td>
<td>73.6 ± 0.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Asymmetric N-methylpyrrole-amidine 192</td>
<td>71.7 ± 0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Chapter II. Results and Discussion/ DNA Melting

Doxorubicin and the symmetric furan-amidine 111 showed high affinity for binding of approximately 20 °C. The amidine groups of the symmetric furan-amidine 111 form H-bonding with thymine keto-groups forming the floor of DNA minor grooves. In addition, the furan ring is pushed slightly away from the floor of DNA minor groove, but it fits well between the walls of the groove, which consists of deoxyribose groups.124

In contrast, all of the asymmetric furan-amidines 110, 144-146, 149-150 showed low affinity for DNA. Their weaker interactions comparing to the symmetric furan-amidine 111 led to a slight increase in DNA melting temperature approximately 2-4.4 °C. This can be explained by the presence of one amidine group, which is capable of making H-bonding with the thymine keto-group inside the minor groove of DNA.

The N-methylpyrrole-amidine 192 showed the lowest binding affinity for DNA with an increase of only 0.9°C in DNA melting temperature. N-Methylpyrrole-amidine 192 is forced from being a planar compound by the methyl group attached to the nitrogen atom, which decreases the ability of this compound to bind in the minor groove of DNA.

The plot of relative absorbance (A/A_{25 °C}) versus the temperature (°C) showed the effect of a compound with a high DNA binding affinity on the T_m when comparing to a compound with low DNA binding affinity. Figure 86 shows the effect of doxorubicin 20, the symmetric furan-amidine 111 and the asymmetric furan-amidine 144 on DNA melting temperatures.
In addition, the melting temperatures of DNA in the presence of doxorubicin 20, 111, 110, 144-146, 149-150 and 192 were measured in high salt solutions (Table 23). The $T_m$ melting temperature of DNA increases with increasing salt concentration as the DNA is more stable and the cations in the solution form ionic interactions with the negatively charged phosphate groups. The $T_m$ value of DNA alone in high salt solution is 88.1 °C compared to 70.8 °C in low salt solution. The same pattern of binding affinities was observed for the compounds in high salt solution, with only small $\Delta T_m$. 

Figure 86. DNA melting temperature: The plot of relative absorbance versus temperature.
**Chapter II. Results and Discussion/ DNA Melting**

**Table 23.** DNA melting temperatures of doxorubicin, symmetric and asymmetric furan-amidines and asymmetric N-methylpyrrole-amidine in high salt solution.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>$T_m$ (°C)</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>88.1 ± 1.0</td>
<td>0</td>
</tr>
<tr>
<td>Doxorubicin 20</td>
<td>92.9 ± 0.6</td>
<td>4.8</td>
</tr>
<tr>
<td>111</td>
<td>90.6 ± 0.6</td>
<td>2.5</td>
</tr>
<tr>
<td>110</td>
<td>87.7 ± 0.7</td>
<td>-0.4</td>
</tr>
<tr>
<td>144</td>
<td>87.5 ± 0.1</td>
<td>-0.7</td>
</tr>
<tr>
<td>145</td>
<td>88.2 ± 0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>146</td>
<td>88.7 ± 1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>149</td>
<td>88.3 ± 0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>150</td>
<td>87.8 ± 1.1</td>
<td>-0.3</td>
</tr>
<tr>
<td>192</td>
<td>88.5 ± 0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**3. DNA binding: Reversed amidines and DNA binding affinities**

Unsymmetrical reversed amidines 235 and 236 were designed as potential NQO2 inhibitors with low DNA binding affinities. Aryl reversed amidine has a larger amidine-phenyl dihedral angle than aryl amidine, making aryl reversed amidines posses 10-fold lower DNA binding affinity than aryl amidine.\(^{124}\) The amidine-phenyl dihedral angle in the reversed amidine is 90° compared with 40-45° in aryl amidine, which leads to unfavourable interactions of the reversed amidine inside the minor groove of DNA.\(^{124}\)

As the reversed amidines 235 and 236 did not show any inhibitory activity against NQO2; their DNA binding affinities and DNA melting temperatures have not been measured.
1. Conclusion on synthesis and evaluation of novel NQO2 inhibitors

An efficient total synthesis of the lead asymmetric furan-amidine 110 and a series of asymmetric furan-, pyrrole-, N-methylpyrrole-, thiophene-, imidazole, N-methylimidazole- and oxazole-amidines have been successfully carried out using multi-step synthetic pathways. The synthesis of the target compounds started from the preparation of different substituted 1,4-diketone intermediates. The 1,4-diketone intermediates were cyclized to different 5-membered heterocycles using a variety of reagent. The nitrile and nitro groups served as the precursor for amidine and N-aryl amidine (reversed amidine) and amide groups, respectively.

The substitution of the phenyl ring in the case of furan-amidines, specifically with a nitro-group resulted in the most active inhibitors 147 and 148 (Figure 87) of NQO2 activity. In contrast, structural modification of the amidine group in the parent asymmetric furan-amidine 110 resulted in loss of the NQO2 inhibitory activity.

![Figure 87. Structures of the most active asymmetric furan-amidines 147 and 148.](image)

The isosteric replacement of the furan ring into more water-soluble 5-membered heterocycles, namely, pyrrole 191, N-methylpyrrole 192, imidazole 193, N-methylimidazole 194 and oxazole 195, led to the decrease in the NQO2 inhibitory activities when compared to the lead asymmetric furan-amidine 110 (Figure 88).
Chapter II. Results and Discussion/ Conclusion

![Chemical structures](image)

**Figure 88.** Structures of the asymmetric furan-amidine 110 and its 5-membered heterocycle analogues.

One compound with the *N*-hydroxy amidine group 240 was synthesized (Figure 89), which is considered as a potential pro-drug for the asymmetric furan-amidine 110. The pro-drug approach can provide a solution for the low water solubility as all the isosteric modifications led to a decrease or total loss of the inhibitory activity.

![Chemical structure](image)

**Figure 89.** Structure of the potential prodrug 240 of the asymmetric furan-amidine 110.

The asymmetric furan-amidines showed no binding affinity towards DNA when compared to the symmetric furan-amidines, which are known as DNA intercalators. This improves the selectivity of the asymmetric furan-amidines towards NQO2.

In conclusion, the synthesis of potent selective NQO2 inhibitors with the asymmetric furan-amidine scaffold has been achieved. They can be further investigated as potential drugs in chemotherapy and chemoprevention.
Chapter II. Results and Discussion/ Future Work

1. Isosteric replacement of the basic amidine group with the acidic carboxylic acid

   A targeted compound with a carboxylic acid 265 as an isostere of the amidine group 110 is proposed for synthesis (Figure 90). The presence of a highly acidic group can give an indication if the basicity of amidine group was an important reason for the high NQO2 inhibition activity of the furan-amidines.

   ![Figure 90. Structure of asymmetric furan-amidine 110 and its carboxylic acid isostere 265.](image)

2. Synthesis of 3-fluoro diarylimidazole-amidine

   In general, the synthesized furan-amidines showed poor water solubility. An imidazole-amidine 193 (Figure 88) was synthesized as an analogue for the asymmetric furan-amidine 110 (calculated log P value of 2.8) with expected improved water solubility (calculated calculated log P value of 1.8 for 193). The imidazole-amidine 193 inhibited the NQO2 enzyme with an IC\textsubscript{50} value of approximately 1.0 µM compared to a value of 68.0 nM for the furan-amidine 110.

   As the fluoro-substitution on the meta-position 144 (Figure 91) of the phenyl ring of the asymmetric furan-amidine 110 led to the conservation of the inhibition activity, an-imidazole-amidine with meta-fluoro substituent 266 (Figure 91) is proposed to be synthesized.

   ![Figure 91. Structure of asymmetric furan-amidine 144 and 3-fluoro diarylimidazole-amidine 266.](image)

3. Positron Emission Tomography (PET): study of NQO2 biological role and the consequence of its inhibition in tumour cells

   Positron emission tomography (PET) is a non-invasive technology that is used
to monitor the biological systems at the molecular level by using a camera that can image high-energy gamma rays emitted from inside the subject. The technology depends on labelling natural biological molecules or drugs with isotopes capable of producing two gamma-rays through the emission of a positron from their nuclei. The isotopes that are frequently used in this technology are $^{11}$C, $^{15}$O, $^{13}$N or $^{18}$F. $^{157}$

PET technology can be used to monitor the changes in cancer cells at the molecular level during the pharmacological and radiation therapy to get more information that aids the drug design focused on chemoprevention or chemotherapy. $^{157}$

The limitation of the use of this technology is the short half-lives of the isotopes used; for example, the half-life of $^{18}$F is 110 m. In order to benefit from this technology, the rapid and efficient synthesis of the labelled molecules first and their safe administration to the subject are essential.$^{157}$

PET technology can be used to study the biological role of NQO2 and the consequences of its inhibition in tumour cells by introducing labelled drugs that selectively and potently inhibit the enzyme. The asymmetric furan-amidines 144 and 149 (Figure 92) were chosen as the target compounds to be used. The aim is the label of 144 and 149 with the isotopes $^{18}$F and $^{11}$C, respectively, then the administration of these drugs \textit{in vivo}. As, the isotopes $^{18}$F and $^{11}$C are radioactive isotopes; the handling and synthesis must be done in a special purpose laboratory.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/figure92.png}
\caption{Structures of PET candidate compounds 144 and 149.}
\end{figure}
Chapter III. Experimental/ Chemistry

1. Chemicals and materials

Chemicals were purchased from Sigma-Aldrich, solvents were purchased from Fisher Scientific, and deuterated solvents were purchased from Goss. Melting points were measured using a Stuart Scientific melting point apparatus SAMP10. Infrared spectra were recorded in the solid state using a J.A.S.C.O fourier transform infrared spectrophotometer. A Bruker Avance 300 and 400 MHz spectrometer was used to record $^1$H and $^{13}$C NMR spectra. Chemical shifts are quoted in parts per million (ppm) and referenced to tetramethylsilane ($\delta = 0$). Mass spectrometry was carried out in the School of Chemistry, University of Manchester using a Micromass Platform II instrument. Molecular ions peaks are reported as mass/charge (m/z) ratios. Solvents were evaporated on a Buchi rotavapor R-200 equipped with a Buchi heating bath B-490. Thin layer chromatography (TLC) was performed using silica gel 60 on aluminum sheets with F254. The spots were visualized using a UV Mineralight lamp (254/365) UVGL-58. Column chromatography was performed using silica gel particle size 40-63 microns. A BECKMAN DU 7400 spectrophotometer was used to determine enzyme activity. A Grant JB series water bath was used to heat the buffer to 37 °C.

2. Synthesis

The detailed syntheses of the compounds mentioned in the results and discussion chapter are discussed in the following sections.

2.1. Mannich bases 121 and 125

Concentrated hydrochloric acid in ethanol (20 drops of 10.0 % (w/v)) was added to a mixture of 4-acetylbenzonitrile 120 (Mannich base 121 synthesis) or acetophenone 124 (Mannich base 125 synthesis) (1.0 mmol), paraformaldehyde (1.5 mmol) and dimethylammonium hydrochloride (1.5 mmol) in absolute ethanol (20 ml). The mixture was heated at reflux for 3 h and monitored by TLC (10.0 % MeOH/CHCl$_3$). The workup was as described for each Mannich base compound.
Chapter III. Experimental/ Chemistry

2.1.1. N,N-Dimethyl 1-(4-cyanophenyl)-1-oxopropan-3-ammonium chloride 121

Compound 121 was crystallized from ethanol by adding 20 ml acetone. Colourless crystals were collected by filtration and rinsed with acetone to yield 0.96 g (58.0 %) of 121: IR (cm\(^{-1}\)): 3405 (N-H), 2380, 2227 (CN), 1686 (C=O), 1408, 1332, 1220, 962, 858, 786; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 2.79 (6H, s, 2 × CH\(_3\)), 3.40 (2H, t, J = 7.5 Hz, CH\(_2\), H-2), 3.67 (2H, t, J = 7.2 Hz, CH\(_2\), H-3), 8.07 (2H, d, J = 8.1 Hz, 2 × CH, H-2'), 8.16 (2H, d, J = 8.1 Hz, 2 × CH, H-3'); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 33.6 (CH\(_3\)), 33.9 (CH\(_3\)), 42.1 (CH\(_2\), C-2), 51.4 (CH\(_2\), C-3), 115.5 (C, C-4'), 118.1 (CN), 128.6 (2 × CH, C-2'), 132.8 (2 × CH, C-3'), 139.0 (C, C-1'), 196.1 (C=O).

2.1.2. N,N-Dimethyl 1-oxo-1-phenylpropan-3-ammonium chloride 125

Compound 125 was crystallized from ethanol by adding 20 ml acetone. Colourless crystals were collected by filtration and rinsed with acetone to yield 4.20 g (47.0 %); mp 145 °C (Lit. mp 152-153 °C\(^{158}\)); IR (cm\(^{-1}\)): 3486, 3411 (NH), 2662, 1674 (C=O), 1334, 1223, 958, 689; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 2.79 (6H, s, 2 × CH\(_3\)), 3.63 (2H, t, J = 7.2 Hz, CH\(_2\), H-2), 3.39, (2H, t, J = 7.4 Hz, CH\(_2\), H-3), 7.57 (2H, t, J = 7.5 Hz, 2 × CH, H-3'), 7.69 (1H, t, J = 7.2 Hz, CH, H-4'), 8.02 (2H, d, J = 7.5 Hz, 2 × CH, H-2'); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 33.1 (CH\(_3\)), 34.1 (CH\(_3\)), 42.3 (CH\(_2\), C-2), 51.8 (CH\(_2\), C-3), 128.0 (2 × CH, C-3'), 128.8 (2 × CH, C-2'), 133.7 (CH, C-4'), 135.8 (C, C-1'), 196.8 (C=O).

2.2. Aryl 1,4-diketones 123 and 126

The aryl 1,4-diketones, named 1,4-bis(4-cyanophenyl)-1,4-butanedione 123 and 1-(4-cyanophenyl)-4-phenyl-1,4-butanedione 126 were synthesized using two synthetic pathways. The two compounds were synthesized under Stetter reaction conditions starting from the Mannich bases or through the coupling between methyl aryl ketones and α-bromomethyl aryl ketones.
2.2.1. General procedure for Stetter reaction

Triethylamine (2.0 mmol) was added to a suspension of Mannich base (1.0 mmol), 4-formylbenzonitrile 122 (1.0 mmol) and 19 (0.2 mmol) in dry THF (20.0 ml). The mixture was heated at reflux under an atmosphere of N₂ conditions for 48 h and monitored by TLC (20.0% EtOAc/Hexane). After the reaction mixture was cooled to room temperature, triethylammonium chloride (the identity was confirmed by ¹H NMR spectroscopy) was filtered off and THF was evaporated. Aryl 1,4-diketones 123 and 126 were purified from the crude reaction mixture by flash column chromatography using EtOAc–hexane (1:9) as the mobile phase.

2.2.1.1. 1,4-Bis(4-cyanophenyl)-1,4-butadione 123

Light yellow fine solid; 257 mg (21.2 %): mp 247-249 °C (lit. mp 260-265 °C)¹¹⁰; IR (cm⁻¹): 2227 (CN), 1682 (C=O), 1404, 1321, 1195, 1011, 860, 785; ¹H NMR (300 MHz; DMSO-d₆): 3.48 (4H, s, 2 × CH₂, H-2, H-3), 8.04 (4H, d, J = 8.1 Hz, 4 × CH, H-2’), 8.16 (4H, d, J = 8.4 Hz, 4 × CH, H-3’); ¹³C NMR (75 MHz; DMSO-d₆) (assignments made using DEPT-135): 32.7 (2 × CH₂, C-2, C-3), 115.2 (2 × C, C-4’), 118.1 (CN), 128.5 (4 × CH, C-2’), 132.8 (4 × CH, C-3’), 139.5 (2 × C, C-1’), 198.1 (2 × C=O). The spectroscopic data (IR, ¹H and ¹³C NMR) were identical to the reported in the literature.¹¹⁰

2.2.1.2. 1-(4-Cyanophenyl)-4-phenyl-1,4-butadione 126

Light yellow fine solid; 88.0 mg (36.0 %): mp 126-128 °C; IR (cm⁻¹): 2225 (CN), 1673 (C=O), 1334, 1223, 959, 753; ¹H NMR (300 MHz; CDCl₃): 3.44 (2H, t, J = 5.4 Hz, CH₂, H-3), 3.51 (2H, t, J = 5.4 Hz, CH₂, H-2), 7.49 (2H, t, J = 7.2 Hz, 2 × CH, H-3’), 7.60 (1H, t, J = 7.5 Hz, CH, H-4’); 7.80 (4H, d, J = 8.1 Hz, 2 × CH, H-2’), 8.03 (2H, d, J = 7.5 Hz, 2 × CH, H-2’), 8.20 (2H, d, J = 8.1 Hz, 2 × CH), ¹³C NMR (75 MHz, CDCl₃) (assignments made using DEPT-135): 32.6 (CH₂, C-3), 32.8 (CH₂, C-2),
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116.4 (C, C-4’), 118.0 (CN), 128.1 (2 × CH), 128.6 (2 × CH), 128.7 (2 × CH), 132.5 (2 × CH), 133.4 (CH, H-4”), 136.5 (C), 139.8 (C), 197.5 (C=O), 198.2 (C=O); -ESIMS m/z (relative intensity): 262 (100%, [M - H]); +ESIMS m/z (relative intensity): 286 (100%, [M + Na]+); found by +ESIMS 286.0844, C_{17}H_{13}NO_{2}Na [M + Na]^+, requires 286.0839, error 1.8 ppm.

2.2.1. 3.5-(2-Hydroxyethyl)-3,4-dimethyl-1,3-thiazolium iodide catalyst 128

To a solution of 5-(2-hydroxyethyl)-4-methylthiazole 127 (5.00 g, 35.0 mmol) in dry acetonitrile (6.0 ml) was added iodomethane (7.45 g, 53.0 mmol). The mixture was heated at reflux for 24 h. The reaction mixture was cooled at room temperature. Acetonitrile was evaporated and the crude residue was mixed with ethyl acetate (50.0 ml) at room temperature for 6 h. A tan solid 9.77 g (98.0 %) was collected by filtration and rinsed with ethyl acetate. The catalyst was used in the synthesis of 1,4-diketone compounds without further purification: IR (cm\(^{-1}\)): 3283 (O-H), 3023, 2360, 2341, 1056, 812; \(^1\)H NMR (300 MHz; DMSO-\(d_6\)): 2.44 (3H, s, CH\(_3\)), 3.03 (1H, t, \(J = 5.4\) Hz, CH, H-1’), 3.64 (1H, q, \(J = 5.4\) Hz, CH, H-2’), 4.09 (3H, s, NCH\(_3\)), 5.15 (1H, t, \(J = 4.8\), OH), 9.96 (1H, s, CH, H-2); \(^{13}\)C NMR (75 MHz; DMSO-\(d_6\)) (assignments made using DEPT-135): 11.3 (C-1’), 29.4 (CH\(_3\)), 40.2 (CH\(_2\), C-2’), 59.7 (NCH\(_3\)), 134.7 (C), 142.1 (C), 156.4 (CH, C-2).

2.2.2. General procedure for the coupling between methyl aryl ketones and \(\alpha\)-bromomethyl aryl ketones

Zinc chloride (2.0 mmol) was added to dry toluene (5.0 ml), absolute ethanol (1.5 mmol) and triethylamine (1.5 mmol). The mixture was stirred at room temperature for 1-2 h. 4-Cyanoacetophenone 120 (1.5 mmol) and 2-bromo-4’-cyanoazobenzonitrile 136 (aryl 1,4-diketones 123 synthesis) or 2-bromo-acetophenone 133 (aryl 1,4-diketones 126 synthesis) (1.0 mmol) were added and the mixture was stirred at room temperature for 3-4 days and monitored by TLC (20.0 % EtOAc/ hexane). The workup was as described for each 1,4-diketone compound.
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2.2.2.1. 1,4-Bis(4-cyanophenyl)-1,4-butadione 123

Toluene was decanted and the pale yellow residue gum was dissolved in DMF (~80 °C, 50.0 ml). Compound 6 was crystallized from DMF as a pale yellow solid by the addition of methanol (10.0 ml), filtered under vacuum and washed with cold methanol to yield 0.81 g (61.0 %). The $^1$H NMR spectrum was identical to that reported in the previous synthesis of 123 (section 2.2.1.1).

2.2.2.2. 1-(4-Cyanophenyl)-4-phenyl-1,4-butadione 126

The reaction mixture was quenched with cold 10.0% (v/v) aqueous sulfuric acid (10.0 ml). The organic layer was extracted with brine (10.0 ml), dried over magnesium sulfate and concentrated. Compound 126 was crystallized from methanol to give 1.51 g (39.0%) as light-yellow crystals. The $^1$H NMR spectrum was identical to that reported in the previous synthesis of 126 (section 2.2.1.2).

2.3. General procedure for the synthesis of 2,5-diarylfurans 118 and 127

A solution of 1,4-bis(4-cyanophenyl)-1,4-butadione 123, (2,5-diarylfuran 118 synthesis) or 1-(4-cyanophenyl)-4-phenyl-1,4-butadione 126 (2,5-diarylfuran 127 synthesis) in acetic anhydride was heated at reflux. A few drops of concentrated sulfuric acid in acetic anhydride (1.0 ml) was added to the solution. The solution became dark in colour, was removed from heating and allowed to cool to room temperature.

2.3.1. 4,4’-(Furan-2,5-diyl)benzonitrile 118

The yellow crystals of 4,4’-(furan-2,5-diyl)benzonitrile 118 were filtered off under vacuum and washed with hexane: $^1$H NMR (300 MHz; DMSO-d$_6$): 7.44 (2H, s, 2 × CH, H-3’), 7.92 (4H, d, J = 8.1 Hz, 4 × CH, H-3), 8.06 (4H, d, J = 8.4 Hz, 4 × CH, H-2) ; $^{13}$C NMR (75 MHz; CDCl$_3$) (assignments made using DEPT-135): 109.8 (C), 111.9 (2 × CH, C-3’), 118.8 (CN), 124.3 (4 × CH, C-3), 132.9 (4 × CH, C-2), 133.4 (2 x C, C-4), 152.2 (2 x C, C-2’); the spectroscopic data (IR, $^1$H- and $^{13}$C-NMR) were identical to those reported in the literature. 110
2.3.2. 4-(5-Phenylfuran-2-yl)benzonitrile 127

The reaction mixture was poured into iced water (75.0 ml) and the yellow precipitate was collected and re-crystallized from methanol to give 0.61 g of a light yellow fine solid (78.0%): mp 122-124 °C; IR (cm\(^{-1}\)): 2224 (CN), 1738, 1606 (furan C=O), 1023 (C-O), 795; \(^1\)H NMR (300 MHz; CDCl\(_3\)): 6.81 (1H, d, J = 3.6 Hz, furan-CH), 6.93 (1H, d, J = 3.3 Hz, furan-CH), 7.34 (1H, t, J = 7.5 Hz, CH, H-4”), 7.45 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.70 (2H, d, J = 8.4, 2 x CH), 7.78 (2H, d, J = 7.2 Hz, 2 x CH, H-2”), 7.83 (2H, d, J = 8.4, 2 x CH); \(^{13}\)C NMR (100 MHz; CDCl\(_3\)) (assignments made using DEPT-135): 107.7 (C-H), 110.1 (C), 110.5 (CH), 119.1 (C, CN), 123.8 (CH), 124.1 (CH), 128.1 (CH), 128.9 (CH), 130.1 (C), 132.7 (CH), 134.5 (C), 151.2 (C), 155.1 (C); +APCI m/z (relative intensity): 246.2 (100%, [M + H])

2.4. General procedure for the synthesis of furan-imidates 119 and 143

Acetyl chloride (24.0 mmol for 119 or 16.0 mmol for 143 syntheses) was added dropwise to a suspension of 123 or 124 (1.0 mmol) in absolute ethanol (48.0 mmol for 119 or 24.0 mmol for 143 syntheses) in dry chloroform at 0 °C. The reaction was allowed to warm up to room temperature and stirring was continued for 1-3 days. The precipitated product was filtered off under vacuum and rinsed with hexane or diethyl ether.

2.4.1. Diethyl 4,4’-(furan-2,5-diyl)dibenzimidate dihydrochloride 119

The orange precipitate was filtered off under vacuum and rinsed with hexane. The precipitate was added to boiling ethanol and filtered while it was hot to give 0.84 g (42.0%) of 119: \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.52 (6H, t, J = 6.9 Hz, CH\(_3\)), 4.66 (4H, q, J = 6.9 Hz, CH\(_2\)), 7.54 (2H, s, 2 x CH, H-3’), 8.15 (4H, d, J = 8.1 Hz, 4 x CH), 8.25 (4H, d, J = 8.4 Hz, 4 x CH).
2.4.2. Ethyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 143

The yellow-coloured precipitate was filtered off under vacuum and rinsed with hexane. The precipitate was added to boiling ethyl acetate and filtered while it was hot to give 0.47 g (76.0%) of 143: mp 233-235 °C; IR (cm\(^{-1}\)): 2777, 1628, 1606, 1435 (Ar C=C), 1069 (C–O), 781, 751, 601; \(^1\)H-NMR (300 MHz; DMSO-d\(_6\)): 1.52 (3H, t, J = 6.9 Hz, \(\text{C}_3\H_3\)), 4.63 (2H, q, J = 6.9 Hz, \(\text{C}_2\H_2\)), 7.22 (1H, d, J = 3.3 Hz, furan-CH), 7.37 (1H, t, J = 7.5 Hz, CH, H-4”), 7.45 (1H, d, J = 3.6 Hz, furan-CH), 7.49 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.90 (2H, d, J = 7.8 Hz, 2 x CH, H-2”), 8.08 (2H, d, J = 8.7 Hz, 2 x CH), 8.17 (2H, d, J = 8.4 Hz, 2 x CH); \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\)) (assignments made using DEPT-135): 13.5 (CH\(_3\)), 69.5 (OCH\(_2\)), 108.4 (CH), 109.8 (CH), 123.6 (CH), 123.8 (C-4”), 127.8 (CH), 128.2 (CH), 128.9 (CH), 129.8 (C), 132.4 (C), 132.7 (C), 151.8 (C), 153.3 (C), 167.3 (C=\(\equiv\)N); +ESIMS m/z (relative intensity): 292.3 (100%, \([\text{M} + \text{H}]^+\)); found by +ESIMS 292.1331, C\(_{19}\)H\(_{18}\)NO\(_2\)[M + H]\(^+\), requires 292.1338, error 2.2 ppm.

2.5. General procedure for the synthesis of furan-amidines 110 and 111

Ammonium acetate (4.0 mmol for 110 and 8.0 mmol for 111 syntheses) was added to a stirred suspension of furan-imidates 143 or 119 (1.0 mmol) in anhydrous ethanol. The mixture was stirred at room temperature for 1-3 days. The workup was as described for each furan-amidine compound.

2.5.1. 4-(5-Phenylfuran-2-yl)benzamidine acetate 110

4-(5-Phenylfuran-2-yl)benzamidine acetate 110 was collected by filtration and rinsed with ethanol to give 0.28 g (62.0%) of 110 as a fine pale yellow solid: mp 234-237 °C; IR (cm\(^{-1}\)): 2924 (C-H), 1613, 1487, 1440, 796; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.74 (3H, s, CH\(_3\)), 7.17 (1H, d, J = 3.6 Hz, furan-CH), 7.34 - 7.38 (2H, m, 2 x CH
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including furan-C\textsubscript{H}, 7.48 (2H, t, \(J = 7.8\) Hz, 2 x CH, H-3'), 7.87 – 7.90 (4H, m, 4 x CH), 8.01 (2H, d, \(J = 8.4\) Hz, 2 x CH); \^{13}C NMR (75 MHz; DMSO-\textsubscript{d6}) (assignments made using DEPT-135): 22.3 (CH\textsubscript{3}), 108.6 (CH), 111.3 (CH), 123.4 (CH), 123.8 (CH), 126.2 (C), 128.1(CH), 128.7 (CH), 129.0 (CH), 129.6 (C), 134.5 (C), 151.0 (C), 154.0 (C), 164.9 (C, C-amidine), 173.9 (C=O); ESIMS m/z (relative intensity): 263 (100\%, [M + H]\textsuperscript{+}).

2.5.2. 4,4'-((Furan-2,5-diyl)dibenzamidine acetate 111

\[
\text{H}_2\text{C} = \text{O} \quad \text{H}_2\text{N}^+ \\
\text{H}_2\text{N} \\
\text{1} \quad \text{2} \\
\text{3} \quad \text{4} \\
\text{5} \quad \text{C} \\
\text{6} \quad \text{NH} \\
\text{NH}_2
\]

4,4'-((Furan-2,5-diyl)dibenzamidine acetate 111 was collected by filtration, added to boiling ethanol and filtered whilst hot to yield 0.37 g (46\%) of an olive-green solid; IR (cm\textsuperscript{-1}): 3030 (Ar C-H), 2360, 2341, 1668, 1606 (C-O), 1488; \textsuperscript{1}H NMR (300 MHz; DMSO-\textsubscript{d6}): 1.80 (3H, s, CH\textsubscript{3}), 7.46 (2H, s, 2 x CH, H-3'), 7.95 (4H, d, \(J = 8.4\) Hz, 4 x CH), 8.11 (4H, d, \(J = 8.4\) Hz, 4 x CH); \textsuperscript{13}C NMR (75 MHz; DMSO-\textsubscript{d6}) (assignments made using DEPT-135): 22.6 (CH\textsubscript{3}), 111.6 (2 x CH, C-3'), 123.8 (4 x CH), 126.8 (C), 128.8 (4 x CH), 134.1 (C), 152.4 (C), 164.8 (C, C-amidine), (C, C=O) was not observed; ESIMS m/z (relative intensity): 305 (50.0 \%, [M + H]\textsuperscript{+}).

2.6. General procedure for the synthesis of the asymmetric aryl 1,4-diketones 165-174

Zinc chloride (9.0 mmol) was added to dry toluene or dry THF (5.0 ml), absolute ethanol (6.8 mmol) and triethylamine (6.8 mmol). The mixture was stirred at room temperature for 1-2 h. Methyl aryl ketone (6.8 mmol) and 2-bromo-4'-cyanobenzonitrile 136 (4.5 mmol) were added and the mixture was stirred at room temperature for 3-7 days and monitored by TLC (20.0 \% EtOAc/ hexane). The workup was as described for each 1,4-diketone compound.
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2.6.1. 1-(4-Cyanophenyl)-4-(3-fluorophenyl)-1,4-butadione 165

![Chemical Structure Image]

Toluene was evaporated leaving an oily residue. Compound 165 was crystallized from methanol as pale yellow solid to give 0.59 g (16.0 %): mp: 143-147 °C; IR (cm⁻¹): 2220 (CN), 1679 (C=O), 1317, 1201 (C-F), 1006, 781 (C-F), 677; ¹H NMR (300 MHz; CDCl₃): 2.47 (4H, s, 2 × CH₂, H-2, H-3), 7.33 (1H, t, 3JHF = JHH = 8.4 Hz, CH, H-4’), 7.50 (1H, dd~q, 2JHF = JHH = 6.0 Hz, CH, H-5’), 7.71 (1H, d, 3JHF = 9.6 Hz, CH, H-2”), 7.81-7.85 (3H, m, 3 x CH, H-6”), 8.14 (2H, d, J = 8.4 Hz, 2 × CH); ¹³C NMR (100 MHz, CDCl₃) (assignments made using DEPT-135): 32.68 (CH₂), 32.7 (CH₂), 114.9 (d, 2JCF = 22.0 Hz, CH, C-4”), 116.5 (C, C-4”), 118.0 (CN), 120.4 (d, 2JCF = 21.0 Hz, CH, C-2”), 123.9 (d, 4JCF = 3.0 Hz, C-6”), 128.8 (CH), 130.4 (d, 3JCF = 8.0 Hz, C-5”), 132.61 (C), 138.5 (d, 3JCF = 6.0 Hz, C-1”), 139.6 (C), 162.9 (d, 1JCF = 247.0 Hz, C-3”), 197.0 (C=O), 197.3 (C=O); ¹⁹F-NMR (376.5 MHz, CDCl₃): -111.7; -ESIMS m/z (relative intensity): 280 (100%, [M - H]); found by -ESIMS 280.0789, C₁₇H₁₁NO₂F[M - H], requires 280.0779, error 3.6 ppm.

2.6.2. 1-(4-Cyanophenyl)-4-(4-fluorophenyl)-1,4-butadione 166

The reaction mixture was diluted by the addition of ethyl acetate (10 ml). Ethyl acetate and toluene were decanted and evaporated to give a yellow solid. Compound 166 was re-crystallized from methanol to give 0.34 g (26.0 %) of a bright yellow crystals: mp 162-164 °C; IR (cm⁻¹): 2222 (CN), 1677 (C=O), 1593, 1405, 1295, 1186,1001 (C-F), 854, 708 (C-F), 590; ¹H NMR (300 MHz; CDCl₃): 3.35-3.44 (4H, m, 2 × CH₂, H-2, H-3), 7.09 (2H, dd-t, 3JHF = JHH = 8.4 Hz, 2 × CH, H-3”), 7.73 (2H, d, J = 8.4 Hz, 2 × CH), 7.98 (2H, dd-t, 4JHF = JHH = 7.8 Hz, 2 × CH, H-2”), 8.06 (2H, d, J = 8.4 Hz, 2 × CH); ¹³C NMR (75 MHz; CDCl₃) (assignments made using DEPT-135): 32.4 (CH₂), 32.8 (CH₂), 115.8 (d, 2JCF = 22.0, 2 × CH, C-3”), 116.5 (C, C-4”), 118.0 (CN), 128.6 (2 × CH), 130.8 (d, 3JCF = 9.0 Hz, 2 × CH, C-2”), 132.6 (2 × CH), 132.9 (d, 4JCF = 3.0 Hz, C-1”), 139.7 (C), 165.9 (d, 1JCF = 253.0 Hz, C-4”), 196.6 (C=O), 197.4
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(C=O); $^{19}$F-NMR (376.5 MHz, CDCl$_3$): -104.7; +ESIMS m/z (relative intensity): 320 (100%, [M + K]$^+$), -ESIMS m/z (relative intensity): 280 (100%, [M - H]$^-$); found by -ESIMS 280.07829, C$_{17}$H$_{11}$NO$_2$F [M - H]$^-$, requires 280.0779, error 1.1 ppm.

2.6.3. 1-(4-Cyanophenyl)-4-(4-bromophenyl)-1,4-butadione 167

![Chemical Structure of 167](image)

The reaction was diluted by the addition of hexane (20 ml). Hexane was decanted and compound 167 was re-crystallized from MeOH/ CHCl$_3$ to give 1.38 g (56.0 %) of colourless crystals: mp 158-160 °C; IR (cm$^{-1}$): 2227 (CN), 1678 (C=O), 1583, 995, 780 (C-Br); $^1$H NMR (300 MHz; CDCl$_3$): 3.45 (4H, s, 2 × C$_2$H$_2$), 7.64 (2H, d, $J = 8.1$ Hz, 2 × CH), 7.80 (2H, d, $J = 8.1$ Hz, 2 × CH), 8.12 (2H, d, $J = 8.4$ Hz, 2 × CH); $^{13}$C NMR (75 MHz; CDCl$_3$) (assignments made using DEPT-135): 32.5 (CH$_2$), 32.7 (CH$_2$), 116.5 (C, C-4'), 118.0 (CN), 128.6 (2 × CH), 129.6 (2 × CH), 131.6 (C), 132.0 (2 × CH), 132.6 (2 × CH), 135.2 (C), 139.7 (C), 197.2 (C=O), 197.3 (C=O); -ESIMS m/z (relative intensity): 340 (90%, 79Br, [M - H]$^-$), 342 (100%, 81Br); found by -ESIMS 339.9977, C$_{17}$H$_{11}$NO$_2$Br [M - H]$^-$, requires 339.9978, error 0.3 ppm.

2.6.4. 1-(4-Cyanophenyl)-4-(3-nitrophenyl)-1,4-butadione 168

![Chemical Structure of 168](image)

Toluene was decanted from the reaction mixture and the pale yellow residue gum was dissolved in DMF (~80 °C, 50.0 ml). Compound 168 was crystallized from DMF as pale yellow crystals by the addition of methanol (10.0 ml), filtered under vacuum and washed with cold methanol to yield 0.66 g (47.0 %): mp 174-176 °C; IR (cm$^{-1}$): 2229 (CN), 1680 (C=O), 1655, 1527 (NO$_2$), 1348, 1313 (NO$_2$), 1302, 1192, 793, 736, 671; $^1$H NMR (300 MHz; CDCl$_3$): 3.52 (4H, s, 2 × CH$_2$, H-2, H-3), 7.73 (1H, t, $J = 8.1$ Hz, CH, H-5"), 7.82 (2H, d, $J = 8.4$ Hz, 2 × CH), 8.13 (2H, d, $J = 8.4$ Hz, 2 × CH), 8.36 (1H, d, $J = 7.8$ Hz, CH, H-6"), 8.46 (1H, d, $J = 8.2$ Hz, CH, H-4"), 8.87 (1H, s, CH, H-2"); $^{13}$C NMR (75 MHz; CDCl$_3$) (assignments made using DEPT-135): 32.7
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(CH$_2$), 32.8 (CH$_2$), 116.7 (C, C-4’), 117.9 (CN), 123.1 (CH), 127.6 (CH), 128.6 (2 × CH), 130.0 (C, C-4’), 132.6 (2 × CH), 133.6 (C, C-2’), 137.8 (C), 139.5 (C), 148.5 (C, C-3’), 196.2 (C=O), 197.0 (C=O); +ESIMS m/z (relative intensity): 347 (100%, [M + K]$^+$); -ESIMS m/z (relative intensity): 307 (100%, [M - H]); found by -ESIMS 307.0722, C$_{17}$H$_{11}$NO$_4$ [M - H]$^-$, requires 307.0710, error 0.6 ppm.

2.6.5. 1-(4-Cyanophenyl)-4-(4-nitrophenyl)-1,4-butadione 169

The reaction mixture was quenched with 10.0% (v/v) aqueous H$_2$SO$_4$ solution (10 ml) and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10.0 ml), dried over MgSO$_4$ and concentrated. Compound 169 was crystallized from methanol to give 0.71 g (51.0%) of a fine orange solid: mp 173-175 °C; IR (cm$^{-1}$): 2226 (CN), 1683 (C=O), 1521 (NO$_2$), 1316 (NO$_2$), 1310, 1291, 1184, 1002, 860, 794, 741; $^1$H NMR (300 MHz; CDCl$_3$): 3.52 (4H, s, 2 × CH$_2$), 7.83 (2H, d, J = 8.1 Hz, 2 × CH), 8.14 (2H, d, J = 8.4 Hz, 2 × CH), 8.20 (2H, d, J = 8.7 Hz, 2 × CH, H-2’), 8.36 (2H, d, J = 8.7 Hz, 2 × CH, H-3’); $^{13}$C NMR (75 MHz; CDCl$_3$) (assignments made using DEPT-135): 44.8 (2 × CH$_2$, C-2, C-3), 109.5 (C), 115.3 (C), 118.1 (C, C-4’), 118.9 (C≡N), 128.8 (2 × CH), 131.1 (2 × CH), 132.0 (2 × CH, C-2’), 132.8 (2 × CH, C-3’), 139.5 (C, C-1’), 140.6 (C, C-4’), 196.3 (2 × C=O); -ESIMS m/z (relative intensity): 307 (100%, [M - H]); found by -ESIMS 307.0727, C$_{17}$H$_{11}$NO$_4$ [M - H]$^-$, requires 307.0724, error 1.0 ppm.

2.6.6. 1-(4-Cyanophenyl)-4-(4-methoxyphenyl)-1,4-butadione 170

The reaction was quenched by the addition of 10.0% (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10 ml), dried over dry MgSO$_4$ and evaporated. The crude reaction mixture was purified by column chromatography using EtOAc-hexane (1:9) as a mobile phase to
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give 0.35 g (27.0 %) of 170 as a colourless solid: mp 120-122 °C; IR (cm\(^{-1}\)):
3241, 2931, 1676 (C=O), 1606, 1356, 1226, 991, 823; \(^1\)H NMR (300 MHz; CDCl\(_3\)):
3.42-3.49 (4H, m, 2 × CH\(_2\), H-2, H-3), 3.90 (3H, s, OCH\(_3\)), 6.84 (2H, d, J = 8.7 Hz, 2 × CH, H-3”), 7.81 (2H, d, J = 8.4 Hz, 2 × CH), 8.03 (2H, d, J = 8.7 Hz, 2 × CH), 8.15 (2H, d, J = 8.4 Hz, 2 × CH); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) (assignments made using DEPT-135):
32.3 (C\(_2\)H\(_2\)), 32.8 (C\(_2\)H\(_2\)), 55.5 (OCH\(_3\)), 113.8 (CH), 116.4 (C, C-4’), 118.0 (CN), 128.6 (2 × CH, C-3”), 129.6 (C), 130.4 (2 × CH), 132.5 (2 × CH), 139.9 (C), 163.7 (C), 197.6 C=O); +ESIMS m/z (relative intensity): 316 (100%, [M + Na]^+); found by +ESIMS 316.0938, C\(_{18}\)H\(_{15}\)NO\(_3\)Na [M + Na]^+, requires 316.0944, error 1.9 ppm.

2.6.7. 1-(4-Cyanophenyl)-4-(4-methylphenyl)-1,4-butadione 171

The reaction mixture was diluted by the addition of ethyl acetate (10 ml), washed with 0.1 N HCl (10 ml) and water (10 ml). The organic layer was dried over dry MgSO\(_4\) and concentrated. Compound 171 was re-crystallized from methanol to give 0.12 g (9.0 %) of colourless crystals: mp 134-136 °C; IR (cm\(^{-1}\)):
2227 (CN), 1680 (C=O), 1676 (C=O), 1356, 1226, 991, 840, 822, 565; \(^1\)H NMR (300 MHz; CDCl\(_3\)):
2.45 (3H, s, CH\(_3\)), 3.45-3.50 (4H, m, 2 × CH\(_2\), H-2, H-3), 7.31 (2H, d, J = 8.7 Hz, 2 × CH, H-3”), 7.82 (2H, d, J = 8.1 Hz, 2 × CH), 7.95 (2H, d, J = 8.1 Hz, 2 × CH, H-2”), 8.15 (2H, d, J = 8.4 Hz, 2 × CH); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) (assignments made using DEPT-135):
21.7 (CH\(_3\)), 32.5 (CH\(_2\)), 32.8 (CH\(_2\)), 116.4 (C, C-4’), 118.0 (CN), 128.2 (2 × CH, C-3”), 128.6 (2 × CH), 129.4 (2 × CH, C-2”), 132.5 (2 × CH), 134.0 (C), 139.8 (C), 144.2 (C), 197.6 (C=O), 197.8 (C=O); +ESIMS m/z (relative intensity): 316 (100%, [M + K]^+), -ESIMS m/z 276 (100%, [M - H]^+); found by +ESIMS 300.0997, C\(_{18}\)H\(_{15}\)NO\(_2\)Na [M + Na]^+, requires 300.0995, error 0.5 ppm.
2.6.8. 1-(4-Cyanophenyl)-4-(4-ethylphenyl)-1,4-butanone 172

The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10.0 ml), dried over dry MgSO₄ and concentrated. Compound 172 was crystallized from methanol to give 0.19 g (15.0 %) of colourless crystals: mp 126-128 °C; IR (cm⁻¹): 2222 (CN), 1671 (C=O), 1224, 992, 801, 568; ¹H NMR (300 MHz; CDCl₃): 1.20 (3H, t, J = 7.5 Hz, CH₃), 2.65 (2H, q, J = 7.5 Hz, CH₂), 3.36-3.41 (4H, m, 2 × CH₂, H-2, H-3), 7.24 (2H, d, J = 7.8 Hz, 2 × CH, H-3"), 7.73 (2H, d, J = 7.8 Hz, 2 × C), 7.88 (2H, d, J = 7.8 Hz, 2 × CH), 8.06 (2H, d, J = 7.8 Hz, 2 × CH); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 15.2 (CH₃), 29.0 (CH₃CH₂), 32.5 (CH₂), 32.8 (CH₂), 116.4 (C, C-4'), 118.0 (CN), 128.2 (2 × CH, C-3''), 128.4 (2 × CH), 128.6 (2 × CH), 132.5 (2 × CH), 134.2 (C), 139.9 (C), 150.4 (C), 197.7 (C=O), 197.9 (C=O); +ESIMS m/z (relative intensity): 292.3 (100%, [M + H]+), -ESIMS m/z 290.4 (100%, [M - H]-); found by +ESIMS 292.1331, C₁₉H₁₈NO₂ [M + H]+, requires 292.1332, error 0.4 ppm.

Compound 172 was also synthesized using an alternative synthetic pathway starting from 4-acetylbenzonitrile 120 and 2-bromo-1-(4-ethylphenyl)ethanone 175. The procedure for this synthetic pathway: triethylamine (0.6 ml, 4.1 mmol) was added to a suspension of zinc chloride (0.74 g, 5.4 mmol) in dry toluene (5.0 ml) and absolute ethanol (0.2 ml, 4.1 mmol). The reaction mixture was stirred at room temperature for 1-2 h. 4-Acetylbenzonitrile 120 (0.61 g, 2.7 mmol) and 2-bromo-1-(4-ethylphenyl)ethanone 175 (0.60 g, 4.1 mmol) were added and the reaction mixture was stirred at room temperature for 7 days and monitored by TLC (20.0 % EtOAc/ hexane). The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10 ml), dried over anhydrous MgSO₄ and evaporated. The crude reaction mixture was purified by column chromatography using EtOAc-hexane (1:9) as the mobile phase to give 0.14 g (18.0 %) of 172 as a colourless solid.
2.6.9. 1-(4-Cyanophenyl)-4-(4-isopropylphenyl)-1,4-butanedione 173

The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was rinsed with brine (10.0 ml), dried over anhydrous MgSO₄ and evaporated. The crude reaction mixture was purified by column chromatography using EtOAc-hexane (1:9) as the mobile phase to give 0.28 g (11.0 %) of 173 as a colourless solid: mp 92-93 °C; IR (cm⁻¹): 2222 (CN), 1675 (C=O), 1001, 788, 582; ¹H NMR (300 MHz; CDCl₃): 1.28 (6H, d, J = 6.9 Hz, 2 × CH₃), 2.98 (1H, septet, J = 6.9 Hz, CH), 3.43-3.48 (4H, m, 2 × CH₂, H-2, H-3), 7.34 (2H, d, J = 7.8 Hz, 2 × CH, H-3”), 7.79 (2H, d, J = 8.1 Hz, 2 × CH), 7.96 (2H, d, J = 8.1 Hz, 2 × CH), 8.13 (2H, d, J = 8.1 Hz, 2 × CH); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 23.7 (2 × CH₃), 31.1 (CH), 32.5 (CH₂), 32.8 (CH₂), 116.3 (C, C4’), 118.0 (CN), 125.6 (2 × CH), 126.8 (2 × CH), 128.4 (2 × CH), 132.5 (2 × CH), 139.8 (C), 155.0 (C), 157.2 (C), 197.6 (C=O), 197.8 (C=O); +ESIMS m/z (relative intensity): 328 (100%, [M + H]^+); found by +ESIMS 328.1300, C₂₀H₁₉NO₂Na [M + Na]^+, requires 328.1308, error 2.4 ppm.

2.6.10. 1-(4-Cyanophenyl)-4-(4-tert-butylphenyl)-1,4-butanedione 174

The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10 ml), dried over anhydrous MgSO₄ and concentrated. The crude reaction mixture was purified by column chromatography using EtOAc-hexane (1:9) as the mobile phase to give 0.13 g (9.0 %) of 174 as a white solid: mp 133-135 °C; IR (cm⁻¹): 2222 (CN), 1671 (C=O), 1320, 1006, 855, 701, 569; ¹H NMR (300 MHz; CDCl₃): 1.35 (9H, s, 3 × CH₃), 3.43-3.48 (4H, m, 2 × CH₂, H-2, H-3), 7.50 (2H, d, J = 8.4 Hz, 2 × CH, H-3”), 7.79 (2H, d, J = 8.4 Hz, 2 × CH), 7.97 (2H, d, J = 8.1 Hz, 2 × CH), 8.13
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(2H, d, J = 8.1 Hz, 2 × CH); $^{13}$C NMR (100 MHz; CDCl$_3$) (assignments made using DEPT-135): 23.7 (3 × CH$_3$), 32.5 (2 × CH$_2$, C-2, C-3), 35.2 (C), 116.4 (C, C-4$''$), 118.0 (CN), 125.6 (2 × CH, C-3$''$), 126.8 (2 × CH), 128.4 (2 × CH), 132.5 (2 × CH), 134.3 (C), 139.8 (C), 155.0 (C), 197.6 (C=O), 197.8 (C=O); +ESIMS m/z (relative intensity): 342 (100%, [M + Na]$^+$); found by +ESIMS 320.1648, C$_{21}$H$_{22}$NO$_2$ [M + H]$^+$, requires 320.1645, error 0.9 ppm.

2.7. 1-(3-Cyanophenyl)-4-phenyl-1,4-butadione 179

Zinc chloride (1.40 g, 10 mmol) was added to dry toluene (5.0 ml), absolute ethanol (0.4 ml, 7.5 mmol) and triethylamine (1.0 ml, 7.5 mmol). The mixture was stirred at room temperature for 1-2 h. 3-Acetylbenzonitrile 178 (1.10 g, 7.5 mmol) and 2-bromoacetophenone 133 (1.00 g, 5.0 mmol) were added and the mixture was stirred at room temperature for 4 days and monitored by TLC (20.0 % EtOAc/ hexane). The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution and extracted with ethyl acetate (20 ml). The organic layer was washed with brine (10 ml), dried over anhydrous MgSO$_4$ and evaporated. The crude reaction mixture was purified by column chromatography using EtOAc-hexane (1:9) as the mobile phase to give 0.20 g (15.2%) of 179 as a colourless solid: mp 102-104 °C; IR (cm$^{-1}$): 2222 (CN), 1670 (C=O), 750, 680; $^1$H NMR (300 MHz; CDCl$_3$): 3.45 (2H, t, J = 5.7 Hz, CH$_2$), 3.53 (2H, t, J = 6.0 Hz, CH$_2$), 7.51 (2H, t, J = 7.5 Hz, 2 × CH, H-3$''$), 7.60 (1H, d, J = 7.5 Hz, CH); 7.63 (1H, t, J = 7.8 Hz, CH), 7.88 (1H, d, J = 7.8 Hz, CH), 8.05 (2H, d, J = 7.5 Hz, 2 × CH, H-2$''$), 8.28 (1H, d, J = 7.8 Hz, CH), 8.35 (1H, s, CH, H-2'); $^{13}$C NMR (75 MHz, CDCl$_3$) (assignments made using DEPT-135): 31.50 (CH$_2$), 31.52 (CH$_2$), 112.2 (C, C-3$''$), 117.0 (CN), 127.1 (CH), 127.7 (CH), 128.7 (CH), 130.9 (CH), 131.1 (CH), 132.4 (CH), 135.0 (CH), 135.4 (C), 136.5 (C), 195.8 (C=O), 197.2 (C=O); +ESIMS m/z 264 (50%, [M + H]$^+$), 286 (100%, [M + Na]$^+$), 302 (32%, [M + K]$^+$); found by +ESIMS 264.1018, C$_{17}$H$_{13}$NO$_2$ [M + H]$^+$, requires 264.1019, error 0.4 ppm.
2.8. General procedure for the synthesis of methyl aryl ketones 163 and 164

Acetyl chloride (1.8 mmol) was added dropwise to a suspension of AlCl₃ (1.8 mmol) in dry dichloromethane (20.0 ml) at 0 °C. After 10-15 m stirring at 0 °C the alkylbenzene was added dropwise. The reaction mixture was stirred at room temperature for 30 m and monitored by TLC using 20% EtOAc in hexane as a mobile phase. The reaction mixture was cooled to 0 °C and water (60.0 ml) was added slowly. The organic layer was washed with saturated NaCl solution (30 ml), dried over dry MgSO₄ and evaporated to give the product as an oil.

2.8.1. 4-Isopropylacetophenone 163

Yellow oil (1.22 g, 90.0%); ¹H NMR (300 MHz; CDCl₃): 1.28 (6H, d, J = 6.9 Hz, 2 × CH₃), 2.58 (3H, s, CH₃), 2.97 (1H, septet, J = 6.9 Hz, CH), 7.32 (2H, d, J = 8.1 Hz, 2 × CH, H-3), 7.91 (2H, d, J = 7.8 Hz, 2 × CH, H-2); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 23.7 (2 × CH₃), 26.6 (CH₃), 34.3 (CH), 127.7 (C, C-4), 128.6 (2 × CH, C-3), 135.1 (2 × CH, C-2), 154.6 (C, C-1), 197.8 (C=O); +ESIMS m/z 163 (100%, [M + H]+), 185 (800%, [M + Na]+).

2.8.2. 4-tert-Butylacetophenone 164

Yellow oil (1.30 g, 98.0%); ¹H NMR (300 MHz; CDCl₃): 1.34 (9H, s, 3 × CH₃), 2.58 (3H, s, CH₃), 7.48 (2H, d, J = 8.4 Hz, 2 × CH, H-3), 7.91 (2H, d, J = 8.4 Hz, 2 × CH, H-2); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 26.6 (3 x CH₃), 31.1 (CH₃), 35.1 (C), 124.9 (2 x CH, C-3), 128.0 (2 x CH, C-2), 139.4 (C, C-4), 156.8 (C, C-1), 198.3 (C=O).
2.9. 2-Bromo-1-(4-ethylphenyl)ethanone 175

4-Ethylacetophenone 162 (1.00 g, 6.7 mmol) and N-bromosuccinimide (1.20 g, 6.7 mmol) were dissolved in acetonitrile (10.0 ml). para-Toluenesulfonic acid (1.90 g, 10.1 mmol) was added slowly to the reaction mixture and the reaction mixture was heated at reflux and monitored by TLC (10.0 % EtOAc/hexane). Acetonitrile was evaporated and the oily residue was dissolved in dichloromethane, washed with water and dried over anhydrous MgSO₄. The organic layer was evaporated to give 1.06 g (69.7 %) of 175 as a light yellow oil, which was used in the further step without any further purification: ¹H NMR (300 MHz; CDCl₃): 1.26 (3H, t, J = 6.6 Hz, CH₃), 2.71 (2H, q, J = 6.6 Hz, CH₂), 4.43 (2H, s, CH₂), 7.30 (2H, d, J = 6.9 Hz, 2 × CH, H-3'), 7.90 (2H, d, J = 6.9 Hz, 2 × CH, H-2'); +ESIMS m/z (relative intensity): 227 (30%, ⁷⁹Br, [M - H]), 229 (28 %, ⁸¹Br). ¹H NMR data for compound 175 are identical to those reported in literature.¹⁵⁹

2.10. 4-(5-Phenyl-1H-pyrrol-2-yl)benzonitrile 198

Ammonium acetate (0.31 g, 4.0 mmol) was added to a stirred solution of 126 (0.20 g, 0.8 mmol) in absolute ethanol (7.0 ml) and chloroform (10.0 ml). The reaction mixture was stirred at room temperature and monitored by TLC (10 % EtOAc/hexane) for the consumption of the starting material. The reaction mixture was concentrated and kept in the fridge overnight. Compound 198 was collected by filtration and rinsed with hexane to give 0.06 g (32.0 %) of yellow needle crystals; mp 158-161 °C; IR (cm⁻¹): 3400 (NH), 2220 (CN), 725; ¹H NMR (300 MHz; DMSO-d₆/D₂O): 6.65 (1H, d, J = 3.9 Hz, pyrrole-CH), 6.81 (1H, d, J = 3.6 Hz, pyrrole-CH), 7.23 (1H, t, J = 7.2 Hz, CH, H-4’), 7.39 (2H, t, J = 7.8 Hz, 2 × CH, H-3”), 7.56-7.62 (4H, m, 4 × CH), 7.89 (2H, d, J = 8.4 Hz, 2 × CH); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 107.0 (C, C-1), 108.4 (CH), 110.7 (CH), 119.3 (CN), 123.9 (2 × CH), 124.3 (2 × CH), 126.4 (CH), 128.6 (2 × CH), 131.1 (C), 131.9 (C), 132.6 (2 × CH), 135.1 (C),
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136.6 (C); m/z (-ES) 243 (100, [M - H]); found by -ES 243.0929, C_{17}H_{11}N_{2} [M - H], requires 243.0927, error 0.7 ppm.

2.11. 4-(1-Methyl-5-phenyl-1H-pyrrol-2-yl)benzonitrile 199

Methylamine (0.35 ml g, 4.0 mmol) and 15 drops glacial acetic acid were added to a solution of 126 (0.27 g, 1.0 mmol) in chloroform (12.0 ml) and absolute ethanol (8.0 ml). The reaction mixture was stirred at 50 °C and monitored by TLC (10 % MeOH-CHCl₃). After 24 h of heating, the reaction was cooled to room temperature, concentrated on a rotary evaporator and kept in the fridge overnight. The pale yellow crystals were collected by filtration and washed with hexane to give 0.20 g (78.0 %): mp 173-175 °C; IR (cm⁻¹): 2231 (CN), 1461, 755; ¹H NMR (300 MHz; DMSO-d₆): 3.63 (3H, s, NCH₃), 6.35 (1H, d, J = 3.6 Hz, pyrrole-CH), 6.51 (1H, d, J = 3.6 Hz, pyrrole-CH), 7.36 (1H, t, J = 7.2 Hz, CH, H-4”), 7.45-7.54 (4H, m, 4 × CH, H-2”, H-3”), 7.72 (2H, d, J = 8.1 Hz, 2 × CH), 7.90 (2H, d, J = 8.4 Hz, 2 × CH); ¹³C NMR (75 MHz; DMSO-d₆) (assignments made using DEPT-135): 34.5 (NCH₃), 108.2 (C, C-1), 109.2 (CH), 110.9 (CH), 119.1 (CN), 127.2 (CH, C-4”), 128.0 (2 × CH), 128.2 (2 × CH), 128.7 (2 × CH), 132.3 (2 × CH), 132.5 (C), 134.5 (C), 137.2 (C), 138.4 (C); m/z (-ES) 243 (100, [M – CH₄]).

2.12. General procedure for the synthesis of ethyl furan- 180-190, pyrrole- 200 and N-methylpyrrole-imidate hydrochloride 201 intermediates

Acetyl chloride (16.0 mmol for compounds 165-174 and 179 and 8.0 mmol for compounds 200-201) was added dropwise to a suspension of 165-174, 179 or 200-201 (1.0 mmol) in absolute ethanol (24 mmol for compounds 165-174 and 179 and 12.0 mmol for 200-201) in dry chloroform (10 ml) at 0 °C. The reaction was allowed to warm up to room temperature and stirring was continued for 1-3 days.
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2.12.1. Ethyl 4-(5-(3-fluorophenyl)furan-2-yl)benzimidate hydrochloride 180

The yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.36 g (61.0 %): mp 236-238 °C; IR (cm\(^{-1}\)): 2814, 1606, 1433, 1060 (C-F), 846, 752, 686; \(^1\)H NMR (300 MHz; DMSO-\(d_6\)): 1.51 (3H, t, \(J = 6.6\) Hz, \(CH_3\)), 4.63 (2H, q, \(J = 6.9\) Hz, \(CH_2\)), 7.16 (1H, dd-t, \(^3\)J\(_{HF} = J_{HH} = 8.7\) Hz, CH, H-4\(^\prime\)), 7.31 (1H, d, \(J = 3.3\) Hz, furan-CH), 7.45 (1H, d, \(J = 3.3\) Hz, furan-CH), 8.07 (2H, d, \(J = 8.4\) Hz, 2 x CH), 8.10 (2H, d, \(J = 8.7\) Hz, 2 x CH), 8.18 (2H, d, \(J = 8.4\) Hz, 2 x CH), 12.0 (1H, s, N-H); \(^1\)C NMR (75 MHz; DMSO-\(d_6\)): 18.8 (\(CH_3\)), 60.7 (\(CH_2\)), 109.8 (CH), 114.4 (d, \(^2\)J\(_{CF} = 21.2\) Hz), 119.7, 123.2, 128.2, 129.8, 131.0 (d, \(^3\)J\(_{CF} = 8.6\) Hz), 132.1 (d, \(^3\)J\(_{CF} = 8.8\) Hz), 132.2, 132.9, 151.95, 151.99, 162.6 (d, \(^1\)J\(_{CF} = 241.5\) Hz, C-3\(^\prime\)), 167.3 (C-imidate); +ESIMS m/z (relative intensity): 310 (100%, [M + H]\(^+\)); found by +ESIMS 310.1238, C\(_{19}\)H\(_{17}\)NO\(_2\)F [M + H]\(^+\), requires 310.1238, error 0.0 ppm.

2.12.2. Ethyl 4-(5-(4-fluorophenyl)furan-2-yl)benzimidate hydrochloride 181

The yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.22 g (89.0 %): mp 190-192 °C (decomposed); IR (cm\(^{-1}\)): 2910, 1606, 1408, 1229 (C-F), 829, 807, 750 (C-F); \(^1\)H NMR (300 MHz; DMSO-\(d_6\)): 1.51 (3H, t, \(J = 6.9\) Hz, \(CH_3\)), 4.60 (2H, q, \(J = 6.9\) Hz, \(CH_2\)), 7.19 (1H, d, \(J = 3.6\) Hz, furan-CH), 7.34 (2H, t, \(^3\)J\(_{HF} = J_{HH} = 8.4\) Hz, 2 x CH, H-3\(^\prime\)), 7.43 (1H, d, \(J = 3.0\) Hz, furan-CH), 7.95 (2H, dd-t, \(^4\)J\(_{HF} = J_{HH} = 7.2\) Hz, 2 x CH, H-2\(^\prime\)), 8.07 (2H, d, \(J = 8.4\) Hz, 2 x CH), 8.12 (2H, d, \(J = 8.4\) Hz, 2 x CH); +ESIMS m/z (relative intensity): 310.6 (100%, [M + H]\(^+\)); found by +ESIMS 310.1252, C\(_{19}\)H\(_{17}\)NO\(_2\)F [M + H]\(^+\), requires 310.1243, error 2.8 ppm.
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2.12.3. Ethyl 4-(5-(4-bromophenyl)furan-2-yl)benzimidate hydrochloride 182

The orange precipitate was filtered off under vacuum and rinsed with hexane. Compound 182 was re-crystallized from methanol to give 0.73 g (64.0 %) of a bright yellow solid: mp 144-145 °C; IR (cm⁻¹): 2360, 1607, 752; ¹H NMR (300 MHz; DMSO-d₆): 1.52 (3H, t, J = 6.9 Hz, CH₃), 4.62 (2H, q, J = 6.9 Hz, CH₂), 7.28 (1H, d, J = 3.5 Hz, furan-CH), 7.45 (1H, d, J = 3.5 Hz, furan-CH), 7.68 (2H, d, J = 8.4 Hz, 2 x CH), 7.85 (2H, d, J = 8.4 Hz, 2 x CH), 8.08 (2H, d, J = 8.6 Hz, 2 x CH), 8.15 (2H, d, J = 8.6 Hz, 2 x CH); +ESIMS m/z (relative intensity): 370 (100%, ⁷⁹Br, [M + H]⁺), 372 (98%, ⁸¹Br); found by +ESIMS 370.0438, C₁₉H₁₇NO₂Br [M + H]⁺, requires 370.0443, error 1.3 ppm.

2.12.4. Ethyl 4-(5-(3-nitrophenyl)furan-2-yl)benzimidate hydrochloride 183

The precipitate was filtered under vacuum and rinsed with chloroform to give 0.65 g (97%) of a fine bright yellow solid: mp 200-203°C; IR (cm⁻¹): 2981, 1677 (C=N), 1606 (furan C=C), 1527, 1342; ¹H NMR (400 MHz; DMSO-d₆): 1.35 (3H, t, J = 8.0 Hz, CH₃), 4.34 (2H, q, J = 8.0 Hz, CH₂), 7.37 (1H, d, J = 3.6 Hz, furan-CH), 7.47 (1H, d, J = 3.0 Hz, furan-CH), 7.77 (1H, t, J = 8.0 Hz, CH, H-5”), 8.00 (2H, d, J = 8.8 Hz, 2 x CH), 8.04 (2H, d, J = 8.8 Hz, 2 x CH), 8.17 (1H, d, J = 8.0 Hz, CH, H-6”), 8.31 (1H, d, J = 7.6 Hz, CH, H-4”), 8.59 (1H, s, CH, H-2”); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 14.2 (CH₃), 60.8 (CH₂), 111.0 (CH), 111.1 (CH), 117.8 (CH), 122.2 (CH), 123.7 (2 x CH), 128.6 (CH), 129.8 (CH), 129.9 (2 x CH), 130.7 (CH), 131.2 (C), 133.6 (C), 148.5 (C, C-3””), 151.4 (C), 152.6 (C), 165.3 (C, C- imidate); +ESIMS m/z (relative intensity): 337.4 (100%, [M + H]⁺); found by +ESIMS 337.1200, C₁₉H₁₇NO₄[M + H]⁺, requires 337.1188, error 3.5ppm.
2.12.5. Ethyl 4-(5-(4-nitrophenyl)furan-2-yl)benzimidate hydrochloride 184

The orange precipitate was filtered under vacuum and rinsed with hexane. Compound 184 was re-crystallized from DMF/H$_2$O to give 0.32 g (53.3 %): mp 250-252°C; IR (cm$^{-1}$): 2981, 1677 (C=N), 1606, 1527, 1342; $^1$H NMR (300 MHz; DMSO-d$_6$): 1.34 (3H, t, J = 7.2 Hz, CH$_3$), 4.33 (2H, q, J = 7.2 Hz, CH$_2$), 7.41 (1H, d, J = 3.6 Hz, furan-CH), 7.51 (1H, d, J = 3.6 Hz, furan-CH), 8.00-8.03 (4H, m, 4 x CH), 8.11 (2H, d, J = 8.7 Hz, 2 x CH), 8.31 (2H, d, J = 8.7 Hz, 2 x CH).

2.12.6. Ethyl 4-(5-(4-methoxyphenyl)furan-2-yl)benzimidate hydrochloride 185

The reaction mixture was diluted with hexane and the yellow-coloured precipitate was collected and rinsed with hexane to give 0.014 g (57.0 %) of 185: $^1$H NMR (300 MHz; DMSO-d$_6$): 1.51 (3H, t, J = 6.6 Hz, CH$_3$), 2.35 (3H, s, CH$_3$), 4.53 (2H, q, J = 6.4 Hz, OCH$_2$), 7.13 (1H, d, J = 3.6 Hz, furan-CH), 7.82 (2H, d, J = 8.1 Hz, 2 x CH, H-2’”), 8.03 (2H, d, J = 7.8 Hz, 2 x CH), 8.11 (2H, d, J = 7.8 Hz, 2 x CH); $^{13}$C NMR (75 MHz; DMSO-d$_6$): 14.2, 55.2, 60.7, 106.9, 110.9, 114.2, 114.4, 123.0, 125.3, 127.9, 129.8, 150.6, 159.1, 165.4.

2.12.7. Ethyl 4-(5-(4-methylphenyl)furan-2-yl)benzimidate hydrochloride 186

The yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.8 g (65.0 %) of 186: $^1$H NMR (300 MHz; DMSO-d$_6$): 1.50 (3H, t, J = 7.2 Hz, CH$_2$CH$_3$), 2.35 (3H, s, CH$_3$), 4.53 (2H, q, J = 6.4 Hz, OCH$_2$), 7.13 (1H, d, J = 3.6 Hz, furan-CH), 7.30 (2H, d, J = 7.8 Hz, 2 x CH, H-3’”), 7.40 (1H, d, J = 3.3 Hz, furan-CH), 7.78 (2H, d, J = 8.1 Hz, 2 x CH, H-2’”), 8.04 (2H, d, J = 9.0 Hz, 2 x CH), 8.09 (2H, d, J =...
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\[ \text{Assignments made using DEPT-135: } 14.2 \text{ (CH}_3\text{), } 20.9 \text{ (OCH}_2\text{CH}_3\text{), } 60.0 \text{ (OCH}_2\text{), } 109.8 \text{ (CH), } 110.8 \text{ (C), } 122.9 \text{ (CH), } 123.2 \text{ (CH), } 123.6 \text{ (CH), } 123.7 \text{ (CH), } 128.2 \text{ (C), } 129.5 \text{ (CH), } 129.8 \text{ (C), } 132.5 \text{ (C), } 151.4 \text{ (C), } 153.5 \text{ (C), } 167.6 \text{ (C, C-imidate).} \]

2.12.8. Ethyl 4-(5-(4-ethylphenyl)furan-2-yl)benzimidate \(^{187}\)

The reaction was cooled to 0 °C and saturated solution of sodium bicarbonate was added dropwise until the gas evolution ceased. The organic layer was extracted, washed with water, dried over anhydrous MgSO\(_4\) and evaporated to give 0.10 g (89.0 %) of \(^{187}\) as a yellow solid: mp 250-252 °C; IR (cm\(^{-1}\)): 3438 (N-H), 2891, 1606 (furan C=C), 1441, 1384, 1060, 675; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.21 (3H, t, J = 7.5 Hz, \text{CH}_2\text{CH}_3), 1.51 (3H, t, J = 7.2 Hz, OCH\(_2\text{CH}_3\)), 2.65 (2H, q, J = 7.5 Hz, C\(_2\text{H}_5\text{CH}_3\)), 4.64 (2H, q, J = 6.9 Hz, OCH\(_2\)), 7.14 (1H, d, J = 3.6 Hz, furan-CH), 7.32 (2H, d, J = 8.1 Hz, 2 x CH), 8.05 (2H, d, J = 8.4 Hz, 2 x CH), 8.20 (2H, d, J = 7.8 Hz, 2 x CH), 11.75 (1H, s, N-H); +ESIMS m/z (relative intensity): 320.6 (100%, [M + H]\(^{+}\)); found by +ESIMS 320.1644, C\(_{21}\)H\(_{22}\)NO\(_2\)[M + H]\(^{+}\), requires 320.1651, error 2.0 ppm.

2.12.9. Ethyl 4-(5-(4-isopropylphenyl)furan-2-yl)benzimidate hydrochloride \(^{188}\)

The reaction mixture was diluted with diethyl ether and the precipitate was filtered off under vacuum, rinsed with diethyl ether and re-crystallized from ethanol to give 0.11 g (32.4 %) of \(^{188}\): mp 231-233 °C; IR (cm\(^{-1}\)): 3136, 3049, 1606, 1408, 750, 677; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.24 (6H, d, J = 6.9 Hz, 2 x CH\(_3\)), 1.51 (3H, t, J = 6.9 Hz, CH\(_3\)), 2.88-2.94 (1H, septet, J = Hz, CH), 4.61 (2H, q, J = 6.9 Hz, CH\(_2\)), 7.14 (1H, d, J = 3.9 Hz, furan-CH), 7.36 (2H, d, J = 8.4 Hz, 2 x CH, H-3”), 7.42 (1H, d, J = 3.6 Hz, furan-CH), 7.81 (2H, d, J = 8.1 Hz, 2 x CH), 8.06 (2H, d, J = 8.7 Hz, 2 x CH), 8.15 (2H, d, J = 8.4 Hz, 2 x CH); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)): 14.2, 23.7, 33.2, 202
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60.7, 107.7, 109.8, 122.9, 123.7, 126.8, 128.2, 129.8, 132.46, 132.52, 148.2, 151.5, 153.5, 167.3; +ESIMS m/z (relative intensity): 334.6 (100%, [M + H]+); found by +ESIMS 334.1800, C\textsubscript{22}H\textsubscript{24}N\textsubscript{2}O\textsubscript{2} [M + H]+, requires 334.1802, error 0.5 ppm.

2.12.10. Ethyl 4-(5-(4-tert-butylphenyl)furan-2-yl)benzimidate hydrochloride 189

The reaction mixture was diluted with diethyl ether; The precipitate was filtered off under vacuum and rinsed with diethyl ether. Compound 189 was re-crystallized from ethanol to give 0.08 g (19.0 %): mp: IR (cm\textsuperscript{-1}): 3128, 2965, 1604, 1407, 1071, 807, 750; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.32 (9H, s, 3 x CH\textsubscript{3}), 1.51 (3H, t, J = 6.9 Hz, CH\textsubscript{3}CH\textsubscript{2}), 4.62 (2H, q, J = 6.9 Hz, OCH\textsubscript{2}), 7.14 (1H, d, J = 3.3 Hz, furan-CH), 7.43 (1H, d, J = 3.6 Hz, furan-CH), 7.50 (2H, d, J = 8.4 Hz, 2 x CH, H-3′′), 7.81 (2H, d, J = 8.4 Hz, 2 x CH); \textsuperscript{13}C NMR (75 MHz; DMSO-d\textsubscript{6}): 18.5, 31.0, 34.4, 60.7, 109.8, 110.9, 122.9, 123.2, 123.6, 125.7, 128.0, 128.2, 129.8, 134.1, 153.5, 154.0, 167.3; +ESIMS m/z (relative intensity): 348.8 (100%, [M + H]+); found by +ESIMS 348.1960, C\textsubscript{23}H\textsubscript{24}N\textsubscript{2}O\textsubscript{2} [M + H]+, requires 348.1964, error 1.0 ppm.

2.12.11. Ethyl 3-(5-phenylfuran-2-yl)benzimidate hydrochloride 190

The green-yellow precipitate was filtered off under vacuum and rinsed with diethyl ether to give 0.22 g (88.0 %) of 190: mp 174 ºC; IR (cm\textsuperscript{-1}): 2827, 2362, 1738, 1635 (C=N), 1464, 1353, 1077, 782, 677; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.52 (3H, t, J = 6.9 Hz, CH\textsubscript{3}), 4.66 (2H, q, J = 6.9 Hz, CH\textsubscript{2}), 7.16 (1H, d, J = 3.3 Hz, furan-CH), 7.32-7.37 (2H, m, 2 x CH including furan-CH), 7.47 (2H, t, J = 7.5 Hz, 2 x CH, H-3′′), 7.71 (1H, t, J = 7.8 Hz, CH, H-5), 7.89 (2H, d, J = 7.5 Hz, 2 x CH, H-2′′), 7.96 (1H, d, J = 7.8 Hz, CH), 8.22 (1H, d, J = 7.8 Hz, CH), 8.64 (1H, s, CH, H-2), 11.97 (s, NH); \textsuperscript{13}C NMR (100 MHz; DMSO-d\textsubscript{6}) (assignments made using DEPT-135): 14.2 (CH\textsubscript{3}), 61.0 (OCH\textsubscript{2}), 108.4 (CH), 108.8 (CH), 122.3 (CH), 123.5 (CH), 126.0 (CH), 126.5 (CH), 203
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126.9 (C), 127.7 (CH), 127.9 (CH), 128.9 (CH), 129.5 (C), 130.5 (C), 151.4 (C), 152.9 (C), 167.4 (C, C-imidate); +ESIMS m/z (relative intensity): 292 (100%, [M + H]+); found by +ESIMS 292.1324, C_{19}H_{18}NO_{2} [M + H]^+ requires 292.1332, error 2.8 ppm.


The precipitate was filtered off under vacuum and rinsed with hexane. Compound 200 was re-crystallized from MeOH/CHCl\textsubscript{3} to give 0.16 g (30.8 %) of a brown solid: mp 232-235 °C; IR (cm\textsuperscript{-1}): 3343 (N\textsubscript{H}), 3172, 3002, 1601, 1383, 744; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.51 (3H, t, J = 6.9 Hz, CH\textsubscript{3}), 4.62 (2H, q, J = 6.6 Hz, CH\textsubscript{2}), 6.72 (1H, s, pyrrole-C\textsubscript{H}), 6.97 (1H, s, pyrrole-C\textsubscript{H}), 7.25 (1H, t, J = 6.9 Hz, C\textsubscript{H}, H-4\textsuperscript{b}), 7.42 (2H, t, J = 7.5 Hz, 2 x CH\textsubscript{2}, H-3\textsuperscript{b}), 7.84 (2H, d, J = 7.5 Hz, 2 x CH\textsubscript{2}, H-2\textsuperscript{b}), 8.06 (2H, d, J = 8.4 Hz, 2 x CH\textsubscript{2}), 8.13 (2H, d, J = 8.1 Hz, 2 x CH\textsubscript{2}); +ESIMS m/z (relative intensity): 291.7 (100%, [M + H]+); found by +ESIMS 291.1488, C\textsubscript{19}H\textsubscript{19}N\textsubscript{2}O [M + H]^+ requires 291.1497, error 3.2 ppm.

2.12.13. Ethyl 4-(1-methyl-5-phenyl-H-pyrrol-2-yl)benzimidate hydrochloride 201

The reaction mixture was concentrated and kept in the fridge overnight. The yellow-green precipitate was filtered off under vacuum and rinsed with diethyl ether to give 0.10 g (58.8 %) of 201; IR (cm\textsuperscript{-1}): 2842, 1647, 1604, 1460, 1078, 700; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.51 (3H, t, J = 6.9 Hz, CH\textsubscript{3}), 3.67 (3H, s, NCH\textsubscript{3}), 4.64 (2H, q, J =6.9 Hz, CH\textsubscript{2}), 6.38 (1H, d, J = 3.9 Hz, pyrrole-CH\textsubscript{2}), 6.60 (1H, d, J = 3.9 Hz, pyrrole-CH\textsubscript{2}), 7.38 (1H, t, J = 6.9 Hz, CH\textsubscript{2}, H-4\textsuperscript{b}), 7.46-7.55 (4H, m, 4 x CH\textsubscript{2}, H-2\textsuperscript{b}, H-3\textsuperscript{b}), 7.81 (2H, d, J = 8.1 Hz, 2 x CH\textsubscript{2}), 8.17 (2H, d, J = 8.4 Hz, 2 x CH\textsubscript{2}); +ESIMS m/z (relative intensity): 305.7 (100%, [M + H]+); found by +ESIMS 305.1649, C\textsubscript{20}H\textsubscript{21}N\textsubscript{2}O [M + H]^+ requires 305.1648, error 0.2 ppm.
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2.13. General procedure for the synthesis of furan-144-154, pyrrole-191 and N-methylpyrrole-amidine acetates 192

Ammonium acetate (4.0 mmol) was added to a stirred suspension of furan-180-190, pyrrole 200 and N-methylpyrrole-imidates 201 (1.0 mmol) in anhydrous ethanol. The mixture was stirred at room temperature for 1-3 days.

2.13.1. 4-(5-(3-Fluorophenyl)furan-2-yl)benzamidine acetate 144

The light yellow fine solid was filtered off under vacuum and rinsed with ethanol to give 0.21 g (64.0%) of 144: mp 234-236 °C; IR (cm\(^{-1}\)): 2965, 1738, 1610; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.73 (3H, s, CH\(_3\)), 7.17 (1H, t, \(^3\)J\(_{HF}\) = \(J_{HH} = 8.1\) Hz, CH, H-4’), 7.26 (1H, d, \(J = 3.0\) Hz, furan-CH), 7.34 (1H, d, \(J = 3.0\) Hz, furan-CH), 7.51 (1H, ddd~q, \(^4\)J\(_{HF}\) = \(J_{HH} = 7.8\) Hz, CH, H-5’), 7.71-7.75 (2H, m, 2 x CH, H-2’, H-6’), 7.88 (2H, d, \(J = 8.4\) Hz, 2 x CH), 8.03 (2H, d, \(J = 8.4\) Hz, 2 x CH); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 22.9, 109.9, 111.3, 114.7 (d, \(^2\)J\(_{CF}\) = 22.0 Hz), 119.8, 123.6, 126.7, 128.7, 131.3, 131.7 (d, \(^3\)J\(_{CF}\) = 8.6 Hz), 134.2, 151.5, 152.6, 162.6 (d, \(^1\)J\(_{CF}\) = 241.5 Hz, C-3”), 165.1 (C-amidine), 174.5 (C=O); \(^19\)F-NMR (377 MHz; DMSO-d\(_6\)): -112.4; -ESIMS m/z (relative intensity): 279 (90%, [M - H]); +ESIMS m/z (relative intensity): 281 (100%, [M + H]); found by +ESIMS 281.1080, C\(_{17}\)H\(_{14}\)NO\(_2\)F[M + H], requires 281.1085, error 1.7 ppm.

2.13.2. 4-(5-(4-Fluorophenyl)furan-2-yl)benzamidine acetate 145

The white precipitate was filtered off under vacuum and rinsed with hexane. Compound 145 was re-crystallized from ethanol to give 0.05 g (47.0%): mp 219-222 °C; IR (cm\(^{-1}\)): 2970, 1614 (furan C=C), 1401, 1209 (C-F), 1156, 844, 781, 675, 603; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.74 (3H, s, CH\(_3\)), 7.15 (1H, d, \(J = 2.7\) Hz, furan-CH), 7.30-7.35 (3H, m, 3 x CH, including furan-CH), 7.87-8.01 (6H, m, 6 x CH, H-2, H-3, H-2’); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 24.2
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(CH₃), 108.4 (CH), 110.9 (CH), 116.0 (d, \(^3J_{CF} = 21.9\) Hz, 2 × CH, C-3'), 123.3 (CH), 125.9 (d, \(^3J_{CF} = 8.1\) Hz, 2 × CH, C-2''), 126.4 (C), 128.1 (C), 128.2 (CH), 133.8 (C), 152.4 (C), 152.6 (C), (C-4'', C-amidine and C=O were not observed in the \(^{13}\)C NMR spectrum); \(^{19}\)F NMR (377 MHz; DMSO-\(d_6\)): -113.1; +ESIMS m/z (relative intensity): 281 (100 %, [M + H]^+); found by +ESIMS 281.1094, C\(_{17}\)H\(_{14}\)NO\(_2\)F [M + H]^+, requires 281.1085, error 3.1 ppm

2.13.3. 4-(5-(4-Bromophenyl)furan-2-yl)benzamidine acetate 146

The light yellow-coloured precipitate was filtered off under vacuum and rinsed with hexane. Compound 146 was re-crystallized from ethanol to give 0.05 g (18.2 %) of 146: IR (cm\(^{-1}\)): 2915, 1395, 995, 780; \(^1\)H NMR (300 MHz; DMSO-\(d_6\)): 1.80 (3H, s, CH₃), 7.20 (1H, d, \(J = 3.3\) Hz, furan-CH), 7.24 (1H, d, \(J = 3.4\) Hz, furan-CH), 7.67 (2H, d, \(J = 8.3\) Hz, 2 x CH), 7.84 (2H, d, \(J = 8.3\) Hz, 2 x CH), 7.90 (2H, d, \(J = 8.2\) Hz, 2 x CH), 8.04 (2H, d, \(J = 8.2\) Hz, 2 x CH); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) (assignments made using DEPT-135): 14.2 (CH₃), 109.3 (CH), 109.9 (CH), 120.7 (C), 123.3 (CH), 125.5 (CH), 127.4 (CH), 128.9 (C), 129.0 (C), 131.7 (C), 131.9 (CH), 152.2 (C), 164.5 (C-amidine), (C=O and one quaternary C were not observed in the \(^{13}\)C NMR spectrum); +ESIMS m/z (relative intensity): 341 (85 %, \(^{79}\)Br, [M + H]^+), 343 (100 %, \(^{81}\)Br); found by +ESIMS 341.0287, C\(_{17}\)H\(_{14}\)N\(_2\)OBr [M + H]^+, requires 341.0284, error 0.7 ppm.

2.13.4. 4-(5-(3-Nitrophenyl)furan-2-yl)benzamidine acetate 147

The bright yellow precipitate was filtered under vacuum and rinsed with hexane. Compound 147 was added to boiling ethanol and filtered while it is hot to give 0.33 g (58.0 %): mp 253-255°C; IR (cm\(^{-1}\)): 2978, 2362, 1613, 1518, 1487, 1347, 733; \(^1\)H NMR (400 MHz; DMSO-\(d_6\)): 1.75 (3H, s, CH₃), 7.40 (1H, d, \(J = 3.6\) Hz, furan-CH), 7.48 (1H, d, \(J = 3.6\) Hz, furan-CH), 7.77 (1H, t, \(J = 8.1\) Hz, CH, H-5''), 7.90 (2H, d, \(J = 8.4\) Hz, 2
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The poor solubility of compound 147 in DMSO-d$_6$ meant that a good $^{13}$C NMR spectrum could not be recorded; +ESIMS m/z (relative intensity): 308 (100%, [M + H]$^+$); found by +ESIMS 308.1038, C$_{17}$H$_{14}$N$_3$O$_3$ [M + H]$^+$, requires 308.1030, error 2.5 ppm.

2.13.5. 4-((5-(4-Nitrophenyl)furan-2-yl)benzamidine acetate 148

![Chemical Structure]

The dark yellow-coloured precipitate was filtered under vacuum and rinsed with hexane. Compound 148 was added to boiling ethanol and filtered while hot to give 0.45 g (68.0 %): mp 257-260 °C; IR (cm$^{-1}$): 2933, 1598, 1509, 1408, 1325, 851, 751; $^1$H NMR (300 MHz; DMSO-d$_6$): 1.75 (3H, s, CH$_3$), 7.44 (1H, d, J = 3.3 Hz, furan-CH), 7.52 (1H, d, J = 3.0, furan-CH), 7.91 (2H, d, J = 8.7 Hz, 2 x CH), 8.07 (2H, d, J = 8.1 Hz, 2 x CH), 8.14 (2H, d, J = 8.7 Hz, 2 x CH), 8.32 (2H, d, J = 8.4 Hz, 2 x CH); $^{13}$C NMR (75 MHz; DMSO-d$_6$) (assignments made using DEPT-135): 111.4 (CH), 112.9 (CH), 123.9 (2 x CH), 124.3 (2 x CH), 124.5 (2 x CH), 128.6 (C), 129.9 (2 x CH), 133.5 (C), 135.4 (C), 146.1 (C, C-4”), 151.6 (C), 153.4 (C), 165.8 (C-amidine). The carbons of the acetate groups were not observed in the $^{13}$C NMR spectrum, but the methyl group was observed in the $^1$H NMR spectrum; +ESIMS m/z (relative intensity): 308 (100%, [M + H]$^+$); found by +ESIMS 308.1023, C$_{17}$H$_{14}$N$_3$O$_3$ [M + H]$^+$, requires 308.1030, error 2.3 ppm.

2.13.6. 4-((5-(4-Methoxyphenyl)furan-2-yl)benzamidine acetate 149

![Chemical Structure]

The reaction mixture was diluted with hexane and the yellow precipitate was collected and rinsed with hexane to give 0.014 g (57.0 %) of 149: mp 215-218 °C; IR (cm$^{-1}$): 2928, 1610, 1491, 1409, 1022, 789, 746; the spectroscopic data ($^1$H and $^{13}$C NMR) were identical to the reported in the literature. $^{106a}$ +ESIMS m/z (relative...
intensity): 293 (100%, [M + H]+); found by +ESIMS 293.1284, C_{18}H_{17}N_{2}O _{2} [M + H]^{+}, requires 293.1285, error 0.4 ppm.

2.13.7. 4-(5-(4-Methylphenyl)furan-2-yl)benzamidine acetate 150

![Chemical Structure](image)

The white-coloured precipitate was filtered off under vacuum and rinsed with hexane. Compound 150 was re-crystallized from ethanol to give 0.04 g (51.2 %): mp 233-235 °C; IR (cm⁻¹): 3125, 3023, 2969, 1614, 1495, 1409, 790; ¹H NMR (300 MHz; DMSO-d₆): 1.78 (3H, s, OOCCH₃), 2.35 (3H, s, CH₃), 7.08 (1H, d, J = 3.6 Hz, furan-CH), 7.28-7.30 (3H, m, 3 x CH including furan-CH), 7.76 (2H, d, J = 8.1 Hz, 2 x CH, H-2”), 7.87 (2H, d, J = 8.1 Hz, 2 x CH), 7.96 (2H, d, J = 8.4 Hz, 2 x CH); ¹³C NMR (75 MHz, DMSO-d₆): 20.9 (CH₃), 23.5 (OOCCH₃), 107.8, 111.0, 123.2, 123.7, 127.1, 128.4, 129.5, 137.6, 150.8, 154.1. Four carbon were not observed in the spectrum; +ESIMS m/z (relative intensity): 277 (100%, [M + H]+); found by +ESIMS 277.1348, C_{18}H_{17}N_{2}O [M + H]^{+}, requires 277.1336, error 4.4 ppm.

2.13.8. 4-(5-(4-Ethylphenyl)furan-2-yl)benzamidine acetate 151

![Chemical Structure](image)

The light yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.04 g (73.0 %) of 151: mp 249-252 °C; IR (cm⁻¹): 3169, 2963, 1610 (furan C=C), 1495, 1411, 1200, 790; ¹H NMR (300 MHz; DMSO-d₆): 1.20 (3H, t, J = 7.2 Hz, CH₂CH₃), 1.74 (3H, s, CH₃), 2.64 (2H, q, J = 7.2 Hz, CH₂CH₃), 7.09 (1H, d, J = 3.3 Hz, furan-CH), 7.30-7.32 (3H, m, 3 x CH including furan-CH), 7.78 (2H, d, J = 7.8 Hz, 2 x CH, H-2”), 7.87 (2H, d, J = 8.1 Hz, 2 x CH), 7.98 (2H, d, J = 8.4 Hz, 2 x CH); ¹³C NMR (75 MHz, DMSO-d₆) (assignments made using DEPT-135): 15.9 (CH₃), 22.4 (OOCCH₃), 27.9 (CH₂), 123.3 (CH), 123.7 (CH), 127.3 (CH), 127.9 (CH), 128.3 (CH), 128.6 (C), 134.3 (CH), 143.9 (C), 150.7 (C), 154.2 (C), 165.0 (C-amidine), 173.6 (C, C=O), two carbons (C) were not observed or were overlapping in the ¹³C NMR

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spectrum; +ESIMS m/z (relative intensity): 291.3 (100%, [M + H]+); found by +ESIMS 291.1493, C_{19}H_{19}O_{2}[M + H]^{+}, requires 291.1492, error 0.4 ppm.

2.13.9. 4-(5-(4-Isopropylphenyl)furan-2-yl)benzamidine acetate 152

The white precipitate was filtered off under vacuum and rinsed with hexane to give 0.07 g (95.7%) of 152: mp 235-238 °C (decomposed); IR (cm\(^{-1}\)): 2961, 1610 (furan C=C), 1495, 1488, 840, 788; \(^{1}\)H NMR (300 MHz; DMSO-d\(_{6}\)): 1.25 (6H, d, J = 6.9 Hz, 2 x \(\text{CH}_3\)), 1.83 (3H, s, \(\text{OCCH}_3\)), 2.94 (1H, septet, \(J = 6.9\) Hz, \(\text{CH(CH}_3)_2\)), 7.11 (1H, d, \(J = 3.6\) Hz, furan-CH), 7.34-7.37 (3H, m, 3 x \(\text{CH}\) including furan-CH), 7.80 (2H, d, \(J = 8.1\) Hz, 2 x \(\text{CH}\), H-2\(^\prime\)), 7.91 (2H, d, \(J = 8.7\) Hz, 2 x \(\text{CH}\)), 8.02 (2H, d, \(J = 8.4\) Hz, 2 x \(\text{CH}\)); \(^{13}\)C NMR (75 MHz, DMSO-d\(_{6}\)) (assignments made using DEPT-135): 21.0 (2 x \(\text{CH}_3\)), 23.7 (OCO\(\text{CH}_3\)), 33.2 (CH), 108.0 (CH), 111.5 (CH), 123.3 (CH), 123.9 (CH), 125.8 (C), 126.9 (CH), 127.4 (C), 128.9 (CH), 134.7 (C), 148.6 (C), 150.6 (C), 154.3 (C), 164.8 (C, C-amidine), 172.0 (C, C=O); +ESIMS m/z (relative intensity): 305.6 (100%, [M + H]\(^{+}\)); found by +ESIMS 305.1648, C\(_{20}\)H\(_{21}\)N\(_2\)O [M + H]\(^{+}\), requires 305.1648, error 0.0 ppm.

2.13.10. 4-(5-(4-tert-Butylphenyl)furan-2-yl)benzamidine acetate 153

The white precipitate was filtered off under vacuum and rinsed with hexane. Compound 153 was re-crystallized from ethanol to give 0.04 g (97.4%): mp 272-274 °C; IR (cm\(^{-1}\)): 2969, 1738, 1614, 1487, 1407, 1058, 781; \(^{1}\)H NMR (300 MHz; DMSO-d\(_{6}\)): 1.32 (9H, s, 3 x \(\text{CH}_3\)), 1.74 (3H, s, \(\text{OCCH}_3\)), 7.09 (1H, d, \(J = 3.3\) Hz, furan-CH), 7.31 (1H, d, \(J = 3.6\) Hz, furan-CH), 7.49 (2H, d, \(J = 8.4\) Hz, 2 x \(\text{CH}\), H-3\(^\prime\)), 7.79 (2H, d, \(J = 8.1\) Hz, 2 x \(\text{CH}\), H-2\(^\prime\)), 7.88 (2H, d, \(J = 8.4\) Hz, 2 x \(\text{CH}\)), 7.98 (2H, d, \(J = 8.4\) Hz, 2 x \(\text{CH}\)); +ESIMS m/z (relative intensity): 319.7 (100%, [M + H]\(^{+}\)); found by +ESIMS 319.1805, C\(_{21}\)H\(_{23}\)N\(_2\)O [M + H]\(^{+}\), requires 319.1805, error 0.0 ppm.
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2.13.11. 3-(5-Phenylfuran-2-yl)benzamidine acetate 154

The white precipitate was filtered off under vacuum and rinsed with hexane to give 0.08 g (84.6 %) of 154: mp 190-192 °C; IR (cm$^{-1}$): 3136, 3037, 2838, 1407, 758, 681; $^1$H NMR (300 MHz; DMSO-d$_6$): 1.80 (3H, s, CH$_3$), 7.15 (1H, d, J = 3.3 Hz, furan-CH), 7.25 (1H, d, J = 3.3 Hz, furan-CH), 7.34 (1H, t, J = 7.2 Hz, CH, H-4’), 7.47 (2H, t, J = 7.5 Hz, 2 x CH, H-3”), 7.64-7.73 (2H, m, 2 x CH), 7.88 (2H, d, J = 7.8 Hz, 2 x CH), 8.12 (1H, d, J = 7.2 Hz, CH, H-6), 8.24 (1H, s, CH, H-2); $^{13}$C NMR (100 MHz; DMSO-d$_6$) (assignments made using DEPT-135): 23.1 (CH$_3$), 108.4 (CH), 109.8 (CH), 122.5 (CH), 123.6 (CH), 126.5 (CH), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.6 (CH), 129.7 (C), 129.8 (C), 130.6 (C), 151.1 (C), 153.3 (C), 165.6 (C-amidine), (C, C=O) was not observed in the spectrum; +ESIMS m/z (relative intensity): 263 (100%, [M + H]$^+$); found by +ESIMS 263.1167, C$_{17}$H$_{15}$N$_2$ [M + H]$^+$, requires 263.1179, error 4.5 ppm.

2.13.12. 4-(5-Phenyl-H-pyrrol-2-yl)benzamidine acetate 191

The yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.03 g (67.0 %) of 191: mp > 300 °C (decomposed); IR (cm$^{-1}$): 3444 (NH), 3128, 3043, 1601, 1408, 747; $^1$H NMR (300 MHz; DMSO-d$_6$): 1.83 (3H, s, CH$_3$), 6.68 (1H, d, J = 3.6 Hz, pyrrole-CH), 6.89 (1H, d, J = 3.6 Hz, pyrrole-CH), 7.24 (1H, t, J = 7.5 Hz, CH, H-4”), 7.41 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.80-7.86 (4H, m, 4 x CH), 8.01 (2H, d, J = 8.4 Hz, 2 x CH), 11.50 (1H, s, NH); +ESIMS m/z (relative intensity): 262 (100%, [M + H]$^+$), -ESIMS m/z (relative intensity): 260 (98%, [M - H]); found by 262.1338, C$_{17}$H$_{16}$N$_3$ [M + H]$^+$, requires 262.1339, error 0.3 ppm.
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2.13.13. 4-(1-Methyl-5-phenyl-\textit{H}-pyrrol-2-yl)benzamidine acetate 192

![Molecular structure of 4-(1-Methyl-5-phenyl-\textit{H}-pyrrol-2-yl)benzamidine acetate]

The light yellow precipitate was filtered off under vacuum and rinsed with hexane to give 0.04 g (60.0 %) of 192: mp 226-228 °C; IR (cm\(^{-1}\)): 2842, 1647, 1604, 1460, 1078, 700; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 1.73 (3H, s, CH\(_3\)), 3.64 (3H, s, NCH\(_3\)), 6.34 (1H, d, J = 3.9 Hz, pyrrole-CH), 6.47 (1H, d, J = 3.6 Hz, pyrrole-CH), 7.36 (1H, t, J = 7.2 Hz, CH, H-4\(^{\prime}\)), 7.45-7.54 (4H, m, 4 x CH), 7.71 (2H, d, J = 8.1 Hz, 2 x CH), 7.88 (2H, d, J = 8.4 Hz, 2 x CH); \(^{13}\)C NMR (75 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 24.6 (CH\(_3\)), 34.5 (NCH\(_3\)), 109.0 (CH), 110.3 (CH), 127.1 (CH, C-4\(^{\prime}\)), 127.5 (C), 127.7 (2 x CH, C-3\(^{\prime}\)), 127.8 (2 x CH), 128.3 (2 x CH), 128.6 (2 x CH), 132.6 (C), 134.9 (C), 137.1 (C), 137.8 (C), 165.2 (C, C-amidine), 176.1 (C=O); -ESIMS m/z (relative intensity): 279 (90 %, [M - H]); +ESIMS m/z (relative intensity): 276 (100 %, [M + H]\(^+\)); found by +ESIMS 276.1492, C\(_{18}\)H\(_{18}\)N\(_3\) [M + H]\(^+\), requires 276.1496, error 1.3 ppm.

2.14. 4-(4-Phenyl-1\textit{H}-imidazol-2-yl)benzonitrile 203

Phenylglyoxal monohydrate 202 (2.31 g, 15.2 mmol) was dissolved in methanol (30.0 ml) and was added dropwise to a suspension of 4-cyanobenzaldehyde 122 (2.0 g, 15.2 mmol) and ammonium acetate (14.50 g, 76.0 mmol) in methanol (30.0 ml). The reaction mixture was stirred at room temperature for 3 days and was monitored by TLC (20 % EtOAc/ hexane). The reaction mixture was concentrated, kept in the fridge overnight and the precipitate was filtered off under vacuum and rinsed with hexane. Compound 203 was re-crystallized from ethanol to give 2.25 g (66.8 %) of yellow-orange-coloured precipitate: mp 126-129 °C; IR (cm\(^{-1}\)): 3500 (NH), 3039, 2228 (CN), 1591, 1495, 1182, 851, 759, 697; \(^1\)H NMR (300 MHz; DMSO-d\(_6\) + D\(_2\)O): 7.25 (1H, t, J = 7.2 Hz, CH, H-4\(^{\prime}\)), 7.41 (2H, t, J = 7.8 Hz, 2 x CH, H-3\(^{\prime}\)), 7.84-7.94 (5H, m, 5 x CH)
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including H-5'), 8.17 (2H, d, J = 8.4 Hz, 2 x CH); +ESIMS m/z (relative intensity): 246.5 (100 %, [M + H]⁺); found by +ESIMS 246.1023, C₁₆H₁₂N₃ [M + H]⁺, requires 246.1026, error 1.1 ppm.

2.15. 4-(1-Methyl-4-phenyl-1H-imidazol-2-yl)benzonitrile 205A

Ground potassium hydroxide (1.20 g, 20.5 mmol) was added to a solution of 4-(4-phenyl-1H-imidazol-2-yl)benzonitrile 203 (1.0 g, 4.1 mmol) in acetone (50.0 ml) and stirred at room temperature for a few minutes. Iodomethane (0.64 g, 4.5 mmol) was added and the reaction mixture was stirred vigorously for 30 m and monitored by TLC (20 % EtOAc/ Hex). The reaction was quenched with water and diluted with ethyl acetate, washed with brine, dried over dry MgSO₄ and evaporated to give 0.81 g (80.2 %) of a yellow-orange-coloured solid: mp 142-143 °C; IR (cm⁻¹): 2225 (CN), 1606, 1485, 947, 839, 755, 600; ¹H NMR (300 MHz; DMSO-d₆): 3.86 (3H, s, NCH₃), 7.23 (1H, t, J = 7.2 Hz, CH, H-4”), 7.39 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.81 (2H, d, J = 7.2 Hz, 2 x CH, H-2”), 7.86 (1H, s, CH, H-5”), 7.97 (2H, d, J = 8.7 Hz, 2 x CH), 8.00 (2H, d, J = 8.7 Hz, 2 x CH); +ESIMS m/z (relative intensity): 260.4 (100%, [M + H]⁺); found by +ESIMS 260.1180, C₁₇H₁₄N₃ [M + H]⁺, requires 260.1182, error 0.9 ppm.

2.16. 4-(5-Phenyl oxazol-2-yl)benzonitrile 211

To a solution of 4-cyano-N-(2-oxo-2-phenylethyl)benzamide 209 (1.00 g, 3.8 mmol) in acetic anhydride (25.0 ml) was added concentrated sulfuric acid (8 drops) and the reaction mixture was stirred at room temperature for 10 m. The white precipitate was filtered off and rinsed with hexane to give 0.80 g (85.1 %): mp 174-176 °C (Lit. mp 173-175 °C)⁶⁰; IR (cm⁻¹): 2227 (CN), 1642, 1163, 1017, 842, 770, 689; ¹H NMR (300 MHz; DMSO-d₆): 7.43 (1H, t, J = 7.5 Hz, CH, H-4”), 7.53 (2H, t, J = 7.5 Hz, 2 x CH, H-3”), 7.90 (2H, d, J = 7.2 Hz, 2 x CH, H-2”), 7.97 (1H, s, CH, H-4’), 8.04 (2H, d, J =
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8.7 Hz, 2 x CH), 8.26 (2H, 8.4, J = 8.4 Hz, 2 x CH); +ESIMS m/z (relative intensity): 247 (100 %, [M + H]+).

2.16.2. 4-Cyano-N-(2-oxo-2-phenylethyl)benzamide 209

Saturated sodium bicarbonate solution (25.0 ml) and dichloromethane (10.5 ml) were stirred vigorously and cooled to 0 °C. 4-Cyanobenzoyl chloride 207 (1.0 g, 6.0 mmol) and 2-amino-1-phenylethanone hydrochloride 208 (0.69 g, 4.0 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. The organic layer was extracted, dried over dry MgSO₄ and evaporated. Compound 209 was re-crystallized from DMF/H₂O to give white solid 1.40 g (88.0 %): mp 169-173 °C; IR (cm⁻¹): 3361 (NH), 2227 (CN), 1680 (C=O), 1643 (NHC=O), 1524, 1487, 1360, 1222, 859, 680, 597; ¹H NMR (300 MHz; DMSO-d₆): 4.83 (2H, d, J = 5.7 Hz, CH₂, H-1'), 7.58 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.70 (1H, t, J = 7.2 Hz, CH, H-4”), 7.99-8.08 (6H, m, 6 x CH), 9.16 (1H, t, J = 5.7 Hz, NH); +ESIMS m/z (relative intensity): 288 (100 %, [M + Na]+).

2.17. 4-(Methyl-5-phenylfuran-2-yl)benzonitrile 224

Diethylamine (0.52 g, 7.1 mmol) in dry THF (5 ml) was added dropwise to a solution of ethyl magnesium bromide (1.0 M in THF, 7.1 mmol) at 0 °C. The reaction was allowed to warm to 35 °C and stirred at that temperature for 1 h. The reaction mixture was cooled to 0 °C and a solution of 4-acetylbenzonitrile 120 (1.0 g, 7.1 mmol) and 2-bromopropiophenone 221 (0.7 ml, 4.7 mmol) in THF (5 ml) was added. The reaction was kept at 0 °C for 1 h then stirred at room temperature for 24 h and monitored by TLC (20 % EtOAc/ hexane). The reaction mixture was quenched with cold 5% v/v aqueous sulfuric acid solution, extracted with ethyl acetate, dried over dry MgSO₄ and evaporated. Compound 224 was purified from the crude by column chromatography using ethyl acetate-hexane (2:8) as a mobile phase. Compound 224 was re-crystallized from methanol to give 0.41 g (33.6 %) of a yellow solid: mp 149-
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153 °C; IR (cm\(^{-1}\)): 2224 (CN), 1607, 1491, 840, 760, 702; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 2.54 (3H, s, CH\(_3\)), 7.33 (1H, t, J = 7.3 Hz, CH, H-4\(^{\prime}\)), 7.46 (2H, t, J = 7.8 Hz, 2 x CH, H-3\(^{\prime\prime}\)), 7.52 (3H, d, 3 x CH, H-3, H-2\(^{\prime\prime}\)), 7.88 (4H, s, 4 x CH, H-2, H-3); \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): 13.3 (CH\(_3\)), 109.7 (CH, H-3\(^{\prime}\)), 109.8 (CH, C-4\(^{\prime}\)), 119.1 (C, CN), 123.5 (CH), 123.9 (C), 126.9 (CH), 127.5 (CH), 128.8 (CH), 132.6 (CH), 133.3 (C), 134.6 (C), 149.6 (C), 149.7 (C).

2.18. 4-(5-Phenylthiophen-2-yl)benzonitrile 228

Lawesson’s reagent (0.77 g, 1.9 mmol) was added portionwise to a solution of 1-(4-cyanophenyl)-4-phenyl-1,4-butadione 126 (0.25 g, 1.0 mmol) in tetrahydrofuran (10.0 ml). The reaction mixture was heated at 50 °C for 2 days and was monitored by TLC (20 % EtOAc/Hex). The crude product was purified by column chromatography using EtOAc-Hex (1:9) as a mobile phase to give 0.14 g (56.0 %) as a yellow solid: mp 165-166 °C; IR (cm\(^{-1}\)): 2225 (CN), 1600, 1453, 839, 803, 755, 688, 576; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 7.37 (1H, t, J = 7.5 Hz, CH, H-4\(^{\prime}\)), 7.47 (2H, t, J = 8.1 Hz, 2 x CH, H-3\(^{\prime\prime}\)), 7.64 (1H, d, J = 3.9 Hz, thiophene-CH), 7.73 (2H, d, J = 7.5 Hz, 2 x CH, H-2\(^{\prime\prime}\)), 7.79 (1H, d, J = 3.9 Hz, thiophene-CH), 7.90 (4H, s, 4 x CH, H-2, H-3); \(^{13}\)C NMR (100 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 109.6 (C, C-4), 118.8 (C, CN), 125.38 (CH), 125.4 (CH), 125.6 (CH), 127.6 (CH), 128.3 (CH), 129.2 (CH), 133.0 (C), 133.1 (CH), 137.8 (C), 140.2 (C), 144.9 (C); +APCIMS m/z (relative intensity): 262 (100 %, [M + H]+).

2.19. General procedure for the synthesis of N-hydroxy amidines (amidoximes) 204, 206, 212, 225, 233 and 240

Hydroxylamine hydrochloride (10.0 mmol) was suspended in dry DMSO and the mixture was cooled to 0 °C. Potassium tert-butoxide (10.0 mmol) was added portionwise to the reaction mixture under an argon atmosphere and the reaction mixture was stirred at room temperature for 1 h. Diarylimidazole- 203, diaryl N-methylimidazole- 205A, diaryloxazole- 211, diarylthiophene- 228 or diarylfuran-nitrile-
127 (1.0 mmol) was added and the reaction mixture was stirred at room temperature for 1-3 days and monitored by TLC (10.0 % MeOH/ CHCl₃).

2.19.1. N-Hydroxy-4-(4-phenyl-1H-imidazol-2-yl)benzamidine 204

The reaction mixture was poured into iced water and the yellow precipitate was filtered off under vacuum, rinsed with hexane and re-crystallized from ethanol to give 0.37 g (66.1 %) of yellow-coloured solid: mp 150-151 °C (decomposed); IR (cm⁻¹): 3179, 3114, 2798, 1637, 1386, 1022, 946, 846, 804; ¹H NMR (300 MHz; DMSO-d₆): 5.87 (2H, s, NH₂), 7.22 (1H, t, J = 7.2 Hz, CH, H-4”), 7.38 (2H, t, J = 7.2 Hz, 2 x CH, H-3”), 7.77-7.80 (3H, m, 3 x CH, H-5’, H-2”), 7.88 (2H, d, J = 7.8 Hz, 2 x CH), 8.00 (2H, d, J = 7.8 Hz, 2 x CH), 9.72 (1H, s, NOH); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 114.4 (CH), 124.4 (CH), 124.5 (CH), 125.7 (CH), 126.3 (CH), 130.7 (C), 132.6 (C), 134.4 (C), 141.2 (C), 145.3 (C), 150.3 (C, C-amidoxime); +ESIMS m/z (relative intensity): 279.6 (100 %, [M + H]⁺); found by +ESIMS 279.0952, C₁₇H₁₅N₂S [M + H]⁺, requires 279.0950, error 0.6 ppm.

2.19.2. N-Hydroxy-4-(1-methyl-4-phenyl-1H-imidazol-2-yl)benzamidine 206

The reaction mixture was poured into iced water and the white precipitate was filtered off under vacuum, rinsed with hexane and re-crystallized from ethanol to give 0.40 g (71.4 %) of 206: mp 186-189 °C; IR (cm⁻¹): 3502 (N-H), 3384 (N-H), 3060, 2928, 1664, 1456, 1391, 940, 852, 754, 691; ¹H NMR (300 MHz; DMSO-d₆): 3.82 (3H, s, NC₃H₃), 5.91 (2H, s, N-H₂), 7.21 (1H, t, J = 7.2 Hz, CH, H-4”), 7.38 (2H, t, J = 7.5 Hz, 2 x CH, H-3”), 7.77-7.83 (7H, m, 7 x CH, H-2, H-3, H-5’, H-2”), 9.76 (1H, s, NOH); ¹³C NMR (75 MHz; DMSO-d₆): 33.7 (CH₃), 124.8, 126.0, 127.3, 127.7, 128.5,
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129.4, 130.7, 132.9, 134.1, 139.5, 146.4, 150.3 (C, C-amidoxime); +ESIMS m/z (relative intensity): 293.4 (100%, [M + H]+); found by +ESIMS 293.1405, C₁₇H₁₇N₄O [M + H]+, requires 293.1397, error 2.8 ppm.

2.19.3. \textit{N}-Hydroxy-4-(4-phenyloxazol-2-yl)benzamidine 212

The reaction mixture was poured into iced water and the precipitate was filtered off under vacuum, rinsed with hexane, added to boiling ethanol and filtered while it was hot to give 0.45 g (53.6%) of 212: mp 196-198 °C; IR (cm⁻¹): 3368 (N-H and OH), 3165, 1621, 1400, 1139, 790, 753, 676, 601; \(^1\)H NMR (300 MHz; DMSO-d₆): 5.95 (2H, s, N-H), 7.40 (1H, t, \(J = 7.2 \text{ Hz, CH}_2\), H-4”), 7.51 (2H, t, \(J = 7.8 \text{ Hz, 2 x CH, H-3”}\), 7.85-7.89 (5H, m, 5 x CH including H-5’), 8.09 (2H, d, \(J = 8.4 \text{ Hz, 2 x CH}\), 9.87 (1H, s, N-OH).

2.19.4. \textit{N}-Hydroxy-4-(4-methyl-5-furan-2-yl)benzamidine 225

The reaction mixture was poured into iced water and the precipitate was filtered off under vacuum, rinsed with hexane and re-crystallized from ethanol to give 0.16 g (42.1%) of 225: mp 163-166 °C; IR (cm⁻¹): 3448 (N-H), 3357 (N-H), 3326, 1644, 1388, 1117, 1062, 926, 761, 670; \(^1\)H NMR (300 MHz; DMSO-d₆): 2.52 (3H, s, CH₃), 5.89 (2H, s, NH₂), 7.28 (1H, s, CH, H-3’), 7.31 (1H, t, \(J = 7.2 \text{ Hz, CH, H-4”}\), 7.45 (2H, t, \(J = 8.1 \text{ Hz, 2 x CH, H-3”}\), 7.52 (2H, d, \(J = 6.9 \text{ Hz, 2 x CH, H-2”}\), 7.70 (2H, d, \(J = 9.0 \text{ Hz, 2 x CH}\), 7.74 (2H, d, \(J = 9.0 \text{ Hz, 2 x CH}\), 9.71 (1H, s, N-OH); \(^1\)C NMR (75 MHz; DMSO-d₆): 13.2 (CH₃), 108.0 (CH), 122.6 (CH), 125.2 (C), 125.8 (CH), 126.4 (CH), 127.0 (CH), 128.7 (CH), 130.4 (C), 131.8 (C), 133.1 (C), 147.6(C), 150.4 (C), one quaternary carbon has not been observed; +ESIMS m/z (relative intensity): 293.2 (100%, [M + H]+); found by +ESIMS 293.1277, C₁₈H₁₇N₂O₂ [M + H]+, requires 293.1290, error 4.4 ppm.
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2.19.5. *N*-Hydroxy-4-(5-phenylthiophen-2-yl)benzamidine 233

The reaction mixture was poured into iced water and the precipitate was filtered off under vacuum, rinsed with hexane, added to boiling ethanol and filtered while it was hot to give 0.37 g (78.7 %) of white precipitate of 233: mp 217-219 °C (decomposed); IR (cm⁻¹): 3438 (N-H), 3348 (N-H), 3060, 1655, 1592, 1393, 938, 837, 735; ¹H NMR (300 MHz; DMSO-d₆): 5.87 (2H, s, N-H₂), 7.33 (1H, t, J = 7.2 Hz, CH, H-4"), 7.45 (2H, t, J = 7.5 Hz, 2 x CH, H-3"), 7.57 (1H, d, J = 3.6 Hz, thiophene-CH), 7.61 (1H, d, J = 3.3 Hz, thiophene-CH); 7.70-7.76 (6H, m, 6 x CH, H-2, H-3, H-2"), 9.72 (1H, s, NOH), ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 124.8 (CH), 125.1 (CH), 125.2 (CH), 125.3 (CH), 126.0 (CH), 127.8 (CH), 129.2 (CH), 132.4 (C), 133.4 (C), 133.8 (C), 142.0 (C), 142.8 (C), 150.3 (C); +ESIMS m/z (relative intensity): 279 (100 %, [M + H]⁺); found by +ESIMS 295.0900, C₁₇H₁₅N₂O₂S [M + H]⁺, requires 295.0900, error 0.1 ppm.

2.19.6. *N*-Hydroxy-4-(5-phenylfuran-2-yl)benzamidine 240

The reaction mixture was poured into iced water and compound 240 was extracted with dichloromethane, washed with water, dried over MgSO₄ and evaporated to give 0.02 g (87.0 %) of 240 as a yellow solid: mp 179-181 °C (decomposed); IR (cm⁻¹): 3492(N-H), 33548 (N-H), 2362, 1664, 1388, 1022, 927, 791, 754, 680, 670; ¹H NMR (300 MHz; DMSO-d₆): 5.85 (2H, s, N-H₂), 7.11 (2H, d, J = 6.6 Hz, 2 x CH, H-3", H-4"), 7.31 (1H, t, J = 6.9 Hz, CH, H-4"), 7.45 (2H, t, J = 7.2 Hz, 2 x CH, H-3"), 7.74-7.82 (6H, m, 6 x CH, H-2, H-3, H-2"), 9.69 (1H, s, NOH), ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 108.3 (CH), 108.8 (CH), 123.1 (CH), 123.5 (CH), 125.8 (CH), 127.7 (CH), 129.0 (CH), 129.9 (C), 130.3 (C), 132.0 (C), 150.3 (C), 152.2 (C), 152.8 (C); -ESIMS m/z (relative intensity): 277 (100 %, [M - H]⁻); +ESIMS m/z (relative intensity): 279 (100 %, [M + H]⁺); found by +ESIMS 279.1129, C₁₇H₁₅O₂N₂ [M + H]⁺, requires 279.1128, error 0.3 ppm.
2.20. General procedure for the synthesis of amidines 193-195 and 223

Ammonium formate (5.0 mmol) and Pd/C (10.0 % g/ml) were added slowly to a solution of the amidoxime (1.0 mmol) in glacial acetic acid. The reaction mixture was heated at reflux and monitored with TLC (1.0 % MeOH/ CHCl₃). Upon the consumption of the starting amidoxime, the reaction mixture was cooled to room temperature, filtered through Celite 521 and rinsed with glacial acetic acid. The reaction mixture was quenched with 1.0 M NaOH until pH became around 7 and stirred at room temperature for 1 h.

2.20.1. 4-(Phenyl-1H-imidazol-2-yl)benzamidine acetate 193

The precipitate was filtered off under vacuum, rinsed with H₂O and re-crystallized from ethanol to give 0.18 g (27.7 %) of 193 as an orange solid: mp 228-231 °C; IR (cm⁻¹): 2920, 1557, 1409, 1157, 823, 740; ¹H NMR (300 MHz; DMSO-d₆): 1.72 (3H, s, CH₃), 7.24 (1H, t, J = 7.5 Hz, CH, H-4’), 7.40 (2H, t, J = 7.5 Hz, 2 x CH, H-3’), 7.81 (1H, s, CH, H-5’), 7.87-7.92 (4H, m, 4 x CH), 8.20 (2H, d, J = 8.4 Hz, 2 x CH), 8.32 (1H, s, N-H); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 24.7 (CH₃), 124.5 (CH), 124.8 (CH), 126.5 (CH), 128.1 (CH), 128.46 (C), 128.5 (CH), 134.6 (C), 144.8 (C), 165.4 (C, C-amidine), 176 (C=O), two quaternary carbons have not been observed and one CH carbon is overlapping; +ESIMS m/z (relative intensity): 263.4 (100 %, [M + H]⁺); found by +ESIMS 263.1293, C₁₆H₁₅N₄ [M + H]⁺, requires 263.1291, error 0.7 ppm.
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2.20.2. 4-(1-Methyl-4-phenyl-1H-imidazol-2-yl)benzamidine acetate 194

The precipitate was filtered off under vacuum, rinsed with H\textsubscript{2}O and re-crystallized from ethanol to give 0.21 g (21.0 %) of creamy solid; IR (cm\textsuperscript{-1}): 3179, 2965, 1606, 1556, 1511, 1483, 1108, 753, 640; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.70 (3H, s, OCOCH\textsubscript{3}), 3.85 (3H, s, NCH\textsubscript{3}), 7.23 (1H, t, J = 7.5 Hz, CH, H-4"), 7.38 (2H, t, J = 7.8 Hz, 2 x CH, H-3"), 7.81 (2H, d, J = 7.2 Hz, 2 x CH, H-2"), 7.84 (1H, s, CH, H-5"), 7.92 (2H, d, J = 8.4 Hz, 2 x CH), 7.97 (2H, d, J = 9.3 Hz, 2 x CH); \textsuperscript{13}C NMR (75 MHz; DMSO-d\textsubscript{6}) (assignments made using DEPT-135): 24.7 (OCOCH\textsubscript{3}), 35.3 (NCH\textsubscript{3}), 120.8 (CH), 124.2 (CH), 126.5 (CH), 127.8 (CH), 128.2 (CH), 128.6 (CH), 134.1 (C), 134.3 (C), 139.8 (C), 145.5 (C), 165.2 (C, C-amidine), 175.7 (C, C=O); +ESIMS m/z (relative intensity): 277.4 (100 %, [M + H]\textsuperscript{+}); found by +ESIMS 277.1449, C\textsubscript{17}H\textsubscript{17}N\textsubscript{4}[M + H]\textsuperscript{+}, requires 277.1453, error 1.5 ppm.

2.20.3. 4-(4-Phenylloxazol-2-yl)benzamidine acetate 195

The grey-coloured precipitate was filtered off under vacuum, rinsed with H\textsubscript{2}O and re-crystallized from ethanol to give 0.06 g (23.6 %) of 195: mp 240-243 °C; IR (cm\textsuperscript{-1}): 2921, 1556, 1486, 1409, 848, 766, 707, 688; \textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}): 1.76 (3H, s, CH\textsubscript{3}), 7.42 (1H, t, J = 7.2 Hz, CH, H-4"), 7.53 (2H, t, J = 7.8 Hz, 2 x CH, H-3"), 7.90 (2H, d, J = 7.5 Hz, 2 x CH, H-2"), 7.93 (1H, s, CH, H-4"), 7.97 (2H, d, J = 8.4 Hz, 2 x CH), 8.22 (2H, d, J = 8.4 Hz, 2 x CH); \textsuperscript{13}C NMR (75 MHz; DMSO-d\textsubscript{6}) (assignments made using DEPT-135): 23.7 (CH\textsubscript{3}), 123.8 (C), 124.2 (CH), 124.7 (CH), 125.1 (C), 126.1 (CH), 127.1 (C), 128.0 (C), 128.4 (CH), 128.8 (CH), 130.0 (CH), 159.2 (C), 164.7 (C, C-amidine), C=O was not observed; +ESIMS m/z (relative intensity): 264.5
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(100 %, [M + H]⁺); found by +ESIMS 264.1147, C₁₆H₁₄ON₃ [M + H]⁺, requires 264.1137, error 3.8 ppm.

2.20.4 4-(4-Methyl-5-phenylfuran-2-yl)benzamidine acetate 223

The precipitate was filtered off under vacuum, rinsed with H₂O and recrystallized from EtOH to give 0.1 g (21%): mp 224 °C (decomposed); IR (cm⁻¹): 3354 (N-H), 3205, 1571, 1415, 932, 761, 697; NMR (300 MHz; DMSO-d₆): 1.68 (3H, s, CH₃ of acetate), 2.54 (3H, s, CH₃), 7.32 (1H, t, J = 7.2 Hz, CH, H-4’’), 7.43-7.54 (5H, m, 5 x CH, H-3’, H-2”, H-3”), 7.86 (4H, s, 4 x CH, H-2, H-3); +ESIMS m/z (relative intensity): 277.0 (100 %, [M + H]⁺).

2.21. 4-(5-Phenylthiophen-2-yl)benzamidine acetate 226

N-Hydroxy-4-(5-phenylthiophen-2-yl)benzamidine 233 (0.11 g, 0.4 mmol) was dissolved in glacial acetic acid (2.0 ml) and acetic anhydride (0.04 g, 0.4 mmol) and the reaction mixture was stirred at room temperature for 30 m. Triethylsilane (0.06 g, 0.5 mmol) and PdCl₂ (10.0 %) were added and the reaction mixture was refluxed for 12 h and monitored by TLC (1.0 MeOH/CHCl₃). Upon the consumption of the starting amidoxime, the reaction was cooled to room temperature, filtered though Celite 521 and neutralized with saturated sodium bicarbonate solution. The precipitate was filtered off under vacuum, rinsed with acetone, added to boiling ethanol and filtered while it was hot to give 0.06 g (42.6 %): mp > 300 °C; IR (cm⁻¹): 2973, 1487, 1409, 1077, 1012, 808, 750, 691, 670; ¹H NMR (300 MHz; DMSO-d₆): 1.60 (3H, s, CH₃), 7.35 (1H, t, J = 7.2 Hz, CH, H-4’’), 7.46 (2H, t, J = 7.5 Hz, 2 x CH, H-3’’), 7.60 (1H, d, J = 3.9 Hz, thiophene-CH), 7.68 (1H, d, J = 3.9 Hz, thiophene-CH), 7.72 (2H, d, J = 7.2 Hz, 2 x CH, H-2”), 7.85 (2H, d, J = 7.2 Hz, 2 x CH); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 25.9 (CH₃), 125.3
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(CH), 125.7 (CH), 125.8 (CH), 126.7 (CH), 128.3 (CH), 128.5 (CH), 129.7 (CH), 133.8 (C), 136.5 (C), 141.8 (C), 144.1 (C), 175.3 (C), C-amidine and another quaternary carbon have not been observed; +ESIMS m/z (relative intensity): 279.3 (100 %, [M + H]⁺); found by +ESIMS 279.0952, C₁₇H₁₅N₂S [M + H]⁺, requires 279.0950, error 0.6 ppm.

2.22. Methyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 234

\[
\text{H}_3\text{C}^-\text{O} \quad \text{Cl}^-\text{H}_2\text{N}^+ \quad \text{Cl}
\]

Ammonium chloride (0.04 g, 0.8 mmol) was added to a solution of ethyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride 143 (0.20 g, 0.7 mmol) in methanol (15.0 ml) and water (2.0 ml). The reaction mixture was heated at reflux for 3 h. The yellow precipitate was filtered off and rinsed with hexane: ¹H NMR (DMSO-δ₆): 3.90 (3H, s, CH₃), 7.16 (1H, d, J = 3.6 Hz, furan-CH), 7.31 (1H, d, J = 3.6 Hz, furan-CH), 7.36 (1H, t, J = 7.2 Hz, CH, H-4”), 7.48 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.86 (2H, d, J = 7.5 Hz, 2 x CH, H-2”), 7.96 (2H, d, J = 8.1 Hz, 2 x CH), 8.03 (2H, d, J = 8.4 Hz, 2 x CH); ¹³C NMR (75 MHz; DMSO-δ₆) (assignments made using DEPT-135): 52.1 (CH₃), 107.5 (CH), 109.6 (CH), 123.3 (CH), 124.0 (CH), 127.8 (CH), 128.4 (C), 128.8 (CH), 130.2 (CH), 130.4 (C), 134.7 (C), 152.2 (C), 154.5 (C), 166.8 (C, C-imidate).

2.23. General procedure for the synthesis of the aryl 1,4-diketones 241 and 242

Triethylamine (1.5 mmol) was added to a suspension of zinc chloride (2.0 mmol) in dry toluene and absolute ethanol (1.5 mmol). The reaction mixture was stirred at room temperature for 1-2 h. Methyl aryl ketone (1.5 mmol) and α-bromomethyl aryl ketone (1.0 mmol) were added and the mixture was stirred at room temperature for 3-7 days and monitored by TLC (20.0 % EtOAc/ hexane). The workup was as described for each 1,4-diketone.
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2.23.1. 1-(3-Nitrophenyl)-4-phenyl-1,4-butanedione 241

![Chemical structure of 1-(3-Nitrophenyl)-4-phenyl-1,4-butanedione 241]

The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution. The organic layer was washed with brine, dried over MgSO₄ and concentrated. Compound 241 was crystallized from methanol to give 0.87 g (61.3 %) of colourless crystals: mp 93-94 °C (Lit mp 92 °C)¹¹²; IR (cm⁻¹): 1676 (C=O), 1527 (NO₂), 1310 (NO₂), 1191, 756, 730, 692; ¹H NMR (300 MHz; CDCl₃): 3.49-3.55 (4H, m, 2 × CH₂, H-2, H-3), 7.51 (2H, t, J = 7.5 Hz, 2 × CH, H-3’’), 7.62 (2H, t, J = 7.5 Hz, CH, H-4’’), 7.72 (1H, t, J = 8.1 Hz, CH, H-5’’), 8.06 (2H, d, J = 7.5 Hz, 2 × CH, H-2’’), 8.39 (1H, d, J = 7.8 Hz, CH, H-6’’), 8.47 (1H, d, J = 8.1 Hz, CH, H-4’), 8.90 (1H, s, CH, H-2’); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 32.6 (CH₂, C-3), 32.7 (CH₂, C-2), 123.1 (CH), 127.5 (CH), 128.2 (CH), 128.7 (CH), 129.9 (CH), 133.4 (CH), 133.7 (CH), 136.5 (C, C-1’’), 138.1 (C, C-1’), 196.7 (C=O), 198.2 (C=O), C-3’ (C) was not observed in the ¹³C NMR spectrum; +ESIMS m/z 306 (100%, [M + Na]+).

2.23.2. 1-(4-Nitrophenyl)-4-phenyl-1,4-butanedione 242

![Chemical structure of 1-(4-Nitrophenyl)-4-phenyl-1,4-butanedione 242]

The reaction was quenched by the addition of 10.0 % (v/v) aqueous sulfuric acid solution. The organic layer was washed with brine, dried over MgSO₄ and concentrated. Compound 242 was crystallized from methanol to give 0.44 g (31.0 %) of light orange crystals: mp 145-146 °C (Lit mp 140-142 °C)¹¹²; IR (cm⁻¹): 1670 (C=O), 1515 (NO₂), 1318 (NO₂), 701; ¹H NMR (300 MHz; CDCl₃): 3.48-3.54 (4H, m, 2 × CH₂, H-2, H-3), 7.51 (2H, t, J = 7.5 Hz, 2 × CH, H-3’’), 7.62 (1H, t, J = 7.5 Hz, CH, H-4’’), 8.05 (2H, d, J = 7.5 Hz, 2 × CH, H-2’’), 8.21 (2H, d, J = 8.7 Hz, 2 × CH, H-2’), 8.36 (2H, d, J = 8.7 Hz, 2 × CH, H-3’’); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 32.6 (CH₂, C-3), 33.0 (CH₂, C-2), 123.9 (2 × CH, C-3’’), 128.1 (CH, C-4’’), 128.7 (2 × CH, C-2’’), 129.2 (2 × CH, C-2’), 133.4 (2 × CH, C-3’’), 136.4 (C, C-1’’), 141.3 (C, C-1’), 150.4 (C, C-4’’), 197.4 (C=O), 198.2 (C=O); +ESIMS m/z 306 (100%, [M + Na]+).
284 (70%, [M + H]⁺); found by +ESIMS 284.0917, C₁₆H₁₄O₄N [M + H]⁺, requires 284.0917, error 0.1 ppm.

2.24. General procedure for the synthesis of 2,5-diarylfurans 243 and 244

Acetyl chloride (8.0 mmol) was added dropwise to a solution of 1,4-diarylketones 241 or 242 (1.0 mmol) in chloroform and ethanol (12.0 mmol) at 0 °C. The reaction was stirred at room temperature for 1 day and monitored by TLC (20 % EtOAc/ hexane).

2.24.1. 2-(3-Nitrophenyl)-5-phenylfuran 243

The reaction mixture was cooled to 0 °C and saturated sodium bicarbonate solution was added dropwise until the gas evolution ceased. The organic layer was extracted, washed with water, dried over MgSO₄ and evaporated to give 0.79 g (87.8 %) of an orange solid: mp 111-113 °C; IR (cm⁻¹): 1590, 1501 (NO₂), 1327 (NO₂), 844, 794, 688; ¹H NMR (300 MHz; CDCl₃): 6.81 (1H, d, J = 3.3 Hz, CH, H-4), 6.92 (1H, d, J = 3.3 Hz, CH, H-3), 7.34 (1H, t, J = 7.5 Hz, CH, H-4”), 7.46 (2H, t, J = 7.5 Hz, 2 x CH, H-3”), 7.59 (1H, t, J = 8.1 Hz, CH, H-5”), 7.79 (2H, d, J = 7.8 Hz, 2 x CH, H-2”), 8.05 (1H, d, J = 7.8 Hz, CH, H-6”), 8.12 (1H, d, J = 8.4 Hz, CH, H-4”), 8.57 (1H, s, CH, H-2’); ¹³C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 107.5 (CH, C-4’), 109.6 (CH, C-3’), 118.3 (CH), 121.6 (CH), 124.0 (CH), 128.0 (CH), 128.8 (CH), 129.1 (CH), 129.8 (CH), 130.1 (C), 132.3 (C), 148.8 (C), 150.8 (C), 154.7 (C); +ESIMS m/z 288 (100%, [M + Na]⁺).

2.24.2. 2-(4-Nitrophenyl)-5-phenylfuran 244

The reaction mixture was cooled to 0 °C and saturated sodium bicarbonate solution was added dropwise until the gas evolution ceased. The organic layer was extracted, washed with water, dried over MgSO₄ and evaporated to give 0.70 g (88.6 %)
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of an orange solid: mp 136-138 °C (Lit mp 132 °C)\textsuperscript{161}; IR (cm\textsuperscript{-1}): 1590, 1501 (NO\textsubscript{2}), 1327 (NO\textsubscript{2}), 844, 794, 688; \textsuperscript{1}H NMR (300 MHz; CDCl\textsubscript{3}): 6.84 (1H, d, J = 3.6 Hz, CH, H-4), 7.00 (1H, d, J = 3.6 Hz, CH, H-3), 7.36 (1H, t, J = 7.5 Hz, CH, H-4’), 7.47 (2H, t, J = 7.8 Hz, 2 x CH, H-3’), 7.79 (2H, d, J = 7.5 Hz, 2 x CH, H-2’), 7.87 (2H, d, J = 9.0 Hz, 2 x CH, H-2’); \textsuperscript{13}C NMR (100 MHz; CDCl\textsubscript{3}) (assignments made using DEPT-135): 107.9 (C\textsubscript{H}, C\textsubscript{H}-4), 111.4 (C\textsubscript{H}, C\textsubscript{H}-3), 123.7 (CH), 124.2 (CH), 124.4 (CH), 128.3 (CH), 128.9 (CH), 130.0 (C), 136.3 (C), 146.3 (C), 151.0 (C), 155.6 (C). The spectral data (\textsuperscript{1}H, \textsuperscript{13}C NMR and IR) were identical to those reported in the literature.\textsuperscript{161}

2.25. General procedure for the synthesis of 2,5-diarylthiophenes 245 and 246

Lawesson’s reagent (1.1 mmol) was added portionwise to a solution of aryl 1,4-diketones 241 or 242 (1.0 mmol) in tetrahydrofuran. The reaction mixture was heated at 55 °C for 1-2 days and was monitored by TLC (20% EtOAc/ hexane).

2.25.1. 2-(3-Nitrophenyl)-5-phenylthiophene 245

![Structure of 2-(3-Nitrophenyl)-5-phenylthiophene 245](image)

The reaction mixture was concentrated, diluted with hexane and diethyl ether and the yellow precipitate was filtered off under vacuum to give 1.13 g (36.5 %) of 245: mp °C; IR (cm\textsuperscript{-1}): 1516 (NO\textsubscript{2}), 1344 (NO\textsubscript{2}), 798, 757, 734, 687; \textsuperscript{1}H NMR (300 MHz; CDCl\textsubscript{3}): 7.32-7.46 (5H, m, 5 x CH, H-3, H-4, H-3’, H-4’), 7.58 (1H, t, J = 7.8 Hz, CH, H-5’), 7.66 (2H, d, J = 7.5 Hz, 2 x CH, H-2’), 7.94 (1H, d, J = 7.8 Hz, CH, H-6’), 8.14 (1H, d, J = 8.1 Hz, CH, H-4’), 8.49 (1H, s, CH, H-2’); \textsuperscript{13}C NMR (100 MHz; DMSO-d\textsubscript{6}) (assignments made using DEPT-135): 119.2 (CH), 122.1 (CH), 125.3 (CH), 125.4 (CH), 127.1 (CH), 128.2 (CH), 129.2 (CH), 130.8 (CH), 131.4 (CH), 133.1 (C), 135.1 (C), 139.6 (C), 144.3 (C), 148.5 (C).
2.25.2. 2-(4-Nitrophenyl)-5-phenylthiophene 246

Compound 246 was purified by column chromatography using ethyl acetate/hexane (1:9) as the mobile phase to give 0.14 g (56.0%) of 246 as a yellow solid: mp 176-178 °C; IR (cm⁻¹): 1592, 1505 (NO₂), 1335 (NO₂), 844, 804, 790, 740, 691, 564; ¹H NMR (300 MHz; DMSO-d₆): 7.37 (1H, t, J = 7.2 Hz, CH, H-4”), 7.47 (2H, t, J = 7.2 Hz, 2 x CH, H-3”), 7.66 (1H, d, J = 3.9 Hz, CH, H-4), 7.75 (2H, d, J = 7.5 Hz, 2 x CH, H-2”), 7.84 (1H, d, J = 3.6 Hz, CH, H-3), 7.97 (2H, d, J = 8.4 Hz, 2 x CH, H-2’), 8.27 (2H, d, J = 8.4 Hz, 2 x CH, H-3’); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 124.6 (CH), 125.4 (CH), 125.6 (CH), 125.8 (CH), 128.3 (CH), 128.4 (CH), 129.3 (CH), 132.9 (C), 139.7 (C), 145.7 (C), 146.0 (C), one quaternary carbon was not observed in the spectrum; +ESIMS m/z (relative intensity): 304 (100 %, [M + Na]+).

2.26. General procedure for the synthesis of amines 247-250

Copper (II) sulfate (2 M solution in H₂O, 0.1 mmol) was added to a solution of a nitro-compound 243-246 (1.0 mmol) in ethanol. The reaction mixture was cooled to 0 °C and sodium borohydride (5.0 mmol) was added portionwise. The reaction mixture was stirred at room temperature for 1 h and monitored with TLC (20 % EtOAc/hexane). Upon consumption of the starting material, the reaction was diluted with ethyl acetate, washed with water, dried over MgSO₄ and evaporated.

2.26.1. 3-(5-Phenylfuran-2-yl)aniline 247

White solid 0.50 g (66.7 %): mp 139-141 °C; IR (cm⁻¹): 3455 (N-H), 3376 (N-H), 3339 (N-H), 1608, 1487, 1022, 778, 759, 690; ¹H NMR (300 MHz; DMSO-d₆): 5.19 (2H, s, NH₂), 6.52 (1H, d, J = 7.8 Hz, CH, H-6), 6.87 (1H, s, CH, H-2), 6.94 (1H, d, J = 7.5 Hz, CH, H-4), 7.02 (2H, s, 2 x CH, H-3”, H-4’), 7.08 (1H, t, J = 7.8 Hz, CH, H-4”), 7.29 (1H, t, J = 7.5 Hz, CH, H-5), 7.44 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.77 (2H, d, J = 7.8 Hz, 2 x CH, H-2”); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using
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DEPT-135): 107.3 (CH), 108.0 (CH), 108.5 (CH), 111.5 (CH), 113.5 (CH), 123.2 (CH), 127.4 (CH), 128.9 (CH), 129.4 (CH), 130.2 (C), 130.5 (C), 149.0 (C), 151.9 (C), 153.4 (C); +ESIMS m/z (relative intensity): 236 (100 %, [M + H]+); found by +ESIMS 236.1073, C_{16}H_{14}ON [M + H]^+, requires 236.1070, error 1.1 ppm.

2.26.2. 4-(5-Phenylfuran-2-yl)aniline 248

White solid 0.19 g (35.0 %): mp 129-133 °C (decomposed); IR (cm⁻¹): 3463 (N-H), 3372 (N-H), 1620, 1498, 1295, 1020, 823, 795, 694; ¹H NMR (300 MHz; DMSO-d₆): 5.36 (2H, s, N-H₂), 6.62 (2H, d, J = 8.1 Hz, 2 x CH, H-2), 6.67 (1H, s, CH, H-3'), 6.96 (1H, s, CH, H-4'), 7.24 (1H, t, J = 7.5 Hz, CH, H-4''), 7.41 (2H, t, J = 7.2 Hz, 2 x CH, H-3''), 7.47 (2H, d, J = 8.1 Hz, 2 x CH, H-2), 7.73 (2H, d, J = 7.8 Hz, 2 x CH, H-3), 7.77 (2H, d, J = 7.8 Hz, 2 x CH, H-2''); ¹³C NMR (100 MHz; DMSO-d₆) (assignments made using DEPT-135): 104.0 (CH), 108.0 (CH), 113.9 (CH), 122.9 (CH), 124.8 (CH), 126.8 (CH), 128.8 (CH), 130.5 (C), 148.5 (C), 150.6 (C), 154.1 (C) one quaternary carbon was not observed in the spectrum; +ESIMS m/z (relative intensity): 236.2 (100 %, [M + H]^+); found by +ESIMS 236.1071, C_{16}H_{14}ON [M + H]^+, requires 236.1070, error 0.5 ppm.

2.26.3. 3-(5-Phenylthiophen-2-yl)aniline 249

Grey solid 0.20 g (32.0 %): IR (cm⁻¹): 3430 (N-H), 3346 (N-H), 3205, 1596, 1581, 1483, 1454, 808, 775, 756, 288; ¹H NMR (300 MHz; DMSO-d₆): 5.22 (2H, s, NH₂), 6.52 (1H, s, CH, H-2), 6.82-6.86 (2H, m, 2 x CH, H-3', H-4'), 7.06 (1H, t, J = 7.5 Hz, CH), 7.30-7.48 (5H, m, 5 x CH), 7.66 (2H, d, J = 7.2 Hz, 2 x CH, H-2'').
2.26.4. 4-(5-Phenylthiophen-2-yl)aniline 250

Orange solid 0.08 g (64.0 %): IR (cm\(^{-1}\)): 3392 (N-H), 3306 (N-H), 1609, 1516, 1489, 1263, 830, 801, 751; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 6.59 (2H, d, J = 8.4 Hz, 2 x CH, H-2), 7.21-7.44 (7H, m, 7 x CH, H-3, H-4', H-2", H-3", H-4"), 7.63 (d, J = 7.5 Hz, 2 x CH, H-3); \(^{13}\)C NMR (100 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 115.3 (C\(_H\)), 122.1 (C\(_H\)), 123.9 (C\(_H\)), 125.1 (C), 125.5 (C\(_H\)), 126.9 (C\(_H\)), 127.2 (C), 128.9 (C\(_H\)), 134.6 (C), 144.4 (C), 146.1 (C), one primary carbon (CH) was not observed in the spectrum.

2.27. General procedure for the synthesis of \(N\)-aryl amidines (reversed amidines) 235 and 236

Glacial acetic acid (2.0 mmol) and S-2-naphthylmethylthioacetimidate hydrobromide 251 (1.0 mmol) were added to a solution of amines, 248 or 250 (1.0 mmol) in chloroform. The reaction was stirred at room temperature overnight and monitored by TLC (20 % EtOAc/ hexane).

2.27.1. \(N\)-(4-(5-Phenylfuran-2-yl)phenyl)acetimidate hydrobromide 235

The brown precipitate was filtered off under vacuum and rinsed with diethyl ether to give 0.10 g (88.8 %) of 235: mp 99 -100 ºC (decomposed); IR (cm\(^{-1}\)): 3023, 2362, 1738, 1672, 1625, 1367, 1215, 768, 685; \(^1\)H NMR (300 MHz; DMSO-d\(_6\)): 7.28 (3H, s, CH\(_3\)), 7.08 (1H, d, J = 3.3 Hz, furan-CH), 7.14 (1H, d, J = 3.6 Hz, furan-CH), 7.28 (1H, t, J = 7.2 Hz, CH, H-4"), 7.34 (2H, d, J = 8.4 Hz, 2 x CH), 7.41 (2H, t, J = 7.8 Hz, 2 x CH, H-3"), 7.78 (2H, d, J = 7.2 Hz, 2 x CH, H-2"), 7.91 (2H, d, J = 8.7 Hz, 2 x CH), 8.58 (1H, s, N-H), 9.44 (1H, s, N-H), 11.14 (1H, s, N-H); \(^{13}\)C NMR (100 MHz; DMSO-d\(_6\)) (assignments made using DEPT-135): 19.0 (CH\(_3\)), 108.4 (CH), 109.2 (CH), 123.5 (CH), 124.9 (CH), 125.8 (CH), 127.8 (CH), 129.0 (CH), 129.8 (C), 129.9 (C), 133.0 (C), 151.6 (C), 153.1 (C), 164.3 (C, C-amidine); -ESIMS m/z (relative intensity):
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275 (60 %, [M - H]); +ESIMS m/z (relative intensity): 277 (100 %, [M + H]+); found by +ESIMS 277.1335, C_{18}H_{17}ON_{2}[M + H]+, requires 277.1335, error 0.1 ppm.

2.27.2. N-(4-(5-Phenylthiophen-2-yl)phenyl)acetimidate hydrobromide 236

![Diagram of molecule 236]

The precipitate was filtered off under vacuum and rinsed with diethyl ether to give 0.09 g (90.0 %) of 236: mp 207-209 ºC (decomposed); IR (cm⁻¹): 3031, 2362, 1670, 1625, 1203, 804, 751, 695; ¹H NMR (300 MHz; DMSO-d₆): 2.33 (3H, s, C₃H₃), 7.32-7.39 (3H, m, 3 x CH), 7.49 (2H, t, J = 7.2 Hz, 2 x CH, H-3”), 7.59 (1H, d, J = 3.6 Hz, thiophene-CH), 7.63 (1H, d, J = 3.6 Hz, thiophene-CH), 7.71 (2H, d, J = 7.5 Hz, 2 x CH), 7.85 (2H, d, J = 8.1 Hz, 2 x CH), 8.65 (1H, s, N-H), 8.75 (1H, s, N-H), 9.48 (1H, s, N-H); ¹³C NMR (100 MHz; DMSO-d₆): 19.0 (CH₃), 125.18 (CH), 125.22 (CH), 125.8 (CH), 126.0 (CH), 126.6 (CH), 128.0 (CH), 129.2 (CH), 133.26 (C), 133.33 (C), 141.2 (C), 143.3 (C), 164.4 (C, C-amidine), one quaternary carbon has not been observed; +ESIMS m/z (relative intensity): 293 (100 %, [M + H]+); found by +ESIMS 293.1113, C_{18}H_{17}N_{2}[M + H]^+, requires 293.1107, error 1.9 ppm.

2.27.3. S-2-Naphthylmethylthioacetimidate hydrobromide 251

![Diagram of molecule 251]

The solution of thioacetamide (0.34 g, 4.5 mmol) and 2-(bromomethyl)naphthalene (1.0 g, 4.5 mmol) in chloroform (10.0 ml) was heated at reflux for 1.5 h. S-2-Naphthylmethylthioacetimidate hydrobromide 251 precipitated from the reaction mixture, which was filtered off under vacuum and rinsed with hexane to give 1.10 g (82.7 %) of a white solid; IR (cm⁻¹): 2849, 1734, 1598, 845, 756, 694; ¹H NMR (300 MHz; DMSO-d₆): 2.64 (3H, s, CH₃), 4.75 (2H, s, CH₂), 7.54-7.56 (3H, m, 3 x CH), 7.91-8.00 (4H, m, 4 x CH), 11.90 (1H, s, N-H); ¹³C NMR data were identical to those reported in the literature.¹⁴¹c
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2.28. General procedure for the synthesis of N-aryl amides 237-239

Acetyl chloride (1.0 mmol) was added dropwise at 0 °C to a solution of amine 247, 248 or 249 (0.01 mmol) in acetonitrile (1.0 mmol) and ethanol (1.0 mmol). The reaction mixture was heated at reflux for 12 h and monitored by TLC with 20 % (EtOAc/hexane).

2.28.1. N-(3-(5-Phenylfuran-2-yl)phenyl)acetamide 237

The reaction mixture was concentrated, kept in a fridge overnight and the white crystals were filtered off under vacuum and rinsed with hexane. Compound 237 was re-crystallized from DMF/H2O: mp 164-165 °C; IR (cm⁻¹): 3310 (N-H), 1665 (H N C=O), 1606, 1415, 1316, 1026, 762, 689; ¹H NMR (400 MHz; DMSO-d6): 2.08 (3H, s, CH₃), 7.01 (1H, d, J = 3.6 Hz, furan-CH), 7.10 (1H, d, J = 3.6 Hz, furan-CH), 7.33-7.39 (2H, m, 2 x CH), 7.46-7.50 (3H, m, 3 x CH), 7.55 (1H, d, J = 7.8 Hz, CH), 7.80 (2H, d, J = 7.8 Hz, 2 x CH), 8.02 (1H, s, C-H), 10.10 (1H, s, N-H); ¹³C NMR (100 MHz; DMSO-d6) (assignments made using DEPT-135): 24.0 (CH₃), 108.2 (furan-CH, 2 x CH), one of the furan CH was observed overlapping with the second furan CH, 113.7 (CH), 118.2 (CH), 118.4 (CH), 123.3 (CH), 127.6 (CH), 128.9 (CH), 129.3 (CH), 130.0 (C), 133.4 (C), 139.9 (C), 152.45 (C), 152.5 (C), 168.5 (C, C-amide); -ESIMS m/z (relative intensity): 277 (100 %, [M - H]⁺).

2.28.2. N-(4-(5-Phenylfuran-2-yl)phenyl)acetamide 238

The reaction mixture was concentrated, kept in a fridge overnight and the white crystals were filtered off under vacuum and rinsed with hexane. Compound 238 was re-crystallized from DMF/H₂O to give 0.023 g (37.0 %); IR (cm⁻¹): 3274 (N-H), 1656 (HNC=O), 1530, 1369, 1023, 830, 782, 755, 573; ¹H NMR (300 MHz; DMSO-d6): 2.06 (3H, s, CH₃), 6.95 (1H, s, furan-CH), 7.05 (1H, s, furan-CH), 7.29 (1H, t, J = 7.2 Hz, CH, H-4”), 7.44 (2H, t, J = 7.8 Hz, 2 x CH, H-3”), 7.65-7.80 (6H, m, 6 x CH, H-2, H-3,
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H-2”), 10.06 (1H, s, N-H); $^{13}$C NMR (100 MHz; DMSO-d$_6$) (assignments made using DEPT-135): 24.0 (CH$_3$), 107.0 (CH), 108.2 (CH), 119.1 (CH), 123.3 (CH), 124.0 (CH), 124.9 (C), 127.3 (CH), 128.9 (CH), 130.1 (C), 138.8 (C), 152.0 (C), 152.7 (C), 168.3 (C, C-amide); +ESIMS m/z (relative intensity): 277 (100 %, [M + H]$^+$).

2.28.3. N-(3-(5-Phenylthiophen-2-yl)phenyl)acetamide 239

The reaction mixture was diluted with dichloromethane, extracted and washed with saturated sodium bicarbonate solution and water. The organic extracted, dried over dry MgSO$_4$ and evaporated. The crude product was purified by column chromatography using ethyl acetate and hexane (2:8) as the mobile phase to give 0.026 g (23.0 %) of 239 as a yellow solid: mp 166-167 ºC; IR (cm$^{-1}$): 1661, 1606, 1482, 1374, 1327, 779, 755; $^1$H NMR (400 MHz; DMSO-d$_6$): 2.06 (3H, s, CH$_3$), 7.32-7.54 (8H, m, 8 x CH including H-3’ and H-4’), 7.69 (1H, d, J = 7.2 Hz, CH), 7.95 (1H, s, CH, H-2), 10.06 (1H, s, N-H); $^{13}$C NMR (75 MHz; DMSO-d$_6$): 24.0 (CH$_3$), 115.5, 118.3, 119.9, 124.8, 124.9, 125.1, 127.8, 129.2, 129.5, 133.4, 133.8, 140.0, 142.46, 142.54, 186.7 (HNC=O); +ESIMS m/z (relative intensity): 316 (100 %, [M + Na]$^+$).

2.29. Furan analogue of resveratrol

2.29.1. 4’-(Acetyloxy)acetophenone 253

Triethylamine (0.6 g, 5.6 mmol) and 4-(dimethylamino)pyridine (0.01 g, 0.04 mmol) were added to a solution of 4’-hydroxyacetophenone 259 (0.5 g, 3.7 mmol) in dichloromethane (20.0 ml). The reaction mixture was cooled to 0 ºC and acetic anhydride (0.40 g, 3.7 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1.5 h and monitored by TLC (10.0 % MeOH/ CHCl$_3$). Upon the consumption of the starting material 259, the reaction was quenched with water and the organic layer was washed with saturated solution of sodium bicarbonate, dried over anhydrous MgSO$_4$ and evaporated to give 0.34 g (53.1 %) of a colourless oil: $^1$H NMR
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(300 MHz; CDCl$_3$): 2.30 (3H, s, OCOCH$_3$), 2.57 (3H, s, COCH$_3$), 7.17 (2H, d, $J = 8.7$ Hz, 2 x CH, H-3), 7.97 (2H, d, $J = 8.7$ Hz, 2 x CH, H-2); $^{13}$C NMR (100 MHz; CDCl$_3$) (assignments made using DEPT-135): 21.1 (OCOCH$_3$), 28.3 (COCH$_3$), 122.0 (2 x CH, C-3), 129.6 (C, C-4), 134.7 (2 x CH, C-2), 168.9 (C, C-1), 174.3 (OC=O), 197.0 (C=O); $^1$H-NMR data were identical to those reported in the literature.$^{147}$

2.29.2. 3',5'-Di(acetyloxy)acetophenone 254

![Chemical Structure]

Triethylamine (1.0 g, 9.96 mmol) and 4-(dimethylamino)pyridine (0.01 g, 0.07 mmol) were added to a solution of 3',5'-dihydroxyacetophenone 257 (1.00 g, 6.6 mmol) in dichloromethane (20.0 ml). The reaction mixture was cooled to 0 °C and acetic anhydride (2.70 g, 24.6 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1.5 h and monitored with TLC (10.0 % MeOH/ CHCl$_3$). Upon the consumption of the starting material 257, the reaction was quenched with water and the organic layer was extracted, dried over anhydrous MgSO$_4$ and evaporated to give 1.10 g (66.0 %) of a white solid: IR (cm$^{-1}$): 2362, 1763 (OC=O), 1683 (C=O), 1363, 1201, 1123, 1018, 677; $^1$H NMR (300 MHz; CDCl$_3$): 2.33 (6H, s, 2 x OCOCH$_3$), 2.59 (2H, s, COCH$_3$), 7.16 (1H, s, CH, H-4), 7.57 (2H, s, 2 x CH, H-2, H-6); $^{13}$C NMR (100 MHz; CDCl$_3$) (assignments made using DEPT-135): 21.1 (OCOCH$_3$), 26.7 (COCH$_3$), 119.0 (CH, C-4), 120.2 (2 x CH, C-2, C-6), 138.9 (C), 151.2 (C), 168.8 (OC=O), 195.9 (C=O).

2.29.3. 2-Bromo-4'-(acetyloxy)acetophenone 255

![Chemical Structure]

Bromine (1.20 g, 7.5 mmol) in tetrahydrofuran (10.0 ml) was added dropwise at 0 °C to a solution of 4'-hydroxyacetophenone acetate 253 (1.0 g, 5.8 mmol) and aluminium chloride (5.00 mg) in tetrahydrofuran (5.0 ml). The reaction mixture was stirred at room temperature for 2 h and monitored with TLC (1.0 % MeOH/ CHCl$_3$).
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Upon the consumption of the starting material 253, tetrahydrofuran was evaporated and the residue dissolved in ethyl acetate, washed with water and brine, and dried over anhydrous MgSO₄. The crude was purified by column chromatography using ethyl acetate and hexane (1:9) as a mobile phase to give 0.25 g (17.0 %) of a white solid: mp 73-76 ºC; IR (cm⁻¹): 3315, 1661 (OC=O), 1597, 1568, 1279, 1173, 851, 763, 710, 593; \(^1\)H NMR (300 MHz; CDCl₃): 2.35 (3H, s, CH₃), 4.44 (2H, s, CH₂), 7.25 (2H, d, J = 7.8 Hz, 2 x CH, H-3'), 8.04 (2H, d, J = 8.7 Hz, 2 x CH, H-2'); \(^1\)C NMR (100 MHz; CDCl₃) (assignments made using DEPT-135): 21.2 (OCOCH₃), 30.7 (CH₂), 122.2 (2 x CH, C-3), 130.5 (2 x CH, C-2), 131.6 (C), 154.9 (C), 168.8 (C, OC=O), 190.1 (C, C=O).

2.30. N-Ribosyl dihydronicotinamide NRH

\[ \text{HO} - \text{N} - \text{Ribosyl dihydronicotinamide NRH} \]

NADH (0.50 g, 0.69 mmol) was dissolved in 20.0 ml of 0.4 M sodium carbonate/bicarbonate buffer, pH 10.0, and incubated at 37°C for 16 h with 0.1 unit of phosphodiesterase 1 type IV (phosphodiesterase I from Crotalus atrox western diamondback rattlesnake) and 500 units of alkaline phosphatase type VII-S. After complete digestion of NADH, the mixture was freeze dried. The dried powder was extracted with methanol (5 × 6 ml), and this methanol extract was dried by rotary evaporation and dissolved in 5.0 ml of water. The NRH was then purified by preparative HPLC performed on a microsorb C18 column (21.2 X 250 mm), eluted with 10 % methanol in water over 15 m at a flow rate of 15.0 ml/m, using a 1.0 ml injection size. The NRH peak was detected at a wavelength of 350 nm. This peak from each injection was collected, freeze-dried and stored at 4°C. \(^1\)H NMR (300 MHz; D₂O): 2.95 (s, CH, H-4) 3.53-3.65 (m, OCH₂), 3.84-3.85 (m, CH), 3.91-4.01 (m, CH), 4.08 (t, J = 6.0 Hz, CH, H-3'), 4.75 (d, J = 6.9 Hz, CH, H-2'), 4.85-4.87 (m, CH, H-4') 5.98 (d, J = 8.1 Hz, CH, H-6), 7.03 (s, CH, H-2); \(^1\)C-NMR (75 MHz; D₂O): 21.9, 61.4, 70.1, 70.9, 83.5, 94.8, 100.9, 105.1, 125.2, 137.8, 172.9 (C, C-amide).
Chapter III. Experimental/ NQO2 Inhibitory Activity

1. Materials and methods

BECKMAN DU 7400 spectrophotometer was used to determine enzyme activity. Water bath Grant JB series was used to heat the buffer to 37 °C. NRH used was synthesized according to the procedure described in Section 2.30.

2. General procedure

The rates of the NQO2 enzyme were determined spectrophotometrically by recording the rates of DCPIP colour change over 1 m at 37 °C in cuvettes of 1.0 cm width containing a final volume of 1.0 ml: 940 µl phosphate buffer (50.0 mM, pH 7.4), 10 µl NQO2 (5 x 10^{-3} mg/ml), 20.0 µl NRH (10.0 mM), 20.0 µl DCPIP (2.0 mM), 10.0 µl DMSO (in the control sample), and 10.0 µl inhibitor (5 samples of different concentration).

All the experiments were performed in triplicates, three independent times. In each independent experiment fresh solutions of NRH (10.0 mM), DCPIP (2.0 mM) and NQO2 (5 x 10^{-3} mg/ml) were prepared. NRH and NQO2 were dissolved in 50 mM phosphate buffer (50 mM, pH 7.4) and kept at 0 °C. DCPIP was dissolved in deionized water. The inhibitor stock solution was prepared by dissolving the inhibitor in DMSO. A 10-fold serial dilution was completed using DMSO to end up with five different concentrations of the inhibitor.

IC_{50} values were determined by the Prism program. The curve was obtained from plotting enzyme activity as a percent of the control versus the final concentration of the inhibitor used. IC_{50} values were determined as 50% reduction in the enzyme activity compared to control (100%).
Chapter III. Experimental/ Docking

1. Materials and methods

The X-ray crystal structure of human NQO2 (PDB code 1QR2, resolution 2 Å) was obtained from the Protein Bank database (PDB). The inhibitors were drawn using MOE 2011.10 software and the docking was performed using GOLD suite 5.1 software.

2. General procedure

Firstly the hydrogen atoms were added to the structure, which included the protein and FAD molecules. The binding affinities of the docked inhibitors were calculated using ChemScore scoring function. NQO2 protein was prepared for docking by determining the active site area. The active site was defined as being any volume within 15 Å of N5 of the FAD cofactor. The hydrophilic amino acid Asn was allowed to be flexible to maximize the ability of the prediction of hydrogen bonding with the amidine group of the docked inhibitors. The top 10 solutions for each ligand were retained and analyzed for favorable interactions within the active site of NQO2, including low protein ligand clash, lipophilic contacts, and hydrogen bonding interactions.

All figures of NQO2 enzyme and docking images were manipulated using Pymol software (for education use only) and the correlation charts and the regression analyses were done using the Excel program.
Chapter III. Experimental/ DNA Melting

1. Materials and methods

Calf thymus DNA was purchased from Sigma-Aldrich Company. The melting temperatures were measured using Thermal software on a Varian spectrophotometer.

2. Calf thymus DNA preparation

Calf thymus DNA lyophilized powder (1.0 mg) was dissolved in deionized water (1.0 ml) and kept at 2-8 ºC overnight. 10.0 µl aliquot of calf thymus DNA solution (1.0 mg/ml) was diluted with 990.0 µl buffer.

2. General procedure for DNA melting temperatures measurement

The melting temperatures of calf thymus DNA were determined spectrophotometrically by recording the absorbance of DNA at 260 nm over a range of 25-98 ºC in cuvettes of 1.0 cm width containing a final volume of 1.0 ml: 980 µl low-salt phosphate buffer (7.5 mM NaH$_2$PO$_4$, 1 mM EDTA, pH 7.4) or high-salt buffer (7.5 mM NaH$_2$PO$_4$, 150.0 mM NaCl, 15.0 mM sodium citrate, pH 7.0), 10 µl calf thymus DNA (10 µg/ml), and 10.0 µl inhibitor (1.0 mM). All the experiments were performed in triplicates, three independent times. DNA melting temperatures ($T_m$) were determined using Excel program. The curve was obtained from plotting DNA absorbance at 260 nm versus the temperature. $T_m$ values were from the first-derivative curves.
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