



LJMU Research Online

Enoch, SJ

The development of an in silico profiler for mitochondrial toxicity

<http://researchonline.ljmu.ac.uk/2053/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Enoch, SJ (2015) The development of an in silico profiler for mitochondrial toxicity. Chemical Research in Toxicology. ISSN 1520-5010

LJMU has developed [LJMU Research Online](#) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

The development of an in silico profiler for mitochondrial toxicity

Mark D Nelms, Claire L Mellor, Mark Cronin, Judith C Madden, and Steve J Enoch

Chem. Res. Toxicol., **Just Accepted Manuscript** • DOI: 10.1021/acs.chemrestox.5b00275 • Publication Date (Web): 16 Sep 2015

Downloaded from <http://pubs.acs.org> on September 21, 2015

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.



The development of an in silico profiler for mitochondrial toxicity

Mark D Nelms*, Claire L Mellor*, Mark T.D Cronin, Judith C Madden, and Steven J Enoch⁺

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool. L3 3AF, UK

*Joint first authors

⁺Corresponding author

Dr Steve Enoch

E: s.j.enoch@ljmu.ac.uk

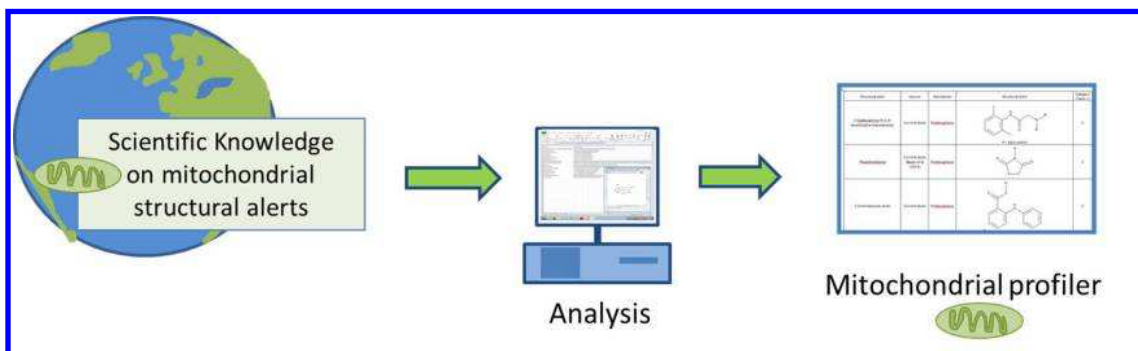
T: +44(0)151 231 2164

School of Pharmacy and Biomolecular Sciences
James Parsons Building
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

Keywords

Structural alerts, mitochondrial toxicity, AOP, profiler, MIE

Table of Contents Graphic



Abstract

This study outlines the analysis of mitochondrial toxicity for a variety of pharmaceutical drugs extracted from Zhang *et al.* These chemicals were grouped into categories based upon structural similarity. Subsequently, mechanistic analysis was undertaken for each category to identify the Molecular Initiating Event driving mitochondrial toxicity. The mechanistic information elucidated during the analysis enabled mechanism-based structural alerts to be developed and combined together to form an *in silico* profiler. This profiler is envisaged to be used to develop chemical categories based upon similar mechanisms as part of the Adverse Outcome Pathway paradigm. Additionally, the profiler could be utilised in screening large dataset in order to identify chemicals with the potential to induce mitochondrial toxicity.

Introduction

Over the past decade a number of changes have occurred in European Union chemicals policy that have led to an increase in efforts to develop alternative methods to traditional animal testing for risk assessment.¹⁻⁷ These alternatives have been developed employing *in silico*, *in chemico* and *in vitro* methods focussing on replacing or reducing animals used in short-term toxicity tests⁸. In order to be relevant, and useful, for regulatory assessment these alternatives should be based upon specific *in vivo* endpoints. Within recent years, interest has grown in developing a greater understanding of toxicity pathways. One such pathway approach is the Adverse Outcome Pathway (AOP) paradigm. An AOP is a framework that means to establish a mechanistic connection between an upstream Molecular Initiating Event (MIE) and a downstream adverse outcome relevant for risk assessment.⁹⁻¹² The MIE is the critical event in the progression of an AOP as it provides insight into the initial interaction(s) between the chemical behaviour of the xenobiotic and the biological system that initiates the perturbation of the normal pathway. Elucidation of the mechanistic information relating to specific MIEs enables the identification of structural (and physico-chemical) features of chemicals that are responsible for the interaction with biological macromolecules, thus, facilitating the development of structural alerts. When utilised together, multiple structural alerts pertaining to the same MIE form the basis of an *in silico* profiler.¹³⁻
¹⁶ The information within an *in silico* profiler can, in turn, be used to develop chemical categories centred on a common MIE (note that multiple MIEs can be initiated by a single chemical). This allows for read-across and data gap filling to be applied. The premise behind these structurally developed categories is that similar chemicals should have similar biological activities and therefore, should have the same MIE. Furthermore, the categories produced using *in silico* profilers can be supported by, and used to prioritise, additional

1
2
3 testing using *in vitro* and/or *in chemico* methods, within an integrated testing strategy. Such
4
5 strategies can be used for hazard identification and risk assessment purposes, as well as being
6
7 incorporated into *in silico* software tools such as the OECD QSAR Toolbox.^{10-11, 16-18}
8
9

10 A number of *in silico* profilers have been developed for a variety of organ-level
11 toxicities, such as skin sensitisation, respiratory sensitisation, genotoxicity and
12 hepatotoxicity.^{13-15, 19-21} However, very few profilers have dealt with toxicity induced by
13 mitochondrial dysfunction.^{1,22,23} This is, in part, due to the number of mechanisms by which a
14 chemical could induce mitochondrial dysfunction²⁴. An additional complication is that a
15 single chemical might have the ability to induce more than one of these mechanisms, making
16 it difficult to define a single MIE within the AOP paradigm. Over the past decade, interest in
17 screening chemicals for an ability to induce mitochondrial dysfunction has increased.^{24,25} This
18 is, in part, due to the withdrawal of a number of pharmaceuticals from the market after
19 observed mitochondrial dysfunctions.²⁶⁻³¹ Toxicity to mitochondria has led to such
20 withdrawals as these are important organelles present within almost every cell type of the
21 body, the exception being mature erythrocytes.^{32,33} The basic structure and function of
22 mitochondria consists of two membranes, the outer and inner membrane, enclosing three
23 compartments, the inter membrane space, the cristae and the mitochondrial matrix. The outer
24 membrane is relatively smooth and permeable to molecules that are less than 5kDa in size. In
25 contrast, the inner membrane contains multiple invaginations (cristae), is impermeable to all
26 molecules except O₂, CO₂, and H₂O and contains each of the protein complexes within the
27 electron transport chain, ATP synthase (Complex V) and various electron carriers. The
28 mitochondria are responsible for a number of tasks vital to a cell's normal functioning and
29 survival. These tasks include the production of approximately 95% of the total amount of
30 Adenosine Tri-Phosphate (ATP) generated by cells during oxidative phosphorylation.
31 Oxidative phosphorylation is a process whereby energy from the transfer of electrons,
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 generated by the oxidation of Nicotinamide Adenine Dinucleotide (NADH) and Flavin
4 Adenine Dinucleotide (FADH₂), along various complexes of the electron transport chain is
5 used to pump protons across the inner mitochondrial membrane generating an
6 electrochemical gradient. The electron transport chain comprises four complexes situated
7 within the inner mitochondrial membrane. Complexes I and II are involved in the oxidation
8 of NADH and FADH₂ respectively, providing the input of electrons into the respiratory chain.
9
10 Complexes I, III and IV use the energy released from the transfer of electrons along the
11 electron transport chain to pump protons out of the mitochondrial matrix into the inter
12 membrane space.^{25,31,34} Complex V, the terminal complex involved in oxidative
13 phosphorylation, utilises the electrochemical gradient produced to transfer protons from the
14 inter membrane space back into the mitochondrial matrix. The energy released from this
15 action is used to phosphorylate adenosine diphosphate into ATP.
16
17
18
19
20
21
22
23
24
25
26
27
28
29

30 Previous research has shown that mitochondrial dysfunction may be induced by a
31 range of chemicals and has been linked to a variety of organ toxicities within kidney, liver
32 and nervous tissues.^{26-31,35} The most susceptible tissues to mitochondrial dysfunction are
33 those containing a higher concentration of mitochondria or those exposed to a higher
34 concentration of chemical: such as the liver, kidneys and heart.^{24,25,28,36} Five general
35 mechanisms of mitochondrial dysfunction have been identified,^{36,37} inhibitors of the electron
36 transport chain and ATP synthase (Complex V), uncouplers of oxidative phosphorylation,
37 opening of the membrane permeability transition pore, inhibition of fatty acid β -oxidation,
38 and oxidation or inhibition of mitochondrial DNA.
39
40
41
42
43
44
45
46
47
48
49

50
51 As an example of the importance of mitochondrial toxicity approximately 35%, of more than
52 500 pharmaceutically relevant chemicals, have been shown to be directly involved in
53 impairing normal mitochondrial functioning by inhibition of the electron transport chain
54 and/or by uncoupling of oxidative phosphorylation.³⁸ Additionally, there are chemicals that
55
56
57
58
59
60

1
2
3 can induce mitochondrial toxicity via alternative mechanisms, such as inducing the
4
5 membrane permeability transition, inhibition of β -oxidation of mitochondrial fatty acids, or
6
7 interfering with mitochondrial DNA. Briefly stated, chemicals that inhibit the electron
8
9 transport chain can do so by either direct binding to the complexes of the electron transport
10
11 chain or ATP synthase or by acting as an alternative electron acceptor.^{27,36,37} The inhibition of
12
13 electron flow along the electron transport chain by both of these mechanisms induces the
14
15 formation of reactive oxygen species resulting in oxidative stress.^{27,36,37} Uncouplers of
16
17 oxidative phosphorylation induce mitochondrial toxicity by shuttling protons into the
18
19 mitochondrial matrix, via the inner mitochondrial membrane, bypassing ATP synthase. This
20
21 assisted transport of protons back into the matrix dissipates the electrochemical potential,
22
23 resulting in the loss of ATP production and, ultimately, cell death.^{27,31,36,37,39-43} Induction of
24
25 the membrane permeability transition increases the permeability of the inner mitochondrial
26
27 membrane to low molecular weight solutes (<1500Da), leading to a disruption of the electron
28
29 transport chain, loss of membrane potential, and swelling of both the inner- and outer
30
31 mitochondrial membranes.^{44,45} Inhibition of β -oxidation of mitochondrial fatty acids reduces
32
33 the amount of NADH and FADH₂ available for oxidative phosphorylation that, in turn,
34
35 reduces ATP production.⁴⁶ Mitochondrial DNA encodes 13 components of the electron
36
37 transport chain, damage that occurs to mitochondrial DNA can have a variety of downstream
38
39 effects depending upon where it occurs.^{36,46} It should be noted, however, that there is the
40
41 potential that multiple, competing, mechanisms could initiate mitochondrial toxicity observed
42
43 for a single (group of) chemical(s), i.e. one chemical may induce several MIEs.
44
45
46
47
48
49

50
51 Given the importance of mitochondria within most cell systems, and the wide range of
52
53 organ-level toxicities that may arise from mitochondrial dysfunction, the aim of the study was
54
55 to identify structural alerts that could be used to form an *in silico* profiler suitable for
56
57 grouping chemicals into mechanism-based categories.
58
59
60

Methods

Data set

A set of 288 chemicals were extracted from Zhang *et al.*¹ This article was chosen for use as it provides one of the largest freely available datasets, for which the chemical list has qualitative mitochondrial toxicity data associated with it. Within this data set 171 chemicals have been reported within the literature as inducing mitochondrial toxicity and were therefore considered to be mitochondrial toxicants. The chemicals with a negative result for mitochondrial toxicity were selected from the FDA-approved drug list, whereby the therapeutic action mechanism, of common and safe oral drugs, was not associated with a mechanism of drug-induced mitochondrial toxicity. Given the lack of supporting mechanistic information to confirm the presence, or absence, of mitochondrial toxicity additional analysis, was carried out as detailed below. The full dataset is available in the supplementary information. Positive and negative chemicals were taken as documented in Zhang *et al* (Table S1 in supporting information).

Category formation based upon structural similarity

All chemical structures were encoded into Simplified Molecular-Input Line-Entry System (SMILES) strings, neutralised and had salts removed. Each of the SMILES strings was extracted from the Royal Society of Chemistry's ChemSpider website.⁴⁷ Similarity calculations were implemented within the freely available Toxmatch software (v1.07) using the atom environment nearest neighbour approach, generating a data matrix with a Tanimoto similarity score for each chemical to all others within the data set. Subsequently, in-house code was implemented within Microsoft Excel that identified analogues with a similarity

1
2
3 index of 0.6 or greater; this was used in order to develop categories for the chemicals within
4
5 the dataset with two, or more, analogues. Further analysis was undertaken upon those
6
7 categories that met the following criteria: they contained three or more chemicals and at least
8
9 one mitochondrial toxic chemical.
10

11 12 *Mechanistic hypothesis and the development of alerts* 13

14
15
16 Once categories had been developed using structural similarity a detailed search of the
17
18 available literature was undertaken to elucidate the mechanistic information behind the
19
20 molecular initiating event, along with other downstream key events, leading to the disruption
21
22 of the mitochondria. This mechanistic information was subsequently utilised to support the
23
24 definition of a structural alert suitable for grouping chemicals. These structural alerts were
25
26 defined by identifying the common fragment present within each of the chemicals found to
27
28 have positive mitochondrial toxicity according to literature information associated with them.
29
30 Any additional information regarding the limits of the fragment found during the literature
31
32 search, such as the requirement for an electron withdrawing group or the type of bond needed
33
34 (e.g. a tertiary amine), was used to further refine the structural alert. The resulting alerts were
35
36 subsequently defined as SMARTS patterns.⁴⁸ A structural alert was only developed if
37
38 information linking category members to mitochondrial toxicity was present within the
39
40 scientific literature. The benefit of undertaking the analysis for each category is that it
41
42 enabled the chemical space associated with a known, and tested, mechanism of mitochondrial
43
44 toxicity to be identified. The development of chemical categories and identification of
45
46 additional mechanistic information from the literature was crucial in addressing the
47
48 limitations of the information in the original dataset. All structural alerts are available in the
49
50 supplementary information and a KNIME workflow containing the structural alerts can be
51
52 found in the COSMOS space which is a freely available tool
53
54 (<http://cosmospace.cosmostox.eu/app/login>).
55
56
57
58
59
60

Results and discussion

The aim of this study was to develop an *in silico* profiler for mitochondrial toxicity based around clearly defined mechanistic information. This was achieved by grouping chemicals based upon their structural similarity, followed by a literature search to elucidate mechanistic information for the chemicals in categories associated with toxicity to mitochondria. Overall, 35 of the 288 chemicals were identified as belonging to categories containing mitochondrial toxic chemicals: local anaesthetics, antianginal, and antiarrhythmic; thiazolidinediones; nonsteroidal anti-inflammatory drugs; anthracycline antibiotics; perfluorinated chemicals; bile acids; phenothiazines; and β -blockers. A summary of the categories developed within this study is shown in Table 1. In total, eight categories were formed: two separate molecular initiating events for the perfluorinated chemical category were identified, whilst no structural alert for the β -blocker category could be defined. Subsequent mechanistic analysis showed the chemical within the eight categories covered five mechanisms of mitochondrial toxicity: inhibition of the electron transport chain, alternative electron acceptance, initiation of the death receptor pathway, uncoupling of oxidative phosphorylation and induction of the membrane permeability transition.

Table 1: Chemicals grouped into categories using structural similarity and their associated mitochondrial toxicity

Category 1: Local anaesthetics, antianginal and antiarrhythmic

The local anaesthetics category consisted of six analogues, four of which are anaesthetics, with ranolazine and tocainide being an antianginal and antiarrhythmic

1
2
3 respectively. All but one of the chemicals, tocainide, has been shown to exhibit toxicity
4
5 towards mitochondria. The category is supported by a number of studies that have suggested
6
7 that such chemicals affect mitochondrial metabolism by uncoupling oxidative
8
9 phosphorylation.^{31,39,49-51} This uncoupling has been suggested to be mediated by both the
10
11 protonophoric properties and the pKa of these chemicals. As the pKa is relatively similar to
12
13 the intracellular pH, the level of protonated and deprotonated chemical is roughly at
14
15 equilibrium. The presence of deprotonated chemical within the inner membrane space means
16
17 that protons can be scavenged. Subsequently, the protonated chemical can combine with a
18
19 hydrophobic anion to form a neutral ion-pair complex, which can then migrate across the
20
21 inner mitochondrial membrane into the matrix, where the complex dissociates and the proton
22
23 is released. Both the chemical and the hydrophobic anion then return to the inner membrane
24
25 space, continuing the cycle. This assisted transport of protons back into the matrix dissipates
26
27 the electrochemical potential, resulting in a loss of ATP production and ultimately cell
28
29 death.^{31,39,41,42,51-53} It has been suggested that bupivacaine, and other highly lipophilic
30
31 anaesthetics, can also act to uncouple oxidative phosphorylation via the mechanism outlined
32
33 above without the need to complex with a lipophilic anion.⁴⁹ The presence, and pKa, of the
34
35 tertiary amine group is thought to be responsible for the ability of these chemicals to
36
37 scavenge protons within the inner membrane space. Therefore, the lack of a tertiary amine
38
39 group offers an explanation as to why no mitochondrial toxicity has been associated with
40
41 tocainide.
42
43
44
45
46
47

48 Category 2: anti-diabetic drugs

49
50
51 This category consists of three thiazolidinediones: pioglitazone, rosiglitazone,
52
53 troglitazone: each of which were identified as inducing mitochondrial toxicity.
54
55 Thiazolidinediones are the major orally administered drugs used in the treatment of Type 2
56
57 (non-insulin dependent) diabetes. These drugs are used to improve insulin sensitivity and
58
59
60

1
2
3 lower blood glucose levels within diabetic patients. Many of the thiazolidinediones have been
4
5 suspected of initiating hepatotoxicity via mitochondrial dysfunction.²⁹ For example,
6
7 troglitazone was withdrawn from the world market in 2000 due to hepatotoxicity observed in
8
9 a number of patients.^{27,51} Research into the thiazolidinediones suggests the chemicals within
10
11 this category elicit their mitochondrial dysfunction by inhibiting the electron transport chain
12
13 and uncoupling oxidative phosphorylation.^{22,26,29,51} These drugs have been shown to inhibit
14
15 the activity of mitochondrial complexes, the main target being Complex I.^{26,27,51,54} These
16
17 chemicals decrease the membrane potential across the inner mitochondrial membrane which
18
19 causes mitochondrial swelling and in turn induces mitochondrial permeability transition.^{54,55}
20
21 Additionally, thiazolidinediones have been shown to uncouple oxidative phosphorylation in a
22
23 manner similar to that described above for the chemicals within category one.^{22,26} It is
24
25 thought that the properties that enable the thiazolidinediones to bind to the nuclear PPAR-
26
27 gamma receptor confers the ability to bind to Complex I.²⁶ Additionally, the heterocyclic
28
29 properties of the ring system are thought to enable the thiazolidinedione to cycle between a
30
31 protonated and deprotonated form conferring the ability to transport protons across the inner
32
33 mitochondrial membrane thereby uncoupling oxidative phosphorylation.²²
34
35
36
37
38

39 Category 3: Nonsteroidal anti-inflammatory drugs

40
41
42 The third category comprises three chemicals: mefenamic acid, flufenamic acid, and
43
44 tolfenamic acid: each of which has been identified as being able to induce mitochondrial
45
46 toxicity. Each of these three chemicals are part of a group of nonsteroidal anti-inflammatory
47
48 (NSAIDs). NSAIDs are some of the most widely used pharmaceutical drugs on the market
49
50 that are used for their analgesic, antipyretic and anti-inflammatory properties to reduce and
51
52 relieve symptoms for a variety of conditions. In order for the anti-inflammatory properties
53
54 associated with NSAIDs to be present a carboxylic acid moiety is needed.⁵¹ The carboxylic
55
56 acid moiety acts to inhibit cyclooxygenase activity, an enzyme responsible for the production
57
58
59
60

1
2
3 of mediators of the inflammatory response, thereby reducing the level of inflammatory
4 signalling. Previous research substantiates the positive mitochondrial toxicity result for each
5 chemical within this category. A variety of literature sources identify each of these chemicals
6 as having the ability to uncouple oxidative phosphorylation via a similar mechanism as that
7 described above for the lidocaine category.⁵⁶⁻⁶⁰ However, due to their lipophilicity, these
8 chemicals do not necessarily need to be associated with a separate hydrophobic anion in order
9 to translocate into the mitochondrial matrix. The carboxylic acid moiety, which is required
10 for the anti-inflammatory properties of the NSAIDs, is believed to also be required to induce
11 the uncoupling ability of this group of chemicals.⁵¹
12
13
14
15
16
17
18
19
20
21
22
23

24 Category 4: Anthracycline antibiotics

25
26
27 Anthracycline antibiotics are a group of hydroxylated tetracycline quinones with a
28 duanosamine sugar sidechain attached. One of the category members, doxorubicin, is one of
29 the most widely used antineoplastic drugs within the U.S.⁶¹ Structural similarity identified
30 three similar chemicals. A number of studies have shown that the anthracycline antibiotics
31 cause mitochondrial dysfunction by acting as alternative electron acceptors interfering with,
32 and inhibiting, the electron transport chain, leading to oxidative stress. This occurs because
33 under normal physiological conditions anthracyclines are usually deprotonated and can
34 permeate across the outer mitochondrial membrane. Once within the inner mitochondrial
35 space these chemicals disrupt the electron transport chain by sequestering an electron from
36 Complex I and are thus reduced to a semiquinone radical intermediate.^{61,62} These
37 semiquinone radicals subsequently interact with molecular oxygen present within the
38 mitochondria, producing reactive oxygen species (ROS), including hydroxyl and superoxide
39 anion radicals. Downstream these ROS lead to a variety of effects such a mitochondrial
40 permeability transition induction and oxidative damage of DNA, proteins and lipids.⁶³⁻⁶⁵ Due
41 to the high level of similarity between the chemicals it can be assumed that the mechanism of
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 action is conserved throughout the category. It should also be noted that quinones are also
4
5 used as antimalarials which are known mitochondrial toxicants.⁶⁶
6
7
8
9

10 11 Category 5: Hypolipodemic drugs 12

13
14 Perfluorinated chemicals have been widely used in a variety of commercial and
15
16 pharmaceutical products, such as flame retardants, surfactants and hypolipidemic drugs.
17
18 These hypolipidemic drugs induce the proliferation of peroxisomes and thus increase β -
19
20 oxidation of fatty acids. Three perfluorinated chemical analogues; perfluorodecanoic acid,
21
22 perfluorooctanoic acid, perfluorooctane sulphonamide; were identified as having a high level
23
24 of similarity. Despite the high levels of similarity between the chemicals. This highlights the
25
26 need to undertake mechanistic analysis of the categories as structural similarity on its own is
27
28 not enough. As is shown with this category slight variations in structure have the potential to
29
30 induce different mechanistic pathways. Accordingly, information in the literature suggests
31
32 that for this category there are two potential mechanisms by which the perfluorinated
33
34 chemicals elicit their mitochondrial toxicity; uncoupling of oxidative phosphorylation and
35
36 induction of the mitochondrial membrane permeability transition pore.
37
38
39
40

41
42 Perfluorooctane sulphonamide has been shown to uncouple oxidative phosphorylation
43
44 *in vitro* via a protonophoric mechanism, similar to that described above, within various
45
46 species^{23,67-69}. In comparison to *p*-trifluoromethoxyphenylhydrazone, one of the most potent
47
48 uncouplers, perfluorooctane sulphonamide has been known to uncouple oxidative
49
50 phosphorylation with a potency of a similar magnitude. It has been suggested that the pKa
51
52 and ionisability of the amino acid moiety, in conjunction with the relatively high lipophilicity
53
54 of the chemical, enables the shuttling of protons across the inner mitochondrial membrane
55
56 into the matrix, dissipating the membrane potential.⁶⁹ Starkov studied the structural activity of
57
58
59
60

1
2
3 perfluoroalkanes and their ability to elicit mitochondrial toxicity via disruption to oxidative
4 phosphorylation.⁶⁹ Their data identified that a protonated nitrogen atom with a favourable
5 pKa is essential to the uncoupling action of perfluorooctane sulphonamides in mitochondria.
6
7
8
9
10 In addition, perfluorooctane sulphonamide is one of a very limited number of uncoupling
11 chemicals that does not contain a ring structure⁶⁸. In contrast, the perfluoroalkyl acids are
12 believed to induce the mitochondrial membrane permeability transition at lower
13 concentrations, whilst higher concentrations can uncouple oxidative phosphorylation⁶⁹⁻⁷¹. It
14 has been observed that perfluorodecanoic acid forms reactive oxygen species (ROS),
15 hydrogen peroxide and peroxyxynitrite anion.⁷² The presence of elevated ROS levels initiates
16 oxidative stress within mitochondria. Oxidative stress has been shown to induce the
17 membrane permeability transition (MPT).^{73,74} The MPT is an increase in permeability of the
18 inner mitochondrial membrane to low molecular weight solutes (<1500 Daltons). The
19 subsequent influx of solutes into the matrix instigates swelling of the inner and outer
20 membranes causing disruption of the electron transport chain and a release of apoptotic
21 proteins such as cytochrome c.^{23,72,75} The uncoupling action of the two perfluoroalkyl acids is
22 similar to that described for category one. Two alerts were defined due to two distinct MIEs
23 being identified (Table 2). Previous research has shown there to be an increase in toxicity
24 concomitant to an increase in alkyl side chain length up to C₁₂, with the most marked increase
25 in toxicity (a five- to ten- fold increase) occurring between C₆ and C₈ perfluoroalkyl acids
26 and sulphates.²³ An unsubstituted amide fragment has been shown to be required in order for
27 uncoupling by perfluorinated sulphonamides to occur: fully substituted sulphonamides, which
28 lack the protonated amide moiety, were found to lack the ability to uncouple oxidative
29 phosphorylation.⁶⁹ The carboxylic acid moiety of the perfluoroalkyl acids chemicals is
30 thought to be responsible for the uncoupling action of these chemicals at higher
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 concentrations. However, it is unclear which fragments are required in the induction of the
4
5 MPT.
6
7

8 Category 6: Bile acids 9

10
11 The bile acid category consists of two primary conjugate bile acids: glycocholic acid
12 and taurocholic acid; and three non-conjugated secondary bile acids: deoxycholic acid,
13 chenodeoxycholic acid and lithocholic acid; all with a high level of similarity to the primary
14 bile acid; cholic acid. Bile acids are one of the main constituents of bile and are synthesised
15 from cholesterol by hepatocytes and are then converted into secondary bile acids via the
16 intestinal bacteria. They play a vital role in multiple functions within both the liver and
17 intestines, the main function being the sequestration of fats within micelles for excretion. Bile
18 acids have been shown to decrease the membrane potential of mitochondria, alongside a
19 decrease in state 3 respiration and an increase in state 4 respiration. The specific cellular
20 mechanism of bile acid-induced toxicity has not been elucidated. However, both the intrinsic
21 (mitochondrial) and extrinsic (death receptor) pathways have been implicated in the
22 disruption of normal mitochondrial function.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 *Intrinsic pathway* 39

40
41 The intrinsic apoptotic pathway results in mitochondrial dysfunction due to an
42 increase in intracellular stress. Hydrophobic bile acids have been shown to inhibit the
43 electron transport chain by decreasing the activity of complexes I, III and IV, resulting in a
44 decrease in state 3 respiration and a concomitant generation of ROS.⁷⁶⁻⁸¹ It has been proposed
45 that the inhibition of complex III leads to a subsequent electron leak through the ubiquinone-
46 complex III site and a concomitant ROS generation.^{80,81} The increased oxidant stress may
47 then cause the induction of the MPT by oxidation of the thiol sites on the membrane
48 permeability transition pore.⁷⁹ Induction of the MPT triggers the release of cytochrome c,
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 thus, stimulating the translocation of Bax to the mitochondrial membrane, stimulating further
4
5 release of cytochrome c.⁸²⁻⁸⁴ Cytochrome c is also able to initiate downstream caspase
6
7 activation events discussed below.^{77,78,81,84,85}
8
9

10 *Extrinsic pathway*

11
12
13 Mitochondrial dysfunction and apoptosis initiated via the extrinsic pathway results
14
15 from extracellular signals triggering downstream caspase activity. The oxidative stress
16
17 generated by bile acids induces an increased presentation of Fas receptor within the plasma
18
19 membrane, following phosphorylation of the Fas receptor by the epidermal growth factor
20
21 receptor.^{78,86,87} Upon presentation of Fas within the plasma membrane Fas agonists can
22
23 interact with the receptor, initiating the formation of the death-inducing signalling complex
24
25 (DISC) and subsequent activation of caspase-8. In turn, caspase-8 activates caspases-3 and -7
26
27 triggering a caspase cascade that culminates in apoptosis.^{78,84-86,88} Additionally, caspase-8 can
28
29 initiate the intrinsic pathway via proteolytic cleavage of Bid. Truncated Bid activates Bax and
30
31 Bak proteins present on the mitochondria via oligomerisation and induction of MPT. The
32
33 activated Bak and Bax proteins form channels within the mitochondria releasing additional
34
35 cytochrome c. Cytosolic cytochrome c causes the assembly of apoptotic protease-activating
36
37 factor-1 (APAF-1) and caspase-9, thus, activating caspase-9. Upon caspase-9 activation a
38
39 proteolytic caspase cascade is initiated ultimately leading to cell death.^{84,85} The generation of
40
41 ROS, induction of MPT and activation of the caspase cascade seem to be essential steps
42
43 within both pathways to initiate mitochondrial perturbation and apoptosis. Therefore, it seems
44
45 likely that both pathways work synergistically to induce mitochondrial dysfunction.
46
47
48
49
50

51 Category 7: Antihistaminic, antipsychotic and antiemetic drugs

52
53
54
55 Phenothiazines are a group of heterocyclic chemicals composed of a nitrogen and a
56
57 sulphur atom joining two benzene rings. These chemicals are widely used in the treatment of
58
59
60

1
2
3 mental disorders, such as schizophrenia, psychosis, and anxiety, as well as conferring
4
5 antihistaminic and antiemetic action. This category comprises seven chemicals:
6
7 chlorpromazine, fluphenazine, mequitazine, methdilazine, promethazine, thiethylperazine,
8
9 and trimeprazine: five of which have been identified have been identified as being non-toxic,
10
11 whilst the remaining two have been shown to be toxic (Table 1). It is important to rationalise
12
13 the mixed toxicity results for the chemicals within this category. A number of studies in the
14
15 literature report toxicity induced by chlorpromazine and fluphenazine that corroborate the data
16
17 in the current study (obtained from Zhang *et al*).^{7,51,89-92}
18
19

20
21 Both chlorpromazine and fluphenazine have been observed to inhibit mitochondrial
22
23 respiration within brain and liver tissues⁹³ This toxicity was induced by binding to, and
24
25 inhibiting, Complex I of the electron transport chain.^{24,27,51} Further investigation revealed that
26
27 chlorpromazine is also capable of impairing mitochondrial function by inhibiting Complex
28
29 IV and acting as an uncoupler of oxidative phosphorylation.^{51,94,95} It has been found that the
30
31 addition of the chlorine atom increases and alters the mechanism by which mitochondrial
32
33 toxicity occurs, i.e. chlorpromazine acts as an uncoupler of oxidative phosphorylation at low
34
35 concentrations and an electron transport chain inhibitor at higher concentrations.⁹⁴ Each
36
37 chemical within this category contains a phenothiazine fragment. This class of drugs were
38
39 found to cause toxicity towards mitochondria by inhibiting oxidative phosphorylation within
40
41 liver mitochondria⁹⁶. Due to this conserved fragment it can be hypothesised that the other
42
43 chemicals within this category elicit their toxicity via a similar mechanism. Research into
44
45 promethazine has shown that it can impede both state 3 and state 4 respiration and
46
47 intramitochondrial potassium ion compartmentalisation at high and low concentrations.^{94,95}
48
49 Work carried out within Terada demonstrated that an acid dissociable group, bulky
50
51 hydrophobic moiety and strong electron-withdrawing group are essential for the induction of
52
53 uncoupling this conclusion can support why this class of drugs may act as uncouplers.
54
55
56
57
58
59
60

1
2
3 Further investigation into chlorpromazine reveals that this chemical elicits its electron
4 transport chain inhibitor action by inhibiting Complex V of the electron transport chain.
5
6 Based upon information in the literature both the tertiary amine moiety and the phenothiazine
7 fragment with an associated electron-withdrawing group are required in order to initiate
8 uncoupling of oxidative phosphorylation.⁵³
9
10
11
12
13
14
15

16 Category 8: β -blockers

17
18
19 Alprenolol, propranolol and atenolol are a group of (non-)selective β -blockers used in
20 the treatment of hypertension. As can be seen in Table 1 two chemicals, atenolol and
21 propranolol, were reported within Zhang *et al* as inducing mitochondrial toxicity, whilst the
22 remaining chemical, alprenolol, was reported as being negative for mitochondrial toxicity.
23
24 Propranolol has been seen to inhibit, via non-competitive binding, Complex V of the
25 respiratory chain.^{97,98} Chemicals that inhibit Complex V can do so by binding to one of two
26 subunits (F_0/F_1) that comprises the ATP synthase enzyme, thus blocking the passage of
27 protons back into the mitochondrial matrix⁹⁸. Together the membrane-bound F_0 and matrix
28 protruding F_1 subunits are responsible for catalysing both the synthesis and hydrolysis of
29 ATP. Wei *et al* have described previously that propranolol binds to the Mg^{2+} -ATPase (F_0
30 subunit) of Complex V inhibiting state 3 respiration.⁹⁸ It has also been seen that the potency
31 of ATPase inhibition induced by propranolol is of the same order of magnitude as its ability
32 to inhibit other membrane-bound enzymes.⁹⁷ Therefore, this inhibitory effect induced by
33 propranolol is due to its membrane stabilising activity and its ability to bind to the lipophilic
34 F_0 subunit of Complex V. In contrast, atenolol, a relatively more hydrophilic drug, has been
35 shown to act via stimulating Complex V activity. The decrease in lipophilicity and, therefore,
36 a decrease in ability to penetrate and interact with membrane macromolecules is pertinent to
37 the decrease in inhibitory potency of atenolol⁹⁷. Additionally, results from Almotrefi suggest
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 that atenolol may interact with the more hydrophilic subunit (F₁) of Complex V, resulting in
4
5 mitochondrial toxicity by stimulating the hydrolysis of ATP to ADP and inorganic
6
7 phosphate.⁹⁷ Further research into how propranolol and atenolol is required in order to
8
9 establish a clear structure-activity relationship for these structurally similar, β -blockers, given
10
11 that they appear to have differing mechanisms of mitochondrial toxicity.
12
13

14 15 16 17 18 *Development of an in silico profiler for mitochondrial toxicity*

19
20
21 The ability to predict organ-level toxicity will become increasingly important to the
22
23 long term goal of replacing animal use in determining a Lowest Observed (Adverse) Effect
24
25 Level (LO(A)EL). Traditionally, LO(A)ELs are identified after undertaking a 28- or 90-day
26
27 repeated dose study, with the lowest dose initiating a treatment related adverse effect in an
28
29 organ(s) producing the LO(A)EL value. However, as no animal testing is permissible for
30
31 cosmetic ingredients in Europe alternatives are required. As discussed previously this has led
32
33 to increased interest in the understanding of toxicity pathways and in the development of
34
35 AOPs. As such the structural alerts that have been developed in this study are intended for
36
37 use in chemical risk assessment within the AOP paradigm. Within the AOP paradigm one of
38
39 the roles of computational toxicology is to define the chemistry associated with key MIEs as
40
41 an *in silico* profiler. To this end the mechanistic chemistry outlined in the current study was
42
43 combined with that previously published by Naven *et al* enabling 17 structural alerts to be
44
45 defined (Table 2).²² Briefly, Naven and co-workers analysed a database of more than 2000
46
47 compounds resulting in the identification of eleven structural alerts associated with the
48
49 uncoupling of oxidative phosphorylation (which was defined by an increase in basal
50
51 respiration). The two sets of structural alerts are, in the most part, complimentary with only
52
53 the thiazolinedione alert being identified in both studies. In addition, the majority of the
54
55
56
57
58
59
60

1
2
3 combined set of alerts identified relate to protonophoric mechanisms, with only two alerts for
4
5 redox cycling (naphthoquinones and anthracene-9,10-diones) and only a single alert related to
6
7 the inhibition of Complexes I-IV (phenothiazines). There is a clear need for additional
8
9 structure-activity studies to define the chemical space for both these mechanisms, especially
10
11 for chemicals capable of inhibiting Complexes I-IV. It should be noted that due to the
12
13 limitations of the data available this study does not aim to create a profiler that would be used
14
15 for pharmaceutical risk assessment, this study has highlighted the need for more
16
17 mitochondrial chemical testing.
18
19

20
21 Table 2: Summary of the structural alerts identified in the current study and from the work of
22
23 Naven *et al.* For the structural alerts acting as protonophores the acidic hydrogen is as
24
25 explicitly drawn.²²
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

AOP development

1
2
3
4
5
6 Recently the development of AOPs has become an integral part of 21st century
7
8 toxicology, with the availability of the OECD AOP knowledgebase making AOP
9
10 development a worldwide focus.⁹⁹ In order for mitochondria-mediated AOPs to be developed,
11
12 further investigation into the organ(s) affected is required; this was, however, beyond the
13
14 scope of the current study. The main outcome from the current study is that the structural
15
16 alerts defined enable chemicals to be grouped into mechanistically-based categories based
17
18 around the knowledge of a number of key MIEs for mitochondrial toxicity. The resulting
19
20 categories can thus be used for either prioritisation of chemicals for further *in vitro* testing or,
21
22 where sufficient *in vivo* data exist, for read-across predictions of organ-level toxicity (from,
23
24 for example, repeat dose toxicity testing). In terms of predicting organ-level toxicity in the
25
26 future it is likely that additional steps in the AOP will need to be investigated within *in vitro*
27
28 assays using a range of organ specific cell lines. For example, the use of primary human renal
29
30 proximal tubule epithelial cells in the MTT assays to investigate nephrotoxicity due to
31
32 mitochondrial dysfunction. This will enable a mechanistically-based weight of evidence to be
33
34 constructed based around the AOP and allow the formation of AOPs based on mitochondrial
35
36 dysfunction. Currently, chemistry-based grouping methods such as those outlined above offer
37
38 the most immediate solution to risk assessment without using animals.
39
40
41
42
43
44

Conclusions

45
46
47 The aim of this study was to develop an *in silico* profiler for mitochondrial toxicity
48
49 based around clearly defined mechanistic information utilising structural similarity and
50
51 chemical category formation. The analysis resulted in the development of eight chemical
52
53 categories and the definition of eight structural alerts. Importantly, these structural alerts were
54
55 derived using mechanistic information in the available literature to elucidate knowledge of a
56
57
58
59
60

1
2
3 number of key Molecular Initiating Events that disrupt the normal functioning of
4 mitochondria. The structural alerts developed within this study have been combined with
5 those from the literature to create a single profiler useful for grouping chemicals into
6 categories, thus, enabling predictions to be made regarding mitochondrial toxicity. It is
7 envisaged that this profiler will help in the formation of a mitochondrial dysfunction AOP.
8
9
10
11
12

13 Funding Information

14
15 The funding from the European Community's Seventh Framework Program (FP7/2007-2013)
16 COSMOS Project under grant agreement n° 266835 and from Cosmetics Europe is gratefully
17 acknowledged.
18
19
20
21
22
23

24 Supporting Information Available

25 The supporting information contains the Zang et al(2010) data set and the structural alerts
26 produced within this study. This material is available free of charge via the internet at
27 <http://pubs.acs.org>.
28
29
30
31
32
33
34
35
36
37

38 Abbreviations

39 Adverse outcome pathway, AOP; Molecular initiating event, MIE; Simplified molecular-
40 input line-entry system, SMILES; Non-steroidal anti-inflammatory drugs, NSAIDs; Reactive
41 oxygen species, ROS; Membrane permeability transition, MPT; Death-inducing signalling
42 complex, DISC; Apoptotic protease-activating factor-1, APAF-1; Lowest observed (adverse)
43 effect level, LO(A)EL.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Zhang H, Chen QY, Xiang ML, Ma CY, Huang Q, Yang SY (2009) In silico prediction of mitochondrial toxicity by using GA-CG-SVM approach. *Toxicol In Vitro* 23:134-140
2. EC (2006a) Corrigenda of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) *Official Journal of the European Union* EC 1907/2006
3. EC (2006b) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) *Official Journal of the European Union* EC 1907/2006
4. ECHA (2008) Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals.
5. ECHA (2009) How to report read-across and categories:1-30
6. ECHA (2010) Practical guide 6: How to report read-across and categories.
7. EC(2003) Directive 2003/15/EC of the European Parliament and of the Council (7th Amendment) *Official Journal of the European Union*
8. Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E, Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S, Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R, Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhrer S, Phrakonkham P, Piersma A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tähti H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar JM.(2011) Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010. *Arch Toxicol* 85:367-485
9. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung M.W, Johnson R.D, Mount D.R, Nichols J.W., Russom C.L., Schmieder P.K, Serrano J.A., Tietge J.E., Villeneuve D.L (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29:730-741
10. OECD (2012) Guidance on developing and assessing Adverse Outcome Pathways.
11. Vinken M (2013a) The adverse outcome pathway concept: a pragmatic tool in toxicology. *Toxicology* 312:158-165
12. Vinken M, Whelan M, and Rogiers V (2014) Adverse outcome pathways: hype or hope? *Arch Toxicol* 88:1-2
13. Enoch SJ, and Cronin MTD (2010) A review of the electrophilic reaction chemistry involved in covalent DNA binding.

1
2
3 *Crit Rev Toxicol* 40:728-748

4
5 14. Enoch SJ, Ellison CM, Schultz TW and Cronin MTD (2011) A review of the electrophilic reaction chemistry involved in
6 covalent protein binding relevant to toxicity. *Crit Rev Toxicol* 41:783-802

7
8
9 15. Hewitt M, Enoch SJ, Madden JC, Przybylak KR, and Cronin MTD (2013) Hepatotoxicity: a scheme for generating
10 chemical categories for read-across, structural alerts and insights into mechanism(s) of action. *Crit Rev Toxicol* 43:537-558

11
12 16. Przybylak KR, and Schultz TW (2013) Informing chemical categories through the development of Adverse Outcome
13 Pathways. In: Cronin MTD, Madden, J. C., Enoch, S. J., and Roberts, D. W. (ed) Chemical Toxicity Prediction: Category
14 Formation and Read-Across, vol 17. The Royal Society of Chemistry, Cambridge, UK

15
16
17 17. OECD QSAR Toolbox, www.qsartoolbox.org

18
19 18. Gutsell S, and Russell P (2013) The role of chemistry in developing understanding of adverse outcome pathways and their
20 application in risk assessment. *Toxicol Res* 2:299

21
22 19. Enoch SJ, Madden JC, Cronin MTD (2008) Identification of mechanisms of toxic action for skin sensitisation using a
23 SMARTS pattern based approach. *SAR QSAR Environ Res* 19:555-578

24
25
26 20. Sakuratani Y, Zhang HQ, Nishikawa S, Yamazaki K, Yamada T, Yamada J, Gerova K, Chankov G, Mekenyan O, Hayashi
27 M. I (2013) Hazard Evaluation Support System (HESS) for predicting repeated dose toxicity using toxicological categories.
28 *SAR QSAR Environ Res* 24:351-363

29
30 21. Vinken M, Landesmann B, Goumenou M, Vinken S, Shah I, Jaeschke H, Willett C, Whelan M, Rogiers V. (2013b)
31 Development of an adverse outcome pathway from drug-mediated bile salt export pump inhibition to cholestatic liver injury.
32 *Toxicol Sci* 136:97-106

33
34 22. Naven RT, Swiss R, Klug-McLeod J, Will Y, and Greene N (2013) The development of structure-activity relationships for
35 mitochondrial dysfunction:phosphorylation. *Toxicol Sci* 131:271-278

36
37 23. Wallace KB, Kissling GE, Melnick RL, Blystone CR (2013) Structure-activity relationships for perfluoroalkane-induced
38 in vitro interference with rat liver mitochondrial respiration. *Toxicol Lett* 222:257-264

39
40 24. Nadanaciva S, and Will Y (2011) New insights in drug-induced mitochondrial toxicity. *Curr Pharm Design* 17:2100-2112

41
42 25. Dykens JA, and Will Y (2008b) Drug-induced Mitochondrial Dysfunction. John Wiley and Sons, Inc., Hoboken, New
43 Jersey, USA

44
45
46 26. Brunmair B, Staniek K, Gras F, Scharf N, Althaym A, Clara R, Roden M, Gnaiger E, Nohl H, Waldhäusl W, Fürnsinn

- 1
2
3 C.(2004) Thiazolidinediones, like metformin, inhibit respiratory complex I. A common mechanism contributing to their
4 antibiabetic actions? *Diabetes* 53:1052-1059
5
6
7 27. Chan K, Truong D, Shangari N, and O'Brien PP (2005) Drug-induced mitochondrial toxicity. *Expert Opin Drug Met*
8 *1*:655-669
9
10
11 28. Dykens JA, and Will Y (2007a) The significance of mitochondrial toxicity testing in drug development *Drug Discov*
12 *Today* 12:777-785
13
14
15 29. Dykens JA, Marroquin LD, and Will Y (2007b) Strategies to reduce late-stage drug attrition due to mitochondrial toxicity.
16 *Expert Rev Mol Diagn* 7:161-175
17
18
19 30. Rolo AP, Palmeira CM, Holy JM, and Wallace KB (2004) Role of mitochondrial dysfunction in combined bile acid-
20 induced cytotoxicity: The switch between apoptosis and necrosis. *Toxicol Sci* 79:196-204
21
22
23 31. Wallace KB, and Starkov AA (2000) Mitochondrial targets of drug toxicity. *Ann Rev Pharm Toxicol* 40:353-388
24
25
26 32. Cohen BH, and Gold DR (2001) Mitochondrial cytopathy in adults: What we know so far. *Clev Clin J Med* 68:625-642
27
28
29 33. Pieczenik SR, and Neustadt J (2007) Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol*
30 83:84-92
31
32
33 34. Hatefi Y (1985) The mitochondrial electron transport and oxidative phosphorylation system. *Annu Rev Biochem* 54:1015-
34 1069
35
36
37 35. Roberts D.W, Patlewicz G, Kern PS, Gerberick F, Kimber I, Dearman RJ, Ryan CA, Basketter DA, Aptula AO (2007)
38 Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem Res Toxicol.*
39 (7):1019-30.
40
41
42 36. Amacher DE (2005) Drug-associated mitochondrial toxicity and its detection. *Curr Med Chem* 12:1829-1839
43
44
45 37. Krahenbuhl S (2001) Mitochondria: important target for drug toxicity? *J Hepatol* 34:334-336
46
47
48 38. Dykens JA (2008a) Preface. In: Dykens JA, and Will, Y. (ed) *Drug-Induced Mitochondrial Dysfunction*. John Wiley and
49 Sons, Inc., Hoboken, New Jersey, USA
50
51
52 39. Cela O, Piccoli C, Scrima R, Quarato G, Marolla A, Cinnella G, Dambrosio M, Capitano N. (2010) Bupivacaine
53 uncouples the mitochondrial oxidative phosphorylation, inhibits respiratory chain complexes I and III and enhances ROS
54 production: results of a study on cell cultures. *Mitochondrion* 10:487-496
55
56
57
58
59
60

- 1
2
3 40. Schonfeld P, Sztark F, Slimani M, Dabadie P, and Mazat JP (1992) Is bupivacaine a decoupler, a protonophore or a proton-
4 leak-inducer. *FEBS Letters* 304:273-276
5
6
7 41. Spycher S, Smejtek P, Netzeva TI, and Escher BI (2008) Towards a class independent Quantitative Structure-Activity
8 Relationship model for uncouplers of oxidative phosphorylation. *Chem Res Toxicol* 21:911-927
9
10
11 42. Sun X, and Garlid KD (1992) On the mechanism by which bupivacaine conducts protons across the membranes of
12 mitochondria and liposomes. *J Biol Chem* 267:19147-19154
13
14
15 43. Terada H (1990a) Uncouplers of oxidative phosphorylation. *Environ Health Persp* 87:213-218
16
17
18 44. Kroemer G, Galluzzi L, and Brenner C (2007) Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87:99-
19 163
20
21
22 45. Lemasters JJ, Theruvath TP, Zhong Z, and Nieminen AL (2009) Mitochondrial calcium and the permeability transition in
23 cell death. *Biochim Biophys ACTA* 1787:1395-1401
24
25
26 46. Pessayre D, Berson A, and Fromenty B (2008) Features and mechanisms of drug-induced liver injury. In: Dykens JA, and
27 Will, Y (ed) Drug-Induced Mitochondrial Dysfunction. John Wiley and Sons, Inc., Hoboken, New Jersey, USA
28
29
30 47. ChemSpider <http://www.chemspider.com/>
31
32
33 48. Daylight, www.daylight.com
34
35
36 49. Dabadie P, Bendriss P, Erny P, and Mazat JP (1987) Uncoupling effects of local anesthetics on rat liver mitochondria.
37 *FEBS Letters* 226:77-82
38
39
40 50. Dippenaar JM (2007) Local anaesthetic toxicity. *SAJAA* 13:23-28
41
42
43 51. Mehta R, Chan K, Lee O, Tafazoli S, and O'Brien PJ (2008) Drug-associated mitochondrial toxicity. In: Dykens JA, and
44 Will, Y (ed) Drug-Induced Mitochondrial Dysfunction. John Wiley and Sons, Inc., Hoboken, New Jersey, USA
45
46
47 52. Sztark F, Ouhabi R, Dabadie P, and Mazat JP (1997) Effects of the local aesthetic bupivacaine on mitochondria energy
48 metabolism, change from uncoupling to decoupling depending on the respiration state. *Biochem Mol Biol Int* 43:997-1003
49
50
51 53. Terada H, Shima O, Yoshida K, and Shinohara Y (1990b) Effects of the local anaesthetic bupivacaine on oxidative
52 phosphorylation in mitochondria. Change from decoupling to uncoupling by formation of a leakage type ion pathway specific
53 from H⁺ in cooperation with hydrophobic anions. *J Biol Chem* 265:7837-7842
54
55
56 54. Nadanaciva S, Dykens JA, Bernal A, Capaldi RA, and Will Y (2007b) Mitochondrial impairment by PPAR agonists and
57
58
59
60

- 1
2
3 statins identified via immunocaptured OXPHOS complex activities and respiration. *Toxicol Appl Pharm* 223:277-287
4
5
6 55. Masubuchi Y (2006) Metabolic and non-metabolic factors determining troglitazone hepatotoxicity: A review. *Drug Metab*
7
8 *Pharmacok* 21:347-356
9
10 56. Boelsterli U (2002) Mechanisms of NSAID-induced hepatotoxicity: Focus on Nimesulide. *Drug Safety* 25:633-648
11
12 57. Masubuchi Y, Saito H, and Horie T (1998) Structural requirements for the hepatotoxicity of nonsteroidal anti-
13
14 inflammatory drugs in isolated rat hepatocytes. *J Pharmacol Exp Ther* 287:208-213
15
16 58. Moreno-Sanchez R, Bravo C, Vasquez C, Ayala G, Silveira LH, and Martinez-Lavin M (1999) Inhibition and uncoupling
17
18 of oxidative phosphorylation by Nonsteroidal Anti-Inflammatory Drugs. *Biochem Pharmacol* 57:743-752
19
20 59. Siraki AG, Chevaldina T, and O'Brien PJ (2005) Application of quantitative structure-toxicity relationships for acute
21
22 NSAID cytotoxicity in rat hepatocytes. *Chem-Biol Interact* 151:177-191
23
24 60. Uyemura SA, Santos AC, Mingatto FE, Jordani MC, and Curti C (1997) Diclofenac sodium and mefenamic acid, potent
25
26 inducers of the membrane permeability transition in renal cortex mitochondria. *Arch Biochem Biophys* 342:231-235
27
28 61. Wallace KB (2003) Doxorubicin-induced cardiac mitochondrionopathy. *Pharmacol Toxicol* 93:105-115
29
30
31 62. Gerwitz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the
32
33 anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol* 57:727-741
34
35 63. Kappus H (1986) Overview of enzyme systems involved in bioreduction of drugs and in redox cycling. *Biochem*
36
37 *Pharmacol* 35:1-6
38
39 64. Kim J-S, He L, and Lemasters JJ (2003) Mitochondrial permeability transition: a common pathway to necrosis and
40
41 apoptosis.:463-470
42
43 65. Ohkuma Y, Hiraku Y, and Kawanishi S (2001) Sequence specific DNA damage induced by carcinogenic danthron and
44
45 anthraquinone in the presence of Cu(II), cytochrome P450 reductase and NADPH. *Free Radical Res* 34:595-604
46
47 66. Nixon G.L, Pidathala C, Shone A.E, Antoine T, Fisher N, O'Neill P.M, Ward S.A, Biagini G.A (2013) Targeting the
48
49 mitochondrial electron transport chain of Plasmodium falciparum: new strategies towards the development of improved
50
51 antimalarials for the elimination era. *Future Med Chem.* ;5(13):1573-91. doi: 10.4155/fmc.13.121.
52
53 67. Schnellmann RG (1990a) The cellular effects of a unique pesticide sulfluramid (N-ethylperfluorooctane sulphonamide) on
54
55 rabbit renal proximal tubules. *Toxicol in Vitro* 4:71-74
56
57
58
59
60

- 1
2
3 68. Schnellmann RG, and Manning RO (1990b) Perfluorooctane sulfonamide: a structurally novel uncoupler of oxidative
4 phosphorylation. *Biochem Biophys ACTA 1016*:344-348
5
6
7 69. Starkov AA, and Wallace KB (2002) Structural determinants of fluorochemical-induced mitochondrial dysfunction.
8 *Toxicol Sci 66*:244-252
9
10
11 70. Keller BJ, Yamanaka H, and Thurman RG (1992) Inhibition of mitochondrial respiration and oxygen-dependent
12 hepatotoxicity by six structurally dissimilar peroxisomal proliferating agents. *Toxicology 71*:49-61
13
14
15 71. Langley AE (1990) Effects of perfluoro-n-decanoic acid on the respiratory activity of isolated rat liver mitochondria. *J*
16 *Toxicol Environ Health 29*:329-336
17
18
19 72. Kleszczynski K, Stepnowski P, and Skladanowski AC (2009) Mechanism of cytotoxic action of perfluorinated acids II.
20 Disruption of mitochondrial bioenergetics. *Toxicol Appl Pharmacol 235*:182-190
21
22
23 73. Battaglia V (2005) Oxidative Stress Is Responsible for Mitochondrial Permeability Transition Induction by Salicylate in
24 Liver Mitochondria. *J Biol Chem 280*:33864-33872
25
26
27 74. Kowaltowski AJ, Castilho RF, Vercesi AE (2001) Mitochondrial permeability transition and oxidative stress. *FEBS Letters*
28 *495*:12-15
29
30
31 75. Kleszczynski K, and Skladanowski AC (2011) Mechanism of cytotoxic action of perfluorinated acids. III. Disturbance in
32 Ca(2)+ homeostasis. *Toxicol Appl Pharmacol 251*:163-168
33
34
35 76. Krahenbuhl S, Talos C, Fischer S, and Reichen J (1994) Toxicity of bile acids on the electron transport chain of isolated rat
36 liver mitochondria. *Hepatology 19*:471-479
37
38
39 77. Palmeira CM, and Rolo AP (2004) Mitochondrially-mediated toxicity of bile acids. *Toxicology 203*:1-15
40
41
42 78. Perez MJ (2009) Bile-acid-induced cell injury and protection. *World J Gastroentero 15*:1677
43
44
45 79. Sokol RJ, Straka MS, Dahl R, Devereaux MW, Yerushalmi B, Gumprich E, Elkins N, Everson G. (2001) Role of oxidant
46 stress in the permeability transition induced in rat hepatic mitochondria by hydrophobic bile acids. *Pediatr Res 49*:519-531
47
48
49 80. Winkhofer-Roob BM, McKim JM, Jr, Devereaux MW, and Sokol RJ (1996) Characterization of site of reactive oxygen
50 species (ROS) generation in mitochondria exposed to glycochenodeoxycholic acid [Abstract] *Hepatology 24*(part2):338A
51
52
53 81. Yerushalmi B, Dahl R, Devereaux MW, Gumprich E, and Sokol RJ (2001) Bile acid-induced rat hepatocyte apoptosis is
54 inhibited by antioxidants and blockers of the mitochondrial permeability transition. *Hepatology 33*:616-626
55
56
57
58
59
60

- 1
2
3 82. Rodrigues CMP, Ma X, Linehan-Stieers C, Fan G, Kren BT, and Steer CJ (1999) Ursodeoxycholic acid prevents
4 cytochrome c release in apoptosis by inhibiting mitochondrial membrane depolarisation and channel formation. *Cell Death*
5 *Differ* 6:842-854
6
7
8
9 83. Spivey JR, Bronk SF, and Gores GJ (1993) Glycochenodeoxycholate-induced lethal hepatocellular injury in rat
10 hepatocytes. Role of ATP depletion and cytosolic free calcium. *J Clinical Invest* 92:17-24
11
12
13 84. Yin X-M, and Ding WX (2003) Death receptor activation-induced hepatocyte apoptosis and liver injury. *Curr Mol Med*
14 3:491-508
15
16
17 85. Taylor RC, Cullen SP, and Martin SJ (2008) Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol*
18 9:231-241
19
20
21 86. Faubion WA, Guicciardi ME, Miyoshi H, Bronk SF, Roberts PJ, Svingen PA, Kaufmann SH, Gores GJ. I (1998) Toxic
22 bile salts induce rodent hepatocyte apoptosis via direct activation of Fas. *J Clin Invest* 103:137-145
23
24
25 87. Qiao L, Studer E, Leach K, McKinstry R, Gupta S, Decker R, Kukreja R, Valerie K, Nagarkatti P, El Deiry W, Molkentin
26 J, Schmidt-Ullrich R, Fisher PB, Grant S, Hylemon PB, Dent P. (2001) Deoxycholic Acid (DCA) causes ligand-independent
27 activation of Epidermal Growth Factor Receptor (EGFR) and Fas receptor in primary hepatocytes: Inhibition of
28 EGFR/Mitogen-activated protein kinase-signalling module enhances DCA-induced apoptosis. *Mol Biol Cell* 12:2609-2645
29
30
31 88. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, and Lemasters JJ (2002) Mechanisms of Hepatotoxicity.
32 *Toxicol Sci* 65:166-176
33
34
35 89. Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol
36 oxidation. *Neuropharmacology* 38:567-577
37
38
39 90. Lucas-Heron B, Schmitt N, Ollivier B (1994) Mdx mouse skeletal muscle: Could a mitochondrial factor be responsible for
40 the absence of progressive necrosis? *Neuroscience* 129:97-100
41
42
43 91. Nadanaciva S, Bernal A, Aggeler R, Capaldi R, and Will Y (2007a) Target identification of drug induced mitochondrial
44 toxicity using immunocapture based OXPHOS activity assays. *Toxicol In Vitro* 21:902-911
45
46
47 92. Saito K, Kakei M, Uchimura S, Kashima T, and Tanaka H (1982) Toxic effects of chlorpromazine on red and white
48 muscles in rats: an ultrastructural study. *Toxicol Appl Pharm* 65:347-353
49
50
51 93. Guth PS, and Sprites MA (1964) The phenothiazine tranquilizers: biochemical and biophysical actions. *Int Rev Neurobiol*
52 7:231-278
53
54
55
56
57
58
59
60

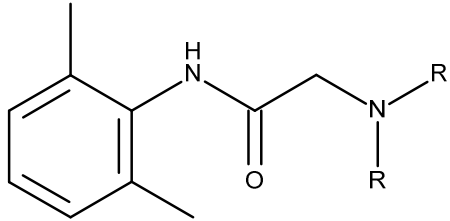
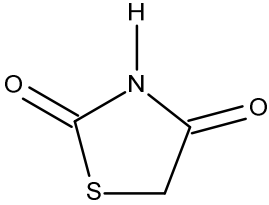
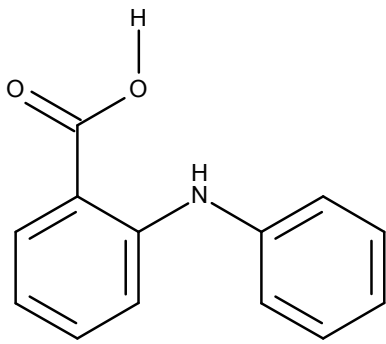
- 1
2
3 94. Eto K, Fukuda T, Araki Y, Inoue B, and Ogata M (1985) Effect of tricyclic drugs on mitochondrial membrane. *Acta Med*
4 *Okayama* 39:289-295
5
6
7 95. Matsubara T, and Hagihara B (1968) Action mechanism of phenothiazine derivatives on mitochondrial respiration. *J*
8 *Biochem* 63:156-164
9
10
11 96. Gallagher CH, Koch JH, and Mann DM (1965) The effect of phenothiazine on the metabolism of liver mitochondria.
12 *Biochem Pharmacol* 14:789-797
13
14
15 97. Almotrefi A, and Dzimir N (1992) Effect of beta-adrenoceptor blockers on mitochondrial ATPase activity in guinea pig
16 heart preparations. *Eur J Pharmacol* 215:231-236
17
18
19 98. Wei Y-H, Lin TN, Hong CY, and Chiang B (1985) Inhibition of the mitochondrial Mg²⁺-ATPase by propranolol. *Biochem*
20 *Pharmacol* 34:911-917
21
22
23 99. AOP-KB (2015) The OECD webpage describing AOPs. [http://www.oecd.org/chemicalsafety/testing/adverse-outcome-](http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm)
24 [pathways-molecular-screening-and-toxicogenomics.htm](http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm). Accessed on 2nd September 2015
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

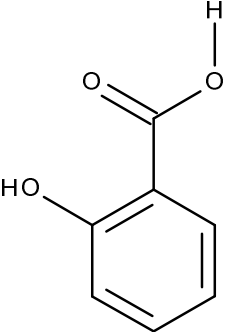
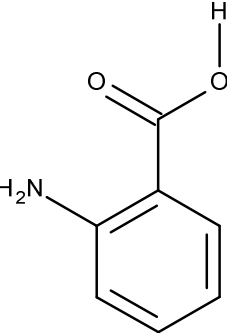
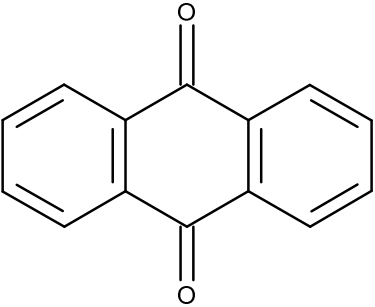
Tables

Table 1

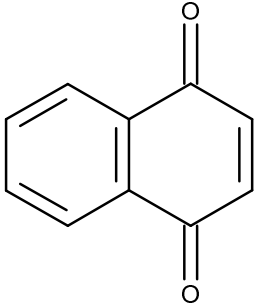
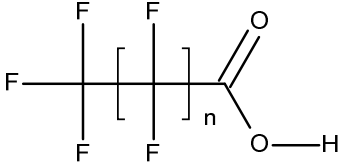
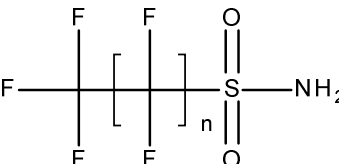
Category	Name	Mitochondrial toxicity
1 (Local anaesthetics, antianginal and antiarrhythmic)	Lidocaine	Positive
	Bupivacaine	Positive
	Etidocaine	Positive
	Ropivacaine	Positive
	Ranolazine	Positive
	Tocainide	Negative
2 (anti-diabetic drugs)	Rosiglitazone	Positive
	Pioglitazone	Positive
	Troglitazone	Positive
3 (Nonsteroidal anti-inflammatory drugs)	Mefenamic acid	Positive
	Flufenamic acid	Positive
	Tolfenamic acid	Positive
4 (Anthracycline antibiotics)	Daunorubicin	Positive
	Doxorubicin	Positive
	Epirubicin	Positive
	Idarubicin	Positive
5 (Hypolipodemic drugs)	Perfluorodecanoic acid	Positive
	Perfluorooctanoic acid	Positive
	Perfluorooctane-sulphonamide	Positive
6 (Bile acids)	Cholic acid	Positive
	Chenodeoxycholic acid	Positive
	Deoxycholic acid	Positive
	Glycocholic acid	Positive
	Lithocholic acid	Positive
	Taurocholic acid	Positive
7 (Antihistaminic, antipsychotic and antiemetic drugs)	Promethazine	Negative
	Chlorpromazine	Positive
	Fluphenazine	Positive
	Mequitazine	Negative
	Methdilazine	Negative
	Thiethylperazine	Negative
	Trimeprazine	Negative
8 (β -blockers)	Alprenolol	Negative
	Atenolol	Positive
	Propranolol	Positive

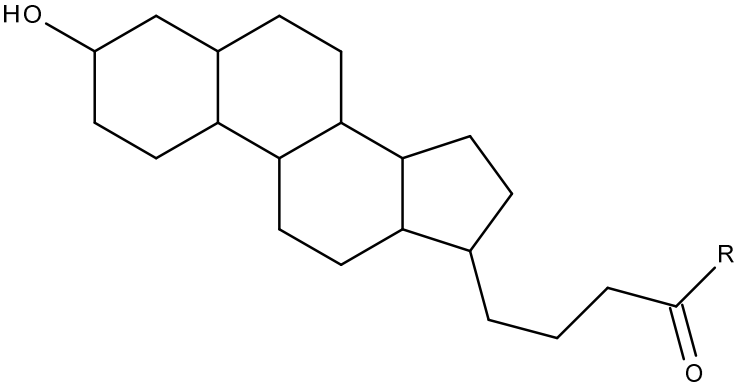
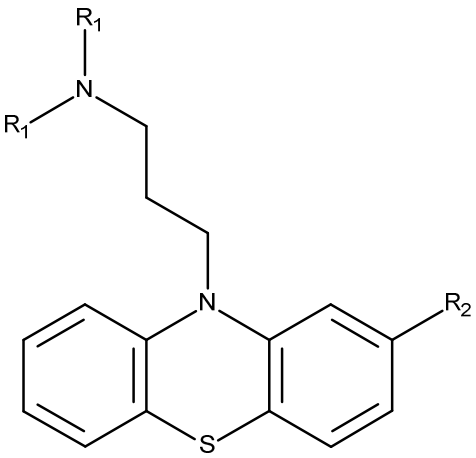
Table 2

Structural alert	Source	Mechanism	Structural Alert	Category (Table 1)
2-(Dialkylamino)-N-(2,6-dimethylphenyl)acetamides	Current study	Protonophore	 <p>R = alkyl carbon</p>	1
Thiazolidinediones	Current study ²²	Protonophore		2
2-Anilinobenzoic acids	Current study	Protonophore		3

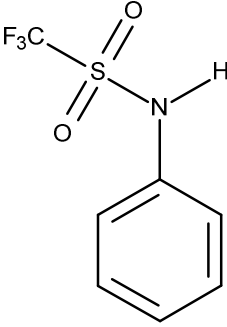
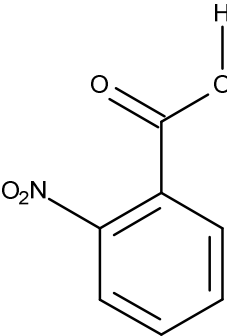
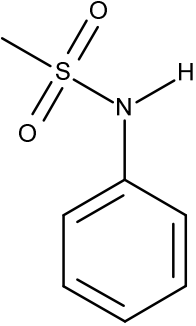
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	Salicylates	22	Protonophore		expands 3
17 18 19 20 21 22 23 24 25 26 27 28	Anthranilic acids	22	Protonophore		expands 3
29 30 31 32 33 34 35 36 37 38 39 40	Anthracene-9,10-diones	Current study	Redox cycling		4

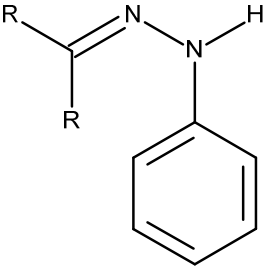
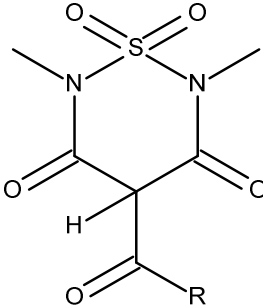
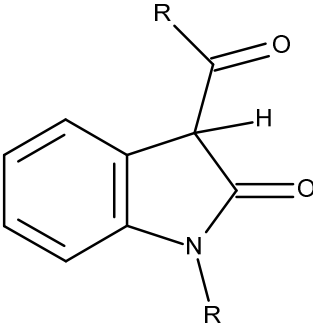
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Naphthoquinones	22	Redox cycling		expands 4
Perfluorinated-carboxylic-acids	Current study	Protonophore	 n = 5-11	5a
Perfluorinated-sulphonamides	Current study	Protonophore	 n = 5-11	5b

Bile acids	Current study	Unclear	 <p>R = alkyl carbon</p>	6
Phenothiazines	Current study	Inhibition of Complexes I-IV	 <p>R₁ = CH₂, CH₃ R₂ = Cl, CF₃</p>	7

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Fluoromethylsulphanilides	22	Protonophore		
Nitrophenols	22	Protonophore		
Nitrosulphonanilides	22	Protonophore		

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	Phenylhydrazones	22	Protonophore	 R = any carbon	
17 18 19 20 21 22 23 24 25 26 27	Thiadiazinedione dioxides	22	Protonophore	 R = any carbon	
28 29 30 31 32 33 34 35 36 37 38 39 40 41	Acylindolones	22	Protonophore	 R = alkyl carbon	

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49