Dicoumarol: a drug which hits at least two very different targets in vitamin K metabolism

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Abstract

Dicoumarol, a symmetrical *biscoumarin* can be considered as the “parent” of the widely used anticoagulant drug, warfarin. The discovery of dicoumarol’s bioactive properties resulted from an investigation into a mysterious cattle disease in the 1940s. It was then developed as a pharmaceutical, but was superseded in the 1950s by warfarin. Both dicoumarol and warfarin antagonise the blood clotting process through inhibition of vitamin K epoxide reductase (VKOR). This blocks the recycling of vitamin K and prevents the γ -carboxylation of glutamate residues in clotting factors. VKOR is an integral membrane protein and our understanding of the molecular mechanism of action of dicoumarol and warfarin is hampered by the lack of a three dimensional structure. There is consequent controversy about the membrane topology of VKOR, the location of the binding site for coumarin inhibitors and the mechanism of inhibition by these compounds. Dicoumarol (and warfarin) also inhibit a second enzyme, NAD(P)H quinone oxidoreductase 1 (NQO1). This soluble, cytoplasmic enzyme may also play a minor role in the recycling of vitamin K. However, its main cellular roles as an enzyme appear to be detoxification and the prevention of the build-up of reactive oxygen species. NQO1 is well characterised biochemically and structurally. Consequently, structure-based drug design has identified NQO1 inhibitors which have potential for the development of anti-cancer drugs. Many of these compounds are structurally related to dicoumarol and some have reduced “off target” effects. Therefore, it is possible that dicoumarol will become the “parent” of a second group of drugs.

Keywords: Warfarin; VKOR; NQO1; quinone cycling; vitamin K; anticoagulant; anti-cancer drug

Introduction: Dicoumarol

Dicoumarol (also dicumarol; 3,3'-methylenebis(4-hydroxy-2*H*-chromen-2-one); CAS # 66-76-2) was first synthesised in 1903 [1]. It was subsequently identified as a fungal metabolite of coumarin and the main cause of sweet clover disease, a haemorrhagic disease of cows and other livestock [2, 3]. The compound is a symmetrical *biscoumarin* and has the potential to exist in three main tautomeric forms (Scheme 1) [4-6]. Further diversity is provided by rotation about the carbon atoms which link the two coumarin units and the possibility of ionisation of the hydroxyl groups [5]. A recent, theoretical, quantum chemical study predicted that dicoumarol will be predominantly in a negatively charged, *syn*-conformation in aqueous solution [7]. This is consistent with the experimentally determined pK_a values (3.9 and 8.0) which predict that approximately 80% of the molecules will be in the singly ionised form at neutral pH [8]. Therefore, it is likely that this form of the compound is the one which predominates in most cells and, therefore, is the one that most commonly interacts with proteins.

Following the discovery that dicoumarol can fatally inhibit clotting in farm animals, it was used pharmacologically as an anticoagulant [9]. It also had some use as a rat poison in agriculture [10]. However, in both applications it was superseded by the related compound warfarin (Scheme 2) [11-15]. Warfarin has the advantage of being more potent than dicoumarol and the sodium salt can be administered orally or by injection [16]. Dicoumarol continued to be used in the laboratory for a variety of applications, including as an uncoupler of the mitochondrial or bacterial membrane proton motive force [17]. In recent years, it has once again been receiving attention as a potential lead molecule in drug discovery – this time for anti-cancer agents, rather than for anticoagulants.

Dicoumarol inhibits VKOR and antagonises blood clotting

Despite their widespread use, the molecular mechanism of action of dicoumarol, warfarin and other coumarin-derived anticoagulants remained elusive for around 40 years. Initial observations demonstrated that the failure of blood clotting resulted from a defect in prothrombin and the effects of

these compounds could be antagonised by vitamin K [18-22]. Over time, it became apparent that these coumarin derivatives act by preventing the vitamin K-dependent γ -carboxylation of glutamate residues in the N-terminal regions of a range of clotting factors including prothrombin, factor VII, factor IX, factor X, protein C and protein S [23-26]. This post-translational modification enables these proteases to bind calcium and anchor themselves to the extracellular side of the cell membranes of platelets and other blood cells [27-32]. This membrane localisation is a critical event in the blood clotting cascade and, thus, a reduction in the extent of γ -carboxylation results in inhibition of clotting [33].

Vitamin K describes a set of related compounds which are a required part of the mammalian diet. In plants, vitamin K₁ (phylloquinone, phytomenadione, or phytonadione; CAS# 84-80-0; Scheme 3) dominates, whereas in animals vitamin K₂ (the menaquinones) is more common. In mammals, gut bacteria can convert vitamin K₁ into menaquinone-4 (CAS# 863-61-6; Scheme 3) [34]. The vitamin is required for the γ -carboxylation of clotting factors. It does not directly donate the carboxyl group, but participates in a cycle in which the vitamin is first reduced to the quinol form [35]. In a series of reactions catalysed by γ -glutamyl carboxylase (GGCX; EC 4.1.1.90), the reduced form of vitamin K is then oxidised by molecular oxygen. The resulting peroxy derivative of the vitamin undergoes rearrangements to form oxy-epoxide vitamin K which can abstract a hydrogen atom from the target protein. The target protein is left negatively charged and reacts with carbon dioxide becoming γ -carboxylated in the process [36]. Vitamin K is left in the oxidised (keto) epoxide form (for structures of the various forms of vitamin K, see Scheme 4). The oxidised form is regenerated by the action of vitamin K epoxide reductase (VKOR; EC 1.1.4.1) [37, 38]. Dicoumarol, warfarin and related compounds inhibit VKOR, thus preventing the recycling of vitamin K, “trapping” the molecule in the epoxide form, blocking the cycle and thus preventing the γ -carboxylation of clotting factors [33, 39-41].

VKOR is a transmembrane protein with three or four membrane spanning regions in the human protein [38, 42-44]. Initially the enzyme was assumed to form part of a larger complex of proteins, hence its alternative name, vitamin K epoxide reductase complex subunit 1 (VKORC1) [38]. No

additional subunits have been definitively identified in humans. However the equivalent bacterial protein has been shown to be fused to DsbA (a protein which catalyses disulphide bond formation) in some species [45]. In mycobacteria, the two proteins may be non-covalently associated and in some species of photosynthetic bacteria VKOR is fused to thioredoxin [46-48]. Despite its relatively small size (18.2 kDa), mammalian VKOR is capable of catalysing two reactions: the conversion of vitamin K epoxide to vitamin K and the reduction of vitamin K to the hydroxy form [49]. The functionally active form of the enzyme is a multimer, most likely a dimer [50].

The catalytic mechanism of epoxide reduction by VKOR involves two cysteine residues (Cys-132 and Cys-135) in the membrane-embedded part of the protein which form a disulphide bond during the course of the reaction [44, 51-53]. The initial step most likely requires the protonation of the epoxide oxygen by one of the two cysteine residues followed by the formation of a covalent enzyme-substrate complex mediated by the second cysteine residue. This carbon-sulphur bond is subsequently broken when a sulphur-sulphur bond between two cysteine residues in VKOR is formed [54]. These two cysteine residues must be re-reduced to regenerate active enzyme; it is assumed that this reduction is catalysed by a second protein, but the identity of this is not known. Warfarin and other coumarins are reported to act as non-competitive inhibitors with respect to vitamin K epoxide [55-57]. True non-competitive inhibitors (i.e. those which affect $V_{\max}$ but not K_m) are considered to be rare [58]. To achieve true non-competitive inhibition, the inhibitor must bind to a site away from the active site with equal affinity to the free enzyme and the enzyme substrate complex [58]. This would be difficult to verify experimentally, but it infers that vitamin K epoxide binding causes little overall change to the conformation of VKOR. Interestingly, warfarin is also reported to act as a competitive inhibitor with respect to the reductant dithiothreitol (DTT) which is used experimentally as the reducing agent in the reaction. Competitive inhibition requires binding at the same site [58]. This suggests that the vitamin K epoxide and reducing agent binding sites may be spatially separated in the enzyme which would require electrons to pass through the enzyme between the two binding sites. It has been postulated that two surface exposed cysteine residues in the cytoplasmic domain may shuttle electrons to the active site in the vitamin K reduction reaction, raising the possibility that something similar

may occur in the reduction of the epoxide [59]. An alternative mechanism has been proposed in which warfarin acts as an irreversible inhibitor of VKOR, forming a covalent adduct with one of the active site cysteine residues [60, 61]. This is consistent with the concept that warfarin (and other coumarins) mimic the action of vitamin K in the reaction cycle and form covalent intermediates with the enzyme. However, there is no direct evidence for a covalent VKOR-warfarin adduct.

A more complete understanding of how warfarin (and other coumarins, including dicoumarol) interacts with VKOR has been hampered by the lack of a high resolution crystal structure of the enzyme. A structure of the equivalent enzyme from the cyanobacterium *Synechococcus* sp. has been solved in complex with thioredoxin (PDB: 3KP9) [47]. The structure revealed four transmembrane helices and a quinone molecule bound in the transmembrane region (Figure 1a). It also suggested an electron transfer pathway in which the thioredoxin subunit first reduces two surface cysteine residues which then transfer their electrons to the active site cysteines. The quinone acts as the final electron acceptor in this model, which is consistent with the enzyme kinetics and non-competitive inhibition evidence described above for mammalian VKOR [47].

There is uncertainty about the membrane topology of mammalian VKOR. Based on its similarity to the cyanobacterial enzyme, it has been suggested that it will have four transmembrane segments [47]. Molecular models based using methods such as Phyre or SwissModel which search the Protein Databank for the most similar proteins and base the models on these will, inevitably, return structures with four transmembrane helices (Figure 1b). However, other experimental and bioinformatics studies suggest that the mammalian enzymes differ and only have three transmembrane segments [42, 43].

There is further uncertainty about the binding site for dicoumarol, warfarin and other coumain inhibitors. Based on the structure of the cyanobacterial enzyme, it was suggested that warfarin binds at the same site as the quinone seen in the structure (and that vitamin K epoxide would also interact at this site) [47]. This is consistent with the locations of some of the mutations which alter the sequence of rat VKOR conferring resistance to warfarin and those which are associated with hereditary clotting

deficiencies in humans [55, 62-67]. Many of these variant forms of VKOR have lower catalytic activity than the wild-type and it is presumed that this loss of fitness is a “price worth paying” for resistance to rat poison [68]. The majority of these mutations affect residues which are predicted to be part of, or close to, the cleft which binds the quinone in the cyanobacterial enzyme (Figure 2). This suggests that the equivalent site in the mammalian enzyme is the binding site for warfarin and, possibly, vitamin K epoxide since the catalytic cysteine residues lie towards the base of this cleft. However, this would predict that warfarin (and other coumarins) should be not be non-competitive inhibitors with respect to vitamin K epoxide.

The uncertainty about the membrane topology and coumarin binding site in VKOR makes molecular modelling studies particularly challenging. Using a homology model based on the cyanobacterial structure, docking with AutoDock Vina as implemented in Chimera [69, 70], predicted that both dicoumarol and warfarin would bind at the base of the cleft with partially overlapping binding sites (Figure 3). These models predict that the ligands bind close to some residues implicated in warfarin resistance, notably Ala-26, Trp-59, Glu-67, His-68, Gly-71, Leu-120 and Tyr-139 [55, 62-67, 71]. However, given the uncertainties in the tertiary structure of the enzyme and the inherent risk of docking into a homology model, these predictions should be viewed with considerable caution. Notwithstanding these difficulties, models based on a membrane topology of four transmembrane helices each embedded in phospholipid to simulate the membrane have been used in computer-aided drug design [72]. The model was used to understand the interaction of a set of 4-hydroxycoumarins with polar substituents. In general, predicted binding energies correlated well with physiological measurements such as the prothrombin time [72]. The most active compound (diethyl [1-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)ethylidene]propanedioate; Scheme 2) had similar pharmacological properties to warfarin and is predicted to form three hydrogen bonds to residues in the active site of VKOR [72]. Based on this work, further compounds were synthesised with polar substituents at the same position. The most of active of this group of compounds was (3*E*)-3-{1-[(2-aminoethyl)amino]ethylidene}-4-hydroxy-3,4-dihydro-2*H*-chromen-2-one (Scheme 2); this molecule caused reduced liver necrosis and oxidative stress compared to warfarin [73]. Additional computational studies suggested that the mode

of interaction of this group of compounds involves non-covalent bonding in which a proton from Cys-135 partially transfers from the protein to the carbonyl oxygen on the ligand [72, 73].

Dicoumarol also inhibits NQO1

Dicoumarol (and warfarin) also inhibit another enzyme implicated in vitamin K metabolism. This enzyme, NAD(P)H quinone oxidoreductase 1 (NQO1, DT-diaphorase; EC 1.6.5.2) has very different structural and physical properties to VKOR. NQO1 is a soluble, cytoplasmic, globular dimeric enzyme with two active sites [74-77]. Each of these active sites is composed of residues from both polypeptide chains [77]. The enzyme catalyses the NADH or NADPH-dependent reduction of a variety of organic compounds, including quinones [78-82]. Its cellular role is not wholly clear, but it seems likely that NQO1 participates in the detoxification of xenobiotic compounds and also in the cycling of quinones in the cell [75]. Since it catalyses the two electron reduction of quinones directly to the corresponding quinols, it avoids the production of potentially damaging semiquinones [83].

Although NQO1 has been shown to catalyse the reduction of menadione (a vitamin K derivative) *in vitro*, the role of the enzyme in vitamin K metabolism is not clear [83]. In addition to VKOR, there appear to be other enzymes which can catalyse the conversion of vitamin K to the reduced form.

However, NQO1's contribution to this process has been questioned: NQO1 deficient mice were still able to generate reduced vitamin K, even in the presence of warfarin suggesting that an enzyme other than VKOR and NQO1 is also able to catalyse this process [84]. The interpretation of these types of experiments is undoubtedly complicated by the fact that warfarin and dicoumarol inhibit both VKOR and NQO1 (and, most likely, other cellular processes).

NQO1 operates by a substituted enzyme ("ping-pong") mechanism in which NAD(P)H reduces a tightly bound FAD cofactor and the resulting NAD(P)⁺ then leaves the active site. This enables the second substrate (e.g. a quinone) to enter and be reduced by the FADH₂ cofactor [85]. Dicoumarol is a competitive inhibitor of NQO1 with respect to NADH [86]. Interestingly, NQO1 displays negative cooperativity towards dicoumarol as an inhibitor, but not towards NAD(P)H as a substrate [86, 87]. A

recent computational study suggested that this cooperativity most likely arises from altered flexibility of “communication pathways” linking the two active sites [87]. This was confirmed by site-directed mutagenesis of a key glycine residue in one of these pathways: alteration of this glycine to the more conformationally restricted serine dramatically reduced the enzyme’s cooperativity towards dicoumarol [87]. The physiological significance of this negative cooperativity is, as yet, unknown. However, it is interesting to note that similar effects have also been observed in the related human enzyme NRH-quinone oxidoreductase 2 (NQO2) and the budding yeast quinone oxidoreductase Lot6p [88, 89]. The crystal structure of NQO1 bound to dicoumarol shows that the ligand binds to both active sites, lying above the isoalloxazine ring of the FAD cofactors [90]. This molecular mechanism of inhibition is consistent with the observation of competitive inhibition since it is expected that NAD(P)H also binds above the FAD molecule in order to facilitate the transfer of electrons [77]. The inhibition of NQO1 by warfarin has received less attention [91, 92]. However, it seems likely that its mode of inhibition and site of interaction will be similar to dicoumarol.

The inhibition of NQO1 has received increased attention recently. The enzyme is highly expressed in several different cancer cell types [93]. One role of NQO1 in these cells appears to be to help defend them against oxidative damage [94, 95]. Therefore, inhibition of the enzyme may directly, or in combination with other therapies, result in the selective death of tumour cells. Indeed, dicoumarol has been shown to inhibit the growth of pancreatic cancer cells [94]. While dicoumarol is unlikely to have great success as an anticancer agent owing to its anticoagulant properties, considerable efforts have been made to identify related structures which combine increased potency of inhibition of NQO1 while reducing the other effects of the drug. Paradoxically, mutations in the gene encoding NQO1 which result in reduced enzymatic activity predispose people to cancer [96]. The best characterised cancer-associated variant is p.P187S (NQO1*2; rs1800566) and approximately a quarter of the human population have at least one allele which codes for this [97-99]. A rarer allele codes for p.R139W (NQO1*3; rs1131341) which is associated with childhood acute lymphoblastic leukaemia [100]. The fundamental biochemical cause of the loss of NQO1 activity in both variants is decreased stability of the protein and, in the case of p.P187S, consequent loss of the FAD cofactor [98, 101-103].

Therefore, there is also interest in discovering reagents which stabilise the cancer-associated variant forms of NQO1.

To date, the majority of efforts to identify novel coumarin-like inhibitors of NQO1 have centred on computational studies backed up by *in vitro* testing. The availability of several high resolution crystal structures of NQO1 makes such a strategy viable [77, 90, 104-106]. Of particular interest is the structure with dicoumarol bound (PDB: 2F1O; Figure 4) [90]. This provides a useful structure to compare modelled structures with since we would expect other coumarins to bind in a similar way to dicoumarol.

In addition to its catalytic roles, NQO1 interacts with the apoptosis regulating protein p53. This interaction stabilises p53 and promotes apoptosis. Curcumin (Scheme 5) is an NQO1 inhibitor which reduces the growth of cancer cells in culture [107]. Curcumin antagonises this interaction, releasing p53 from the complex and promoting its degradation [107]. Dicoumarol has a similar effect on NQO1's interaction with p53 [108-112]. The stability of ornithine decarboxylase and p73 are also regulated by binding to NQO1; in both cases dicoumarol antagonises the interaction [112, 113]. Therefore, any attempts to develop inhibitors of NQO1 catalysis will need to take into account the protein's second role as a stabiliser of a diverse range of cellular proteins.

One of the first attempts to develop novel inhibitors of NQO1 with similar structures to dicoumarol involved the computational screening of the 700,000 compounds then available in the National Cancer Institute's (USA) virtual library [114]. The screening method was validated by docking the known inhibitor, EO9 into the active site of NQO1 and comparing this with the crystal structure of the protein with the ligand bound [106]. Molecules identified in the initial computational screen were reduced in number by eliminating those which were too large (>500 Da) and those which were predicted to have unfavourable pharmacokinetic properties. From this reduced list 21 compounds which were available for testing were assayed *in vitro* for their ability to inhibit NQO1. All 21 inhibited the enzyme's ability to catalyse the reduction of menadione [114]. The compound with the highest experimentally determined IC₅₀, NSC645827 (CAS# 128113-19-9; Scheme 5) shares some

structural features with coumarins, as do many of the other compounds identified in this process [114]. A further study mined the NCI database specifically for compounds with the coumarin scaffold. The highest affinity binders were dicoumarol and its 4-hydroxy-substituted analogue, (3-[(4,7-dihydroxy-2-oxochromen-3-yl)methyl]-4,7-dihydroxychromen-2-one) (NSC682612; CAS# 63844-74-6; Scheme 5) [115]. This compound has a lower affinity for serum albumin compared to dicoumarol. Along with two others – 7-(4-hydroxy-2-oxochromen-3-yl)-7H-chromeno[3,2-c]chromen-6-one (NSC686396) and NSC68639 (Scheme 5) – this compound causes a lower increase in superoxide generation and a lower oxidative respiratory burst compared to dicoumarol [115]. These two effects are likely to arise from dicoumarol's ability to uncouple the mitochondrial proton motive force and, from these data, it can be assumed that these three compounds are less effective in generating this “off-target” effect. All three compounds inhibited the growth of pancreatic cancer cells in a dose-dependent manner [115].

A series of 29 symmetrical and asymmetrical analogues of dicoumarol were synthesised and tested for their ability to inhibit NQO1 [105]. In general the asymmetric series had greater aqueous solubility compared to dicoumarol. Three of the symmetrical analogues had higher affinity for NQO1 than dicoumarol (Scheme 5) [105]. One of the 29 inhibitors was co-crystallised with NQO1 and the resulting structure (PDB: 3JSX) compared to the docked model; the experimental and theoretical structures of the complex were in good agreement, further validating the structure-based drug design approach in this case [105]. These novel compounds inhibited NQO1 in colon, lung and pancreatic cancer cell lines and the degree of cellular inhibition broadly correlated with the *in vitro* affinity [116]. However, the “off target effects” of these compounds (superoxide generation, induction of apoptosis) were generally less than observed with dicoumarol. The magnitude of these effects did not correlate with the *in vitro* affinity suggesting that they are true “off target” effects resulting from the compounds' interactions with other biomolecules and systems within the cells [117].

Conclusions

Dicoumarol inhibits two different enzymes implicated in vitamin K metabolism – VKOR and NQO1. Given the very different nature of the two enzymes and their active sites, this appears to be a coincidence. Attempts to understand the interaction of dicoumarol (and other coumarins) with VKOR have been hampered by the lack of structural information on the enzyme. In contrast, the inhibition of NQO1 is very well understood through a number of biochemical, structural and computational studies. This has enabled the development of novel inhibitors with more promising properties. Another interesting coincidence is that, while dicoumarol was the first well-established inhibitor of both VKOR and NQO1, its structural derivatives of the compound which have greater pharmacological promise. In the case of VKOR, warfarin has been phenomenally successful and is one of the world's bestselling drugs [118]. It has maintained this success for over half a century (although there are now considerable efforts underway to develop improved anticoagulants [119]). While some drugs targeting NQO1 are nearing clinical use (for example, EO9 has shown considerable promise in phase I and II clinical trials for bladder cancer [120]), none of those based on the dicoumarol structure are at this stage. However, given the success of combined computational and biochemical studies in identifying molecules which have improved affinity for NQO1 together with lower off target effects there remains considerable promise in this avenue of research. If it is successful, dicoumarol will be the “parent” of at least two families of drugs targeted at very different groups of diseases.

Conflict of interest statement

The author has no conflicts of interest to declare. No funding was received to support this work.

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Figure and Scheme Legends

Scheme 1: The tautomeric forms of dicoumarol

Scheme 2: The structure of warfarin and two computationally identified analogues

Scheme 3: The structures of common forms of vitamin K₁ and vitamin K₂

Scheme 4: Forms of vitamin K which occur during the γ -carboxylation cycle

Scheme 5: Inhibitors of NQO1 with structures related to dicoumarol

Figure 1: The structure of VKOR. (a) The crystal structure of cyanobacterial VKOR with bound quinone (space filling model, dark grey). The C-terminal thioredoxin-like domain is shown at the top of the figure and VKOR-like subunit at the bottom. The VKOR-like subunit consists largely of four, membrane-spanning helices. The figure was produced using PDB file 3KP9 [47] and PyMol (www.pymol.org). The N- and C-termini are indicated. (b) A homology model of human VKOR using the cyanobacterial VKOR as a template. This protein lacks the thioredoxin-like domain. The model was generated using Phyre2 in the intensive mode [121]. It aligns to the cyanobacterial structure with a root mean square deviation (rmsd) of 4.27 Å over 687 equivalent atoms. The N- and C-termini are indicated. (c) A structure-based sequence alignment (generated in PROMALS3D [122]) of cyanobacterial VKOR (3KP9) and human VKOR (VKOR) showing regions which are α -helical (*h*) and in β -sheets (*e*).

Figure 2: The location of residues in human VKOR which are altered by mutations associated either with disease or with increased tolerance to warfarin. The overall fold of the protein was predicted by homology modelling (see Fig. 1) and the residues affected are shown in stick form and dark grey. The N- and C-termini and the residues which are associated with resistance to warfarin are indicated. The right hand image represents the protein rotated by 90° around the x-axis compared to the left hand one. Images were generated using PyMol.

Figure 3: Docked structures of (a) dicoumarol and (b) warfarin with the VKOR homology model (see Fig. 1). Docking was achieved using AutoDock Vina as implemented in Chimera [69, 70]. The proteins and ligands were prepared using the default settings DockPrep prior to docking. In both (a) and (b) the left hand image shows the overall fold of the protein (generated using PyMol) with the bound ligand (dark grey). The right hand images show the amino acid residues which are predicted to be close to the ligands. These images were generated with Discovery Studio Visualiser 3.5 (Accelrys Software Inc., USA).

Figure 4: The structure of human NQO1 with dicoumarol bound. The left hand image shows the overall, dimeric fold of the protein with the FAD cofactors and dicoumarol ligands bound at both active sites (top right and bottom left of the structure). The two subunits of the dimer are coloured dark and light grey. The N- and C-termini of both subunits are indicated. The right hand image shows the dicoumarol ligand (top) and the FAD cofactor (bottom) only, demonstrating how they stack onto each other. The figures were produced using PDB file 2F1O [90] and PyMol.

Figure 1

Figure 2

Figure 3

(a)

(b)

Figure 4

Scheme 1

Scheme 2

Warfarin

4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2*H*-chromen-2-one

Diethyl [1-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)ethylidene]propanedioate

(3*E*)-3-{1-[(2-aminoethyl)amino]ethylidene}-4-hydroxy-3,4-dihydro-2*H*-chromen-2-one

Scheme 3

Scheme 4

Vitamin K
(oxidised)

Vitamin KH₂
(reduced)

Peroxy form

Oxy-epoxide
form

Vitamin K
epoxide

Scheme 5

Curcumin

NSC645827

NSC 682612

NSC 686396

NSC 686390

Three computationally identified coumarin-like inhibitors of NQO1 with enhanced affinity compared to dicoumarol

Three synthesised analogues of dicoumarol with enhanced affinity for NQO1