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Determining the necessity of phenyl ring π-character in warfarin

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ABSTRACT

Despite the difficulty in administering a safe dose regimen and reports of emerging resistance, warfarin (**1**) remains the most widely-used oral anticoagulant for the prevention and treatment of thrombosis in humans globally. Systematic substitution of the warfarin phenyl ring with either 1,3,5,7-cyclooctatetraene (COT) (**2**), cubane (**3**), cyclohexane (**4**) or cyclooctane (**5**) and subsequent evaluation against the target enzyme, vitamin K epoxide reductase (VKOR), facilitated interrogation of both steric and electronic properties of the phenyl phar-macophore. The tolerance of VKOR to further functional group modification (carboxylate **14**, PTAD adduct **15**) was also investigated. The results demonstrate the importance of both annulene conferred π-interactions and ring size in the activity of warfarin.

Warfarin (**1**) was discovered in the USA 30 years ago and is the most widely-used oral anticoagulant for the prevention and treatment of thrombosis in humans globally.[1] Despite its prevalence in society, warfarin has a number of drawbacks which have yet to be adequately addressed. Although the introduction of monitoring systems and improved dose management have made it easier for practitioners to navigate its narrow therapeutic index,[2] warfarin is still ranked among the top 10 pharmaceuticals with serious adverse drug events.[3] Its continued popularity can be attributed to problems with developments in warfarin pharmacogenetics[4] associated with its target enzyme, vitamin K epoxide reductase (VKOR),[5] as well as shortcomings of a number of warfarin replacements.[6] The controversy regarding the usefulness of treating individual VKOR genotypes[7] and reports of emerging resistance to warfarin[8] compound these unmet medical needs and make the drug an obvious candidate for further scrutiny by the medicinal chemistry community.

Since its seminal synthesis in 1964, cubane has enjoyed a wealth of attention in the areas of medicinal chemistry, material design and fundamental bonding and reactivity.[9] A recent line of enquiry in our laboratories has been into Eaton's 1992 conjecture that cubane can act as a phenyl ring (bio)isostere.[10] Having successfully validated the relationship between the two scaffolds using a portfolio of known pharmaceutical and agrochemical templates,[11] an obvious question arose as to the ability of cubane to confer suitable π-character, an electronic property important for both pharmacodynamic[12] and pharmacokinetic[13] interactions. 1,3,5,7-Cyclooctatetraene (COT) was postulated as a viable (bio)motif in this regard and was subsequently evaluated against its cubane and phenyl counterparts.[14] It was in this context that warfarin (**1**) first came to our attention, where it was demonstrated that a racemic mixture of COT-warfarin (**2**) was a 2-fold more active inhibitor of VKOR than (*S*)-warfarin (the more active enantiomer of the two), whereas (*S*)-cubane warfarin (**3**) was 10-fold less active than racemic warfarin. Encouraged by the increased activity of COT-warfarin relative to warfarin itself (i.e. 3-phenyl substituent), a study to further investigate the importance of annulene conferred π-interactions was initiated with the corresponding saturated systems [e.g. cyclohexyl-(**4**) and cyclooctyl-warfarin (**5**) (Figure 1)] and analogues. The synthesis and evaluation of these analogues against VKOR is herein disclosed.

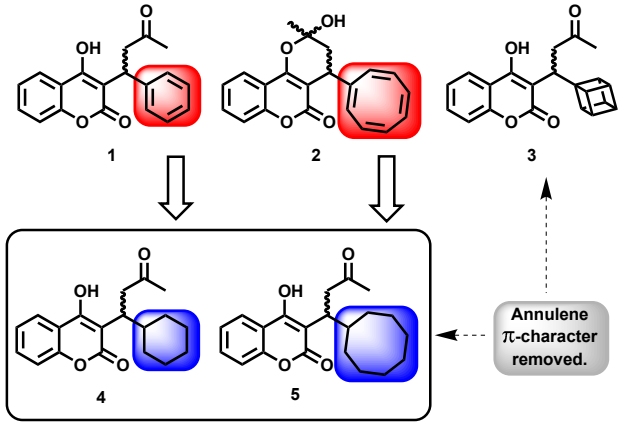
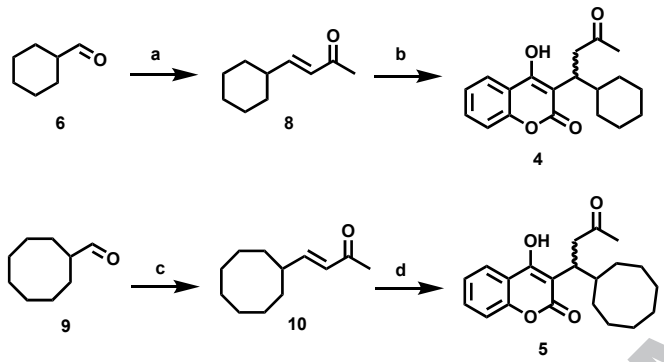


Figure 1: Warfarin [phenyl- (**1**)] along with cyclooctatetraenyl- (**2**), cubyl- (**3**), cyclohexyl- (**4**) and cyclooctyl-warfarin (**5**).

Warfarin (**1**) was purchased from Sigma-Aldrich, whilst COT-warfarin (**2**) was readily accessible from cubyl-warfarin (**3**),[14] via valence isomerisation methodology.[15] Synthesis of cyclohexyl-warfarin (**4**) began with a Wittig olefination between the commercially available cyclohexanecarbaldehyde (**6**) and 1-(triphenylphosphoranylidene)-2-propanone (**7**), and was completed using D-proline mediated conjugate addition between the resulting Wittig alkene **8** and 4-hydroxycoumarin. Cyclooctyl-warfarin (**5**) was accessed in a similar manner starting from the known cyclooctanecarbaldehyde (**9**)[16] (Scheme 1). Phenyl-(**1**), cubyl-(**3**), cyclohexyl-(**4**) and cyclooctyl-warfarin (**5**) all existed partially in the hemi-ketal form in solution (^{1}H NMR, $CDCl_3$), whereas COT-warfarin (**2**) existed solely as the hemi-ketal form (^{1}H NMR, $CDCl_3$ or DMSO:D_2O 3:7). All analogues were isolated and evaluated as racemic mixtures.



Scheme 1: Synthesis of cyclohexyl- (**4**) and cyclooctyl-warfarin (**5**); (a) **7**, DCM, 40 ℃, 66%; (b) 4-hydroxycoumarin, D-proline, DMSO, 72%; (c) **7**, toluene, 110 ℃, 53%; (d) 4-hydroxycoumarin, D-proline, DMSO, 60%.

The half-maximal inhibition concentration (IC_{50}) values for VKOR of cyclohexyl-(**4**) and cyclooctyl-warfarin (**5**) were measured using a mammalian cell-based assay[17] and plotted against previously acquired values for phenyl-(**1**) and COT-warfarin (**2**) (Figure 2).

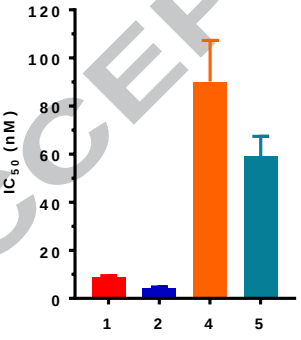
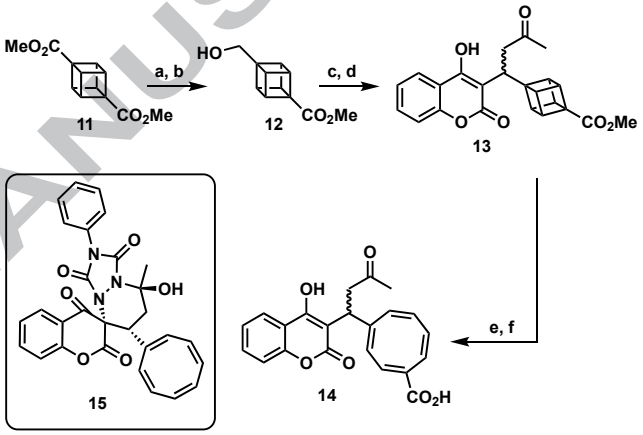


Figure 2: IC_{50} values of phenyl- (**1**), COT- (**2**), cyclohexyl- (**4**) and cyclooctyl-warfarin (**5**) against VKOR using FIXgla-PC/HEK293 reporter cells.

Comparing phenyl-(**1**) to cyclohexyl-warfarin (**4**), complete saturation of the phenyl ring resulted in a 10-fold decrease in VKOR inhibition. This is consistent with the prior observation that removing π-character decreases VKOR inhibition [i.e. (±)-cubyl-warfarin (**3**) was 15-fold less active than (±)-phenyl-warfarin (**1**)].[14] The same trend was observed by comparing COT-(**2**) to cyclooctyl-warfarin (**5**), where complete saturation of the COT ring resulted in a 13-fold decrease in VKOR inhibition between the two analogues.

To further delineate the relationship between structure and VKOR inhibition of the annulene pharmacophore, the ring system of the most active analogue [i.e. COT-warfarin (**2**)] was elaborated to incorporate additional polar functionality. COT-warfarin carboxylate **14** was chosen as a suitable target, and was reached in a synthetic sequence beginning with the commercially available dimethyl 1,4-cubanedicarboxylate (**11**).[18] Half-hydrolysis[19] of **11** followed by borane reduction gave cubane alcohol **12**. Application of our recent developments[20] in the Ley-Griffith oxidation[21] and tandem Wittig[22] reaction to **12** gave an intermediate α,β-unsaturated ketone, which underwent L-proline catalysed[23] conjugate addition of 4-hydroxycoumarin to give coumarin **13**. Finally, rhodium(I) norbornadiene chloride dimer (i.e. $[Rh(nbd)Cl]_2$) mediated valence isomerisation[15] followed by saponification gave the target COT-warfarin carboxylate **14**. PTAD adduct **15**, synthesised as part of our previous study,[14] was also included for testing (Scheme 2). COT-warfarin carboxylate existed solely as the ketone in solution (^{1}H NMR, $CDCl_3$) and was isolated and evaluated as a racemic mixture. PTAD adduct **15** was obtained and used as a single enantiomer.[14]



Scheme 2: Synthesis of additional warfarin analogue **14**, and previously disclosed PTAD adduct **15**: (a) MeOH/NaOH, THF, 95%; (b) BH_3•SMe_2, THF, 0 ℃ → rt 91%; (c) i) TPAP, NMO, 4Å MS, DCM; ii) **7**, DCM, 40 ℃, 40% (two steps); (d) 4-hydroxycoumarin, L-proline, DMSO, 70%; (e) $[Rh(nbd)Cl]_2$, PhMe, 110 ℃, 80%; (f) MeOH/NaOH, THF, 60%.

The IC_{50} values for VKOR of warfarin carboxylate **14** and PTAD adduct **15** were measured and plotted against COT-warfarin (**2**) (Figure 3).

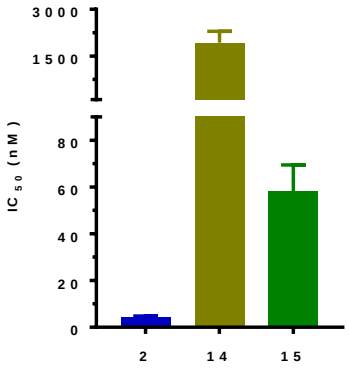


Figure 3: IC_{50} values of warfarin carboxylate **14**, PTAD adduct **15** and COT-warfarin (**2**) against VKOR using FIXgla-PC/HEK293 reporter cells.

Incorporation of additional polar functionality in the form of COT-warfarin-carboxylate (**14**) resulted in a 430-fold decrease in VKOR inhibition compared to COT-warfarin (**2**). Interestingly, this observation is contrary to that observed with acenocoumarol,

which only differs from warfarin by the inclusion of a *p*-nitro group on the phenyl ring, but is much more active than warfarin.[24] Furthermore, preventing the 4-hydroxycoumarin and ketone moieties from undergoing enolization was not tolerated, as demonstrated by the PTAD adduct **15**, which was 13-fold less active than COT-warfarin (**2**).

In summary, π-character contained within the eastern portion, whether aromatic of alkenic, seems essential to mimic the activity of warfarin, as demonstrated by the decreased VKOR inhibition observed for both warfarin analogues with full annulene saturation. Activity against VKOR was enhanced by replacing the phenyl ring for COT as previously reported, a modification that both increased steric bulk and modulated π-character from aromatic to non-aromatic, an improvement which is likely also attributed to the dynamic equilibrium of COT.[14] Overall, modifications of the warfarin scaffold continue to reveal inhibition improvements and subtilties associated with VKOR, and underpin the importance of developing phenyl ring (bio)isosteres, or (bio)motifs, capable of conferring suitable π-character.

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Conflicts of Interest

G. P. S. and C. M. W. have formal and informal commercial relationships with companies developing and supplying cubane intermediates.

Supplementary Data

Supplementary data for this article, which includes experimental procedures and copies of NMR spectra, can be found online at **XXXXXX**.

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