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Design and synthesis of novel diphenacoum-derived, conformation-restricted vitamin K 2,3-epoxide reductase inhibitors

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Abstract—Two novel diphenacoum-derived analogues **5** and **6** are designed, synthesized and tested as potential vitamin K 2,3-epoxide reductase (VKOR) inhibitors. The inhibition studies indicated that **5** is a potent VKOR inhibitor, which confirmed that the replacement of the tetrahydronaphthalene on diphenacoum to a chroman functionality does not have a major impact on inhibition potency. The conformation-restricted compound **6** is a moderate inhibitor which may serve as a lead compound for further study of the mode of action of coumarin-type anticoagulants at the molecular level.

Vitamin K 2,3-epoxide reductase (VKOR, E.C. 1.1.4.2)[1] is a key enzyme involved in the vitamin K cycle,[2,3] the cyclic interconversion of vitamin K metabolites. It catalyzes the conversion of vitamin K 2,3-epoxide to vitamin K hydroquinone, as shown in Figure 1. Vitamin K hydroquinone functions as an essential cofactor for vitamin K-dependent carboxylases,[4] which convert a limited number of glutamic acid residues in targeted proteins to γ-carboxyglumate acid (Gla) residues. These Gla residues enable the proteins to bind Ca^{+2}, as part of the blood coagulation cascade.[5] Concomitant with γ-carboxylation, the hydroquinone cofactor is converted to the metabolite vitamin K 2,3-epoxide, which is reduced back to the hydroquinone by VKOR. Thus, inhibition of the activity of VKOR, with its central position in regulating biosynthesis of biologically active vitamin K-dependent proteins,[6] can prevent the formation of vitamin K and subsequently reduced vitamin K. The lack of carboxylase cofactor will then produce non-functional vitamin K-dependent coagulation factors, which are the basis for clinical drugs for control of thromboembolic disease.[7] It is well-documented that coumarin-type anticoagulants target the vitamin K cycle by inhibition of VKOR activity, which prevents reduction of vitamin K, but their mode of action at the molecular level remains unclear.[8,9] For example, warfarin has long been used as a therapeutic drug for anti-coagulation therapy in humans as well as a poison in rodent pest control. Unfortunately, this sixth most-prescribed cardiovascular drug in North America suffers from high international normalized ratios (varying up to 120-fold), which requires the treated patients to be monitored constantly.[10] Furthermore, the finding of hereditary warfarin resistance in rats represented a major setback in

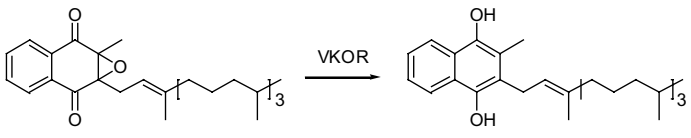

Figure 1. Reaction catalyzed by vitamin K 2,3-epoxide reductase.

Keywords: VKOR inhibitor; Conformation restricted.
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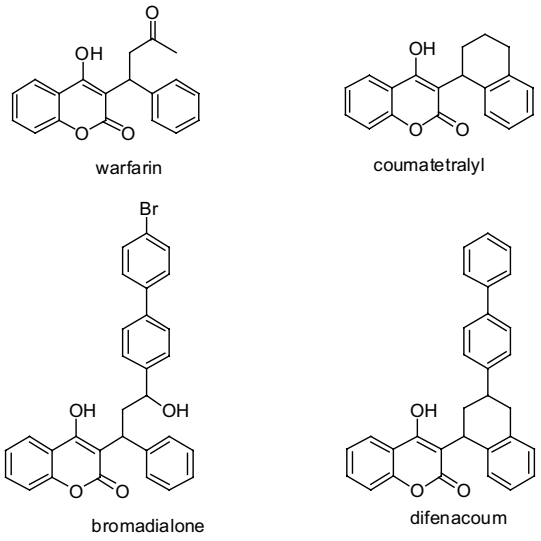
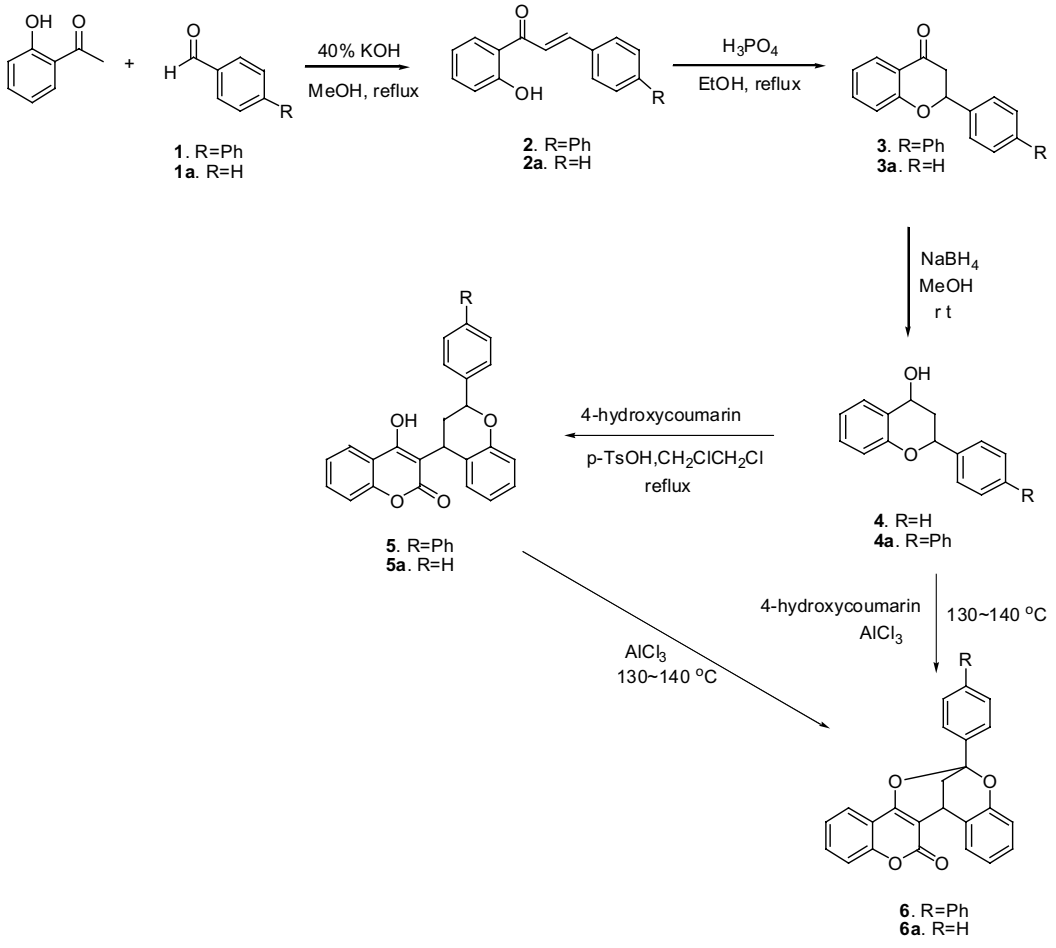


Figure 2. Structures of warfarin and superwarfarins.

rodent control. While the second generation anticoagulants, superwarfarins[11] (coumatetralyl, bromadiolone, and diphenacoum, depicted in Fig. 2), have so far been quite successful in fighting resistant rodents, their extreme toxicity is a danger to humans[12] and other animals. In addition, the commercial preparation of superwarfarins like diphenacoum is laborious and time consuming. Although an improved procedure has been recently reported,[13] large scale production of diphenacoum is still currently unavailable. Thus, developing an easily prepared, potentially less toxic VKOR inhibitor is highly desired. Here, we report the rational design, efficient synthesis, and in vitro evaluation of a diphenacoum derivative and a conformation-restricted dioxabicycle as potential VKOR inhibitors.

Previous studies[14] have demonstrated that the 4-hydroxycoumarin moiety and the right hand phenyl part of superwarfarins are absolutely required for inhibitor potency. Yet the upper right hand moiety has minimal effect and the incorporation of new functional groups will not alter its biological activity dramatically. Hence, we envisioned replacing the tetrahydronaphthalene moiety of diphenacoum with a chroman functionality. The resulting potential VKOR inhibitor presumably will offer benefits of easy preparation, higher water solubility, and lower toxicity when compared with diphenacoum. In addition, it will be more susceptible to further chemical modification, that is, cyclization to give a conformation-restricted molecule, which can reduce the original four possible stereoisomers to two enantiomers. Biological evaluation of these analogues may shed light on the mode of action of coumarin-type VKOR inhibitors.



Scheme 1. Synthesis of compounds **5**, **5a**, **6**, and **6a**.

The preparation of the proposed compound **5** is depicted in Scheme 1. The synthesis started with a base-catalyzed aldol condensation of 2′-hydroxyacetophenone and 4′-phenylbenzaldehyde **1** under reflux conditions in methanol for 3 h to give the α,β-unsaturated ketone **2** with a 75% yield.[15] Ketone **2** was then cyclized by refluxing with phosphoric acid in ethanol for 2 days to yield 2-biphenylchroman-4-one **3** with a 70% yield.[15] Further reduction of the ketone **3** (or the mixtures of **2** and **3**) with sodium borohydride in methanol under room temperature for 30 min gave the corresponding alcohol **4** quantitatively. The final condensation of **4** with 4-hydroxycoumarin in 1,2-dichloroethane in the presence of a catalytic amount of *p*-toluenesulfonic acid under reflux conditions for 6 h afforded the target compound **5** with a 78% yield. This concise synthetic route is highly attractive since no column chromatography is needed. When compound **5** was further heated with aluminum chloride at 130–140 °C for 30 min, a cyclized compound **6** formed as expected. Alternatively, this dioxabicyclic derivative **6** can be prepared directly from condensation of alcohol **4** with 4-hydroxycoumarin in $AlCl_3$.[16] Numerous attempts to obtain a satisfactory crystal structure of **6** proved futile, presumably due to the extended biphenyl moiety in **6** preventing proper crystal formation. Nevertheless, the structure of **6** was indirectly confirmed by X-ray crystallographic analysis of **6a**,[17] as depicted in Figure 3, which was prepared by the exact same procedure as **6** except benzaldehyde **1a** was used as the starting material.

The inhibition studies of **5** and **6** with partially purified VKOR[18] from beef liver indicated that compound **5** remains a potent VKOR inhibitor with IC_{50} value of 0.04 μM, which is 2.5-fold more potent than warfarin.[19] This result confirmed that the replacement of the tetrahydronaphthalene on diphenacoum with a chroman functionality does not have a major impact on inhibition potency. Although further toxicity tests are needed, the four-step, highly efficient synthesis of **5** from relatively inexpensive starting materials and reagents makes it suitable for mass production on an industrial scale. The conformation-restricted compound **6**, on the other hand, was a moderate VKOR inhibitor with IC_{50} value of 1 μM, which is 10-fold less potent than warfarin. This inhibition result is quite encouraging if we consider that the 4-hydroxyl group on the coumarin moiety, which is crucial for potent VKOR inhibition is not available in compound **6**. Since the importance of hydrogen bonding in the enzyme–inhibitor interaction is well-recognized, the inhibition activity of **6** may be attributed, partially at least, to its novel rigid bicyclic skeleton. Therefore, future preparation of 4-hydroxycoumarin conserved as well as conformation-restricted diphenacoum analogues not only can facilitate the development of potent VKOR inhibitors but also can serve as a lead compound for further study of the mode of action of coumarin-type anticoagulants at the molecular level.

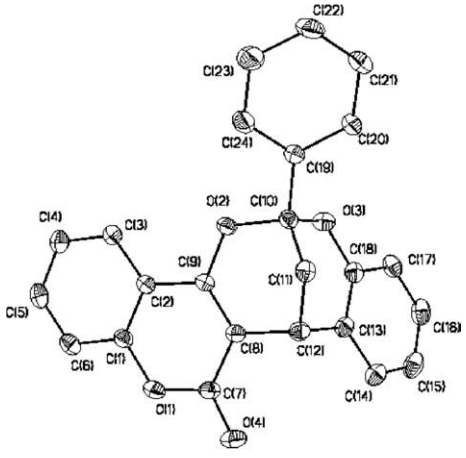


Figure 3. X-ray crystal structure of compound **6a**.

In conclusion, we have rationally designed, chemically synthesized, and characterized two novel diphenacoum-derived, conformation-restricted analogues. Biological evaluation demonstrated that **5** is a potent and **6** is a moderate VKOR inhibitor. Further design and synthesis of a rigid diphenacoum-derived analogue, which retains the 4-hydroxycoumarin functionality is currently underway.

Acknowledgements

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16. For preparation of **5** and **6** from **4**: To a solution of 2-biphenyl-4-yl-chroman-4-ol **4** (0.2 g, 0.66 mmol) and 4-hydroxycoumarin (0.1 g, 0.62 mmol) in 1,2-dichloroethane (25 mL) was added a catalytic amount of *p*-toluenesulfonic acid. This mixture was then gently refluxed for 6 h. After completion of the reaction (monitored by TLC), the

solvent was concentrated in vacuo. This dark red mixture was poured into water and basified with dilute sodium hydride solution. The solution was then extracted with ethyl acetate twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude final product was purified by column chromatography (EtOAc–hexanes = 3:7) to give a white solid 2′-biphenyl-4-yl-hydroxy-3′,4′-dihydro-2′*H*-[3,4′]bichromenyl-2-one **5** in a 78% yield. Mp 225–226 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76–7.00 (m, 17H, Ar H's), 5.18 (t, J = 6.3 Hz, 1H), 4.59 (t, J = 4.2 Hz, 1H), 2.49 (q, J = 1.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 161.6, 158.2, 152.4, 151.3, 142.4, 140.4, 138.6, 132.0, 128.9, 128.4, 128.2, 127.7, 127.4, 127.2, 126.2, 125.2, 124.1, 122.8, 122.1, 116.7, 116.4, 115.1, 106.1, 100.3, 32.9, 27.2. IR (KBr) v 3393, 1673, 1612, 1222, 758 cm^{-1}. HRMS (EI) calcd for $C_{30}H_{22}O_4$ (M^+), 446.1518, found 446.1511. Anal. Calcd for $C_{30}H_{22}O_4$: C, 80.70; H, 4.97; O, 14.33. Found: C, 80.30; H, 5.23; O, 14.23. To a mixture of 2-biphenyl-4-yl-chroman-4-ol **4** (0.2 g, 0.66 mmol) and 4-hydroxycoumarin (0.1 g, 0.62 mmol) was added a catalytic amount of aluminum chloride. This mixture was then gently heated to 130–140 °C. After completion of the reaction within 30 min (monitored by TLC), the stiff solid was allowed to stand 15 min at room temperature. This mixture was poured into water and basified with dilute sodium hydride solution. The solution was then extracted with ethyl acetate twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude final product was purified by column chromatography (EtOAc–hexanes = 1.5:8.5) to give a white solid 6-biphenyl-6,12-methano-6*H*,12*H*,13*H*-[1]benzopyrano[4,3-*d*][1,3]-benzodioxocin-13-one **6** in a 62% yield. Mp 211–212 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90–6.96 (m, 17H, Ar H's), 4.41 (t, J = 2.8 Hz, 1H), 2.51, 2.45 (ABdq, J = 13.6, 2.8 Hz, 1H each); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.0, 161.2, 155.2, 152.6, 141.1, 140.6, 139.1, 132.1, 130.4, 128.7, 128.6, 127.3, 127.1, 127.0, 126.5, 124.0, 123.2, 122.1, 118.9, 118.8, 116.4, 116.1, 107.0, 75.4, 34.7, 32.7, 29.7. IR (KBr) v 1715, 1231, 761 cm^{-1}. HRMS (FAB) calcd for $C_{30}H_{20}O_4$ (MH^+), 445.1449, found 445.1450. Anal. Calcd for $C_{30}H_{20}O_4$: C, 81.07; H, 4.54; O, 14.40. Found: C, 80.78; H, 4.53; O, 14.51.

17. Crystallographic data (excluding structure factors) for **6a** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-236132. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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