Enantioselective Synthesis, Tautomerism, Molecular Docking And Biological Evaluation of 4-Hydroxy-3-(3-Oxo-1-Phenylbutyl)-2H-Chromen-2-One Analogues

Zakia Afzal (zakiafaisal9@gmail.com)
Allama Iqbal Open University https://orcid.org/0000-0002-5166-0434
Naghmana Rashid
Allama Iqbal Open University
Humaira Nadeem
Riphah International University
Arif-ullah Khan
Riphah International University

Research Article

Keywords: Anticoagulant activity, Docking studies, Tautomers, Antifungal, Wafrafin

Posted Date: November 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-982583/v1

Abstract

Keeping in view the aim of better alternatives of 4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one (warfarin), eleven analogs of warfarin have been synthesized with the goal to increase enantioselectivity of (S) enantiomer by using appropriate catalyst and minimize tautomerism by replacing methyl group of the side chain with aryl group. There are many reports of the serious complications with warfarin use, which are associated with the tautomeric forms of warfarin. The key step was highly enantioselective Michael addition of variously substituted chalcone and 4-hydroxycoumarin by using cinchona based 9-amino-9-deoxyepiquinine as chiral catalyst. Synthesized compounds were characterized by IR, ^{1}HNMR,^{13}CNMR, EIMS and CD studies. Enantiomeric excess (%ee) was determined by chiral HPLC which was upto 98%. Synthesized analogues were screened for anticoagulant, antibacterial and antifungal activities. In-vitro anticoagulant activity was evaluated by plasma recalcification time (PRT) method and out of eleven, ten synthesized compounds showed improved IC_{50} values as compaired to IC_{50} values of standard drug warfarin. Compound 4 showed 68.25% inhibation against staphylococcus aureus and compound 7 showed 68% inhibation against bacillus subtillis, gram positive strains of bacteria, compound 6 shows 70% inhibation against fungal strain candida albicans. Furthermore, molecular docking studies were carried out with Vitamin K_1 epoxide reductase VKOR1 receptor 3kp9, a potential target of warfarin for anticoagulant activity.

Introduction

Warfarin is an oral anticoagulant drug generally used in prevention of thrombosis or thrombolism,[1] which is a process of blood clotting and movement of clots in other body parts. Warfarin has been in use as anticoagulant drug since 1950 [2]. It decreases blood clotting by blocking an enzyme vitamin K1 epoxide reductase (VKOR1), that reactivates vitamin K1 [3]. In spite of its advantage, there are many reported complications of warfarin such as internal bleeding [4], tissue damage, purple toe syndrome, interaction with other medicines and food items [5, 6]. One of the major reported reasons of these side effects/unwanted interactions is tautomeric forms of warfarin [7].

Tautomers of any compound mostly have different fingerprints, hydrophobicities, pKa values and electrostatic properties. Such compounds when taken as drug bind with additional proteins resulting into lower drug efficacy and higher toxicity. Warfarin exists in roughly 40 tautomeric forms [8, 9], though two forms, keto and hemiketal, are major contributors (Fig. 1). It has been reported that *(S)* form of warfarin is more active as anticoagulant than *(R)* enantiomer [10], hence the synthesis performed was designed to achieve *(S)* warfarin as dominant product. It is to be noted that in hemiketal form, additional stereogenic centers are also generated.

In the present work, the objective was to synthesize warfarin analogues with decreased tautomerization and increased enantioselectivity especially no hemiketal form and *(S)* enantiomer in excess. The methyl group at 12th position (Fig. 1) was replaced with bulky groups to eliminate/reduce the chances of

tautomerism and cinchona alkaloid based organocatalyst 9-amino-9-deoxyepiquinine (Fig. 2) was used to obtain *(S)* enantiomer as major product.

Docking analysis [11] is a powerful tool used in drug development to evaluate the binding affinity of drugs with specific targets in biochemical pathways. Docking studies also being used by many pharmaceutical companies for drug development [12–14]. Anticoagulant efficiency of newly synthesized compounds was first checked by molecular docking studies with crystal structure of VKOR1 receptor 3kp9 [15] and then *in-vitro* anticoagulant studies were carried out.

In a number of different organisms, including various plants, archaea, bacteria, etc., the homologues of vitamin K epoxide reductase (VKOR) have been identified [16–18]. An active site CXXC motif is present in all homologues, which shifts between reduced (thiol) and oxidized (disulphide-bonded) states as well as pair of cysteine and a conserved serine/threonine, during the course of reaction cycle in mammalian VKOR. Disulphide bridge formation is catalyzed by bacterial VKOR homologues, with the help of Trx-like redox partner, in secreted proteins [17, 18].

Even though for human VKOR a three-TM model has been suggested [18], the structure provided by Li et. al. (PDB ID: 3kp9) [15] and multiple sequence alignment comparison predicted that the core of all VKOR's consist of four TMs including those in mammals. Their topology model placed all enzymatically important residues, including the active site CXXC motif, the conserved serine/threonine in the 1/2-helix, and the two cysteine residues in the 1/2-segment on or close to the extracellular/luminal side of the membrane.

In general, coumarins are naturally occuring medicinally important class of benzopyrones and exibit good antimicrobial activities [19, 20]. Therefore, besides anticoagulant activity synthesized compounds were also screened for antifungal and antibacterial activity.

Results And Discussion

Chemistry

Literature studies shows that chiral derivatives of 4-hydroxycoumarin were synthesized by different catalysts but 9-amino-9-deoxyepiquinine, an organocatalyst, until now reported to have excellent enantioselectivity even upto 99% in certain cases. The reaction conditions were set by reported method [21] using 9-amino-9-deoxyepiquinine 20mol%, additive (TFA) 40mol% in DCM as solvent. The synthesized warfarin analogues are given in Fig. 3.

Stereoselective syntheses of warfarin analogues were carried out through organocatalyzed Michael addition. Differently substituted chalcones (α,β-unsaturated ketones), as shown in Fig. 3 over the arrows, were used as Michael acceptor and 4-hydroxycoumarin as Michael donor. Mechanism involves nucleophilic attack of primary amine of the catalyst to carbonyl group of α,β-unsaturated ketones leading to formation of an intermediate which dehydrate to form trans-iminium cation. Then nucleophilic attack

of 4-hydroxycoumarin takes place from the *si* face [22] of trans-iminium cation to obtain the desired *(S)* product in excess. At the end, the catalyst regenerates by hydrolysis. This mechanism is typical for covalent organocatalysis. Covalent binding of substrate normally requires high catalyst loading, typically ranging from 20-30 mol%.

The characterization of the synthesized compounds was done by UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Enantiomeric excess was determined by chiral stationary phase HPLC using Lux 5 µm cellulose -1, LC Column 250x4.6 mm and configuration of excess enantiomer was determined by CD studies. As already reported in the literature, *(S)* enantiomer is biologically more active as compared to *(R)* enantiomer [10], therefore the corresponding organocatalyst was used and enantiomeric excess (%ee) achieved by the adopted procedure ranged from 23 to 98% and yield range was 60 to 90%.

FTIR spectra of final compounds have shown medium to strong intensity stretching and bending absorption bands of alcoholic O-H between 3200-3500 and 1330-1420cm^{-1}, intense bands of lactam absorption and keto C=O stretching appears between 1700-1745cm^{-1}. Compound 2 gave absorption band of phenolic O-H at 1378cm^{-1}. Compound 6 showed band of N-H stretching at 1585cm^{-1}. Compounds 7-11 showed C-Cl halo bond absorption frequencies between 500-850cm^{1}.

^{1}HNMR results indicated that keto form is in excess as the labile hydrogen of hydroxyl group appeared above 8ppm. Protons of the analogues showed similar values as of reported spectrum of warfarin. Due to different tautomeric forms (keto and hemiketal), the CH_2 signal of position 10 (Fig. 1) appears in two different regions. The one at low field due to hemiketal form, present in less percentage and for keto they appear between 3.00 to 4.00ppm up field than expected value and compound 7 shows a singlet of two protons at 5.61ppm. The characteristic peaks in the ^{13}CNMR also supported the confirmation of synthesized compounds. Due to low solubility of synthesized compounds, ^{13}CNMR spectra gave low intensity signals.

EIMS either show molecular ion peak or typical fragments.

Table 1
CD data of synthesized warfarin analogues

Compound	%ee	CD: λ_{max} [nm] (mdeg)
1	91(S)	215 (-54), 224 (+20)
2	70(S)	206 (-20), 204 (-20), 209 (+20)
3	70(S)	193 (-58), 196(-18), 204(-5)
4	60(S)	190(-7), 203(-5)
5	60(S)	194 (-20), 204(-28)
6	50(S)	195 (-7), 202 (-7), 209 (-4)
7	41(S)	203 (+20), 206 (-12), 222 (-30)
8	24(S)	206 (-25), 216 (-5)
9	23(S)	202 (-30), 204 (+15), 203 (-12), 213 (-26)
10	98(S)	201(-5), 204 (+40), 208 (-30)
11	96(S)	204 (+10), 206 (-30), 210 (+10)

Enantiomeric excess was determined by chiral stationary phase HPLC and configuration of the major enantiomer was determined by CD studies compared with already reported data of warfarin. CD spectrum was measured in range of 180-400nm. The intense band in the range of 190-223nm which is also present in the UV spectrum of the respective compound is due to allowed π-π* transition. As already reported, in the case of *(S)* warfarin this band shows a negative cotton effect. All the synthesized analogues showed a negative cotton effect due to π-π* transition in the range of 190-223nm (Table 1). So the assigned configuration of all the synthesized compounds is based on this comparison.

Biological Evaluation

Anticoagulant activity

As already mentioned that warfarin exists in different tautomeric forms especially two forms, keto and hemiketal are more prominent. The side effects of warfarin are also attributed to the existence of these tautomers being involved in multi-target interactions. Therefore, some analogues of warfarin were designed to restrict the formation of tautomeric forms as in compounds 2, 3 and 4; methyl group is substituted with aryl group in order to decrease rotation across dihedral bond and to avoid formation of hemiketal form. In compound 2 and 3, methyl group is replaced by substituted benzene ring while compound 4 has naphthyl ring. All these three have greater binding affinities than warfarin. Compound 4 showed maximum binding affinity i-e 11.6 kcal/mol, that is much higher than warfarin keto form i-e 8.8

kcal/mol. According to in-vitro anticoagulant studies, compound 4 has higher coagulation time than warfarin but not the highest among all.

In the remaining analogues of warfarin i.e. 5, 6, 7, 8, 9, 10 and 11 both aromatic rings are substituted with different electron withdrawing and electron donating aryl groups. All the compounds showed better activity as compared to warfarin except 10. The compound 10, having methoxy at one aromatic ring and chloro on the other aromatic ring directly bonded to the stereocenter, is showing less coagulation time as compared to warfarin but its IC_{50} values are 316.31μM is better than warfarin (IC_{50}=408.70μM). However, in case of the analogue in which chloro group is substituted on both aromatic ring, the coagulation time was greater than warfarin but its IC_{50} value (419.22μM) was greater than warfarin. Results of docking are also comparable with *in-vitro* anticoagulant studies.

Preliminary docking results showed that out of two tautomeric forms of warfarin keto form showed good binding affinity than hemiketal form. It appears that different tautomeric forms may have different mode of action in body. In almost all the cases, the synthesized compounds showed improved anticoagulant activities both in docking as well as in *in-vitro* studies. Compounds 1 have methyl group like warfarin but benzene ring attached to stereogenic centre is substituted by –OH and –OMe groups. According to docking results compound 1 in which benzene ring is substituted with hydroxyl at para and methoxy at meta position showed greater binding affinities in docking results and its coagulation time is highest among all analogues of warfarin. This added activity can be attributed to the additional methoxy group. IC_{50} values of ten out of eleven synthesized compounds are improved than standard drug warfarin (408.70 μM). Compound 2, 3, 6 and 7 showed much improved IC_{50} values (249.88-299.93μM) than standard drug warfarin.

Antibacterial activity

Compound 4 showed 68.5 % inhibition against *staphylococcus aureus* and compound 7 showed 68% inhibition against *Bacillus subtillis*. Other compounds showed moderate to no activity against different strains of gram positive and gram negative bacteria. Results are summarized in Table 2.

Table 2
Percentage (%) inhibition of synthesized warfarin analogues for antibacterial screening

Compound	% Inhibition *Pseudomonas aeruginosa*	% Inhibition *Bacillus subtillis*	% Inhibition *Staphylococcus aureus*	% Inhibition *Pseudomonas aeruginosa*	% Inhibition *Salmonella typhus*
Warfarin	-	20.9	-	-	-
1	-	22.03	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	24.5	68.25	-	-
5	-	-	-	-	-
6	-	-	25	-	-
7	-	68	-	-	-
8	-	-	34	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	35	-	-	-
Ofloxacin	92	94	87	95	90
"-"means that compound showed no activity.					

Antifungal activity

Compound 6 showed 70% inhibition against *candida albicans* while others showed moderate or no activity. Results are summarized in Table 3.

Table 3
Percentage (%) inhibition of synthesized warfarin analogues for antifungal screening

Compound	%Inhibition *Trichphyton rubrum*	% Inhibition *Candida albicans*	% Inhibition *Aspergillus niger*	% Inhibition *Microsporum canis*	%Inhibition *Fusarium lini*	%Inhibition *Canadida Glabrata*
Warfarin	-	-	-	-	-	2.5
1	-	-	-	2.5	-	-
3	-	-	-	2.5	-	-
6	-	70	-	-	10	-
7	-	-	-	-	15	-
11	-	-	-	-	-	25
Miconazole	113.5	97.8	-	98.1	73.50	49.58
Amphotericin B	-	-	20.70	-	-	-
"-"means that compound showed no activity						

Docking Studies Of Compounds 1-11

Binding affinities of compounds 1-11 in kcal/mol, major interactions and the residues with which compounds interacted are summarized in Table 4.

2D interaction of compound 1 with 3kp9 is shown in Fig. 6.

Table 4
Summary of docking results of compounds 1-12.

Compounds	Docking score kcal/mol	Interactions	Amino Acids
1	-9.3	Hydrogen Bonding, Carbon Hydrogen Bonding, pi-Sigma, pi-Sulphur and pi-Alkyl Bonding, Van der Waals Forces	Alanine (ALA A: 73), Threonine (THR A: 34), Methionine (MET A: 118), Threonine (THR A: 72), Cysteine (CYS A: 133)
2	-9.5	pi-Sigma, pi-Sulphur, pi-pi Stacked and pi-Alkyl Bonding	Methionine (MET A: 118), Cysteine (CYS A: 133), Phenylalanine (PHE A: 114), Leucine (LEU A: 37), Alanine (ALA A: 65 and A: 136)
3	-9.1	Hydrogen Bonding, Carbon Hydrogen Bonding, pi-Alkyl Bonding, Van der Waals Forces	Lysine (LYS A: 41), Alanine (ALA A: 136, A: 73, A: 65), Leucine (LEU A: 37, A 60, A: 121), Valine (VAL A: 59, A: 140)
4	-11.6	Hydrogen Bonding, pi-pi Stacked, Amide pi Stacked, pi-Sulphur and pi-Alkyl Bonding, Van der Waals Forces	Isoleucine (ILE A: 137). Phenylalanine (PHE A: 114), Valine (VAL A: 75, A: 140), Alanine (ALA A: 65, A: 73, A: 136)
5	-8.8	Hydrogen Bonding, Carbon Hydrogen Bonding, pi-Sulphur and pi-Alkyl Bonding, Van der Waals Forces	Lysine (LYS A: 41), Methionine (MET A: 118), Alanine (ALA A: 65, A: 73, A: 136) Isoleucine (ILE A: 137), Valine (VAL A: 59, A: 140), Leucine (LEU A: 37, A: 60, A: 121)
6	-9.4	Hydrogen Bonding, Carbon Hydrogen Bonding, pi-Sulphur and pi-Sigma Bonding, Van der Waals Forces	Arginine (ARG A: 63), Valine (VAL A: 59), Methionine (MET A: 118), Threonine (THR A: 72), Cysteine (CYS A; 133), Alanine (ALA A: 65, A: 73, A: 136)
7	-9.7	Hydrogen Bonding, pi-pi Stacked, pi-Alkyl, pi-Sigma, and pi-Sulphur Bonding	Threonine (THR A: 72), Cysteine (CYS A: 133), Methionine (MET A: 118), Leucine (LEU A: 37), Phenylalanine (PHE A: 114), Methionine (MET A: 122, A: 111)
8	-7.8	Hydrogen Bonding, pi-Cation, pi-pi T-shaped, pi-Alkyl, and Alkyl Bonding, Van dar Waals Forces	Glutamine (GLN A:213), Arginine (ARG A: 63), Glutamic acid (GLU A: 217), Tryptofan (TRP A: 64), Lysine (LYS A: 216), Valine (VAL A: 125), Tyrosine (TYR A: 120)
9	-8.6	Carbon Hydrogen Bonding, pi-Sulphur, pi-Alkyl, pi-Sigma, and pi-Sulphur Bonding, Van dar Waal Forces	Methionine (MET A: 111, A; 118) Cysteine (CYS A: 133), Alanine (ALA A: 65, A: 136), Valine (VAL A: 59, A: 140) Leucine (LEU: 121, A: 126)

Compounds	Docking score kcal/mol	Interactions	Amino Acids
10	-8.9	Hydrogen Bonding, Carbon Hydrogen Bonding, pi-Sigma, pi-Sulphur, Amide pi-Stacked and pi-Alkyl Bonding	Lysine (LYS A: 41), Threonine (THR A: 34), Methionine (MET A: 111), Cysteine (CYS A: 133), Tryptophan (TRP A: 64), Valine (VAL A: 59, A: 140), Alanine (ALA A: 65, A: 136)
11	-9.7	Hydrogen Bonding, Carbon Hydrogen Bonding, Halogen, pi-Sulphur, pi-pi Stacked and pi-Alkyl Bonding	Glycine (GLY A: 76), Methionine (MET A: 118), Cysteine (CYS A: 133) Phenylalanine (PHE A: 114), Alanine A: 73, A: 136), Leucine (LEU A: 37, A: 121, A: 126)

Experimental

Materials And Methods

All reagents and chemicals were purchased from sigma aldrich, fluka, BDH and used as received. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$-*d* on Bruker biospin ICON-NMR spectrometer and AVANCE AV-300 spectrometer (US) using TMS as internal reference. The apparent resonance multiplicity is described as s (singlet), br (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) and m (multiplet). Infrared measurements were recorded in 400-4000cm^{-1} on a spectrum 2000 FTIR spectrophotometer by Perkin Elmer (USA). Melting point was determined in a capillary tube using a Gallenkamp (UK) electrothermal melting point apparatus. The enantiomeric excess was determined by chiral column, Lux 5 µm Cellulose-1, LC Column 250x4.6 mm(USA). The instrument used for this technique was HPLC PerkinElmer (USA). Analysis of sample with an evaporation point of max. 300^{o}C; mass range: m/z 30-800 amu. VG Instruments autospec/EBEE-Geometry was used to record mass spectra. Electron impact (EIMS) yield mostly fragment ions. Molecular ions are not always observed. High resolution features with ca 6000-8000 resolution. CD spectra were measured by JASCO-815 CD spectrometer (USA) in static mode. For CD measurements, the warfarin analogues were dissolved in a mixture of aqueous phosphoric acid and acetonitrile having ratio 4:6 and pH 2.

General procedure for synthesis of analogues of 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one

Scheme 1 presented complete synthetic strategies. In first step α,β unsaturated ketones (chalcones) were synthesised by base catalysed aldol condensation [22, 23] and their structures were confirmed by the comparison of their melting points and IR data with the reported values.

Then to prepare [21] warfarin analogues 1-11; 4-hydroxycoumarin (0.32mmol), α,β unsaturated ketones (0.2mmol), and 20 mol % 9-amino-9-deoxy epiquinine were dissolved in about 20 ml of dry DCM in round

bottom flask, followed by addition of 40 mol % trifluroacetic acid (TFA) as additive. The reaction mixture was stirred at room temperature for 3 to 4 days and the progress of reactions was monitored by TLC visualized under UV lamp and developed in vanillin spray. Crude sample obtained had unreacted reactants and product only and no other by-product, which was purified by column chromatography using different ratios of n-hexane and ethyl acetate. A single spot of product was obtained on TLC after column chromatography and purity was further verified by HPLC.

Circular Dichroism Studies

CD spectra were measured by *JASCO-815* CD spectrophotometer in static mode. For CD measurements, 200µg/ml of samples were prepared by dissolving synthesized compounds in a mixture of aqueous phosphoric acid and acetonitrile (4:6) having pH 2.0.

As regards the measuring parameters, wavelength range was 170-400nm, data interval time 1sec, response time 2sec, spectral band width 1nm, number of accumulations 3, and optical path length 10mm were used as provided in *JASCO* CD spectrometer's instrumental manual for CD measurement of warfarin.

All the synthesized products showed maximum UV absorbance band between 190 to 220nm (Table 1). In CD, the cotton effect of the band at the value of the maximum absorption of UV was compared with the reported cotton effect of warfarin provided in *JASCO* CD spectrometer's instrumental manual. A negative cotton effect in CD in particular absorption indicates the *(S)* enantiomer in excess. The CD spectra are given in Fig. 5.

Chiral HPLC

Enantiomeric excess was determined by chiral stationary phase HPLC using Lux 5 µm cellulose -1, LC column 250x4.6 mm. Mixture of n-hexane and isopropanol in ratio of 60:40 with 0.1% formic acid was used as eluent using flow rate of 1ml/min and data acquisition time was 10min.

4-hydroxy-3-[1-(4-hydroxy-3-methoxyphenyl)-3-oxobutyl]-2H-chromen-2-one (1)
Compound 1 was obtained as dirty white solid (Yield 90%); m.p. 139-141°C; ee 91% *[S]*; IR υmax 1720, 1625, 1510, 1380 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.89 (1H, dd, J_1 = 8.14Hz, J_2 = 1.5Hz, ArH), 7.79 (1H, dd, J_1 = 7.12Hz, J_2 = 1.15Hz, ArH), 7.52 (1H, m, ArH), 7.26 (1H, s, ArH), 6.83 (2H, d, J = 2.35Hz, ArH), 6.69 (1H, m, ArH), 4.38 (1H, dd, keto, J_1 = 6.57Hz, J_2 = 4.13Hz, CH), 4.23 (1H, dd, keto J_1 = 7.2Hz, J_2 = 1.45Hz, CH_2), 4.13 (1H, bs, Ar-OH), 4.08 (1H, m, CH_2, keto), 3.84 (3H, s, OCH_3, keto form), 3.83 (3H, s, OCH_3, hemiketal), 2.52 (1H, dd, ketal J_1 = 14.1Hz, J_2 = 2.85Hz, CH_2), 2.45 (1H, dd, J_1 = 14, J_2 = 6.85, CH_2 hemiketal), 2.33 (1H, m, CH hemiketal), 2.29 (1H, s, OH, keto 43%), 2.23 (1H, s, OH, ketal 57%), 1.72 (3H, s, CH_3 keto form), 1.65 (3H, s, CH_3 hemiketal); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.0 (C=O), 161.0 (C=O), 159.0 (C-OH), 158.0 (ArC), 144.0 (ArC), 135.0 (ArC), 132.0 (ArC-OCH_3), 123.0 (ArC-OH), 117.0 (ArC), 115.0 (ArC),

114.0 (ArC), 100.0 (ArC), 99.0 (CH), 77.0 (CH), 39.0 (OCH_3), 35.0 (CH_3); EI MS M.I peak m/z 354.1 [$C_{20}H_{18}O_6^+$] (40%).

4-hydroxy-3-(3-oxo-1,3-diphenylpropyl)-2H-chromen-2-one (2)

Compound 2 was obtained as yellowish brown solid (Yield 80%); m.p.150^oC; ee 70% *[S]*. IR υmax 1741, 1677, 1570, 1380 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 9.78 (1H, s, OH labile proton of enol 69%), 8.03 (2H, m, ArH), 8.00 (1H, s, ArH), 7.94 (1H, d, *J* = 1.7 Hz, ArH), 7.90 (1H, d, *J* = 1.7 Hz, ArH), 7.54 (1H, m, ArH), 7.46 (1H, d, *J* = 1.4 Hz, ArH), 7.42 (1H, q, *J* = 1.7 Hz, ArH), 7.38 (1H, m, ArH), 7.32 (1H, s, ArH), 7.27 (1H, m, ArH), 7.24 (2H, d, *J* = 1.0 Hz, ArH), 7.14 (1H, m, Ar H), 4.86 (1H, d, *J* = 2.4 Hz, CH_2), 4.05 (1H, m, CH of stereogenic center), 3.77 (1H, d, *J* = 2.4 Hz, CH_2), 3.68 (1H, d, *J* = 2.4 Hz, CH keto 31%); ^{13}C NMR (50 MHz, $CDCl_3$) δ 139.9 (C=O), 134.42 (C-OH), 131.7 (ArC), 128.8 (ArC), 128.19 (ArC), 128 (ArC), 126.7 (ArC), 124 (ArC), 116.2 (ArC), 65.0 (CH), 45.0 (CH), 29.6 (CH_2); EI MS, M.I peak m/z 370.2 [$C_{24}H_{18}O_4^+$] (15%).

4-hydroxy-3-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-phenylpropyl)-2H-chromen-2-one (3)

Compound 3 was obtained as brown solid (Yield 68%); m.p. 154^oC; ee 70% *[S]*; IR υmax 1743, 1607, 1513, 1360 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 9.76 (1H, s, OH labile proton of enol 48%), 7.66 (1H, s, ArH), 7.63 (1H, s, ArH), 7.64 (1H, s, ArH),7.62 (1H, s, ArH), 7.61 (2H, s, ArH), 7.48 (2H, s, ArH), 7.46 (2H, s, ArH), 7.45 (1H, s, ArH), 7.35 (1H, s, ArH), 5.35 (1H, s, Ar-OH), 4.47 (1H, d, CH_2, *J* = 4 Hz), 3.83 (1H, d, CH, *J*=6.0Hz), 3.80 (3H, s, OCH_3), 3.76 (1H, m, CH, keto 52%), 3.55 (1H, d, *J*=6.0Hz, CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 206.5 (C=O), 165.7 (C=O), 161.9 (C-OH), 153.5 (ArC), 132.7 (ArC), 123.9 (ArC), 123.2 (ArC), 116.3 (ArC), 115.8 (ArC), 90.8 (CH), 56.0 (CH), 55.0 (CH_2), 30.0 (OCH_3); EI MS M.I peak m/z 416.1 [$C_{25}H_{20}O_6^+$] (5%).

4-hydroxy-3-(3-(naphthalen-3-yl)-3-oxo-1-phenylpropyl)-2H-chromen-2-one (4)

Compound 4 was obtained as white solid (Yield 60%); m.p. 210 ºC; ee 60% *[S]*. IR υmax 1715, 1670, 1508, 1384 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 9.88 (1H, s, OH labile proton of enol 85%), 8.18 (1H, d, ArH, *J* = 1.8 Hz), 8.05 (1H, m, ArH), 7.86 (1H, d, ArH *J* = 6.0 Hz), 7.79 (2H, d, *J* = 5.3 Hz, ArH), 7.69 (1H, dd, *J* = 8.7, 1.8 Hz, ArH), 7.55 (1H, m, ArH), 7.37 (2H, q, ArH, *J* = 1.6 Hz), 7.31 (1H, m, ArH), 7.27 (2H, q, *J* = 1.5 Hz, ArH), 7.23 (1H, m, ArH), 5.92 (1H, d, *J* = 5.0 Hz, CH_2), 4.70 (1H, d, *J* = 4.9 Hz, CH_2), 2.10 (1H, s, CH of keto 15%), 1.17 (1H, m, CH, steric center);^{13}C NMR ($CDCl_3$, 50 MHz) δ 204.01 (C=O), 167 (C=O), 133.45 (C-OH), 133.0 (ArC), 129.7 (ArC), 127.6 (ArC), 127.2 (ArC), 126.6 (ArC), 123.9 (ArC), 77.1 (CH), 36.7 (CH_2), 32.4 (CH_2). 27.28 (CH); EI MS M.I peak m/z 403.1 [$C_{27}H_{15}O_4^+$] (20%), 402.1 [$C_{27}H_{14}O_4^+$] (65%), 403.1 [$C_{27}H_{15}O_4^+$] (20%), 326.1 [$C_{21}H_{10}O_4^+$] (28%), 325.1 [$C_{21}H_9O_4^+$] (100%), 282.1 [$C_{17}H_{14}O_4^+$] (16%), 252.1 [$C_{16}H_{12}O_3^+$] (12%), 176.1 [$C_{11}H_{12}O_2^+$] (6%), 127 [$C_6H_7O_3^+$] (14%), 121 [$C_7H_5O_2^+$] (12%), 77.1 [$C_6H_5^+$] (6%), 51.1 [$C_4H_3^+$] (4%), 26.9 [$C_2H_3^+$] (6%).

4-hydroxy-3-(1-(4-hydroxy-3-methoxyphenyl)-3-(2-hydroxyphenyl)-3-oxopropyl)-2H-chromen-2-one (5)

Compound 5 was obtained as brownish solid (Yield 70%); m.p 192ºC; ee 60% *[S]*; IR υmax 1700, 1620 1480, 1390 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.94 (3H, d, *J* = 8.4 Hz, ArH), 7.64 (1H, m, ArH), 7.54 (1H, dd, *J* = 7.1, 1.5 Hz, ArH), 7.47 (1H, m, ArH), 7.35 (1H, d, *J* = 5.3 Hz, ArH), 7.32 (2H, s, ArH), 6.80 (1H, d, *J* = 8.2 Hz, ArH), 6.65 (1H, m, ArH), 6.0 (1H, s, OH of enol 60%), 5.25 (1H, m, CH of enol 40%), 4.26 (2H, d, *J* = 4.3 Hz, CH_2), 4.21 (1H, s, OH), 4.15 (1H, dd, *J* = 1.7, 5.9 Hz, CH), 4.09 (1H, s, Ar-OH), 3.79(1H, d, CH), 3.68 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 200.0 (C=O), 164.8 (C=O), 152.35 (C-OH), 146.7 (ArC), 144.6 (ArC), 132.8(ArC), 126.8(ArC), 124.9(ArC), 119.5(ArC), 116.7(ArC), 114.5(ArC), 109.5(CH), 60.4(CH_2), 56.1, 35.7(CH), 30.9(OCH_3); EI MS M.I peak m/z 432.1 [$C_{25}H_{20}O_7^+$] (3%).

3-[3-(4-aminophenyl)-3-oxo-1-phenylpropyl]-4-hydroxy-2H-chromen-2-one (6)

Compound 6 was obtained as black solid (Yield 80%); m.p. 137 ºC; ee 50% *[S]*; IR υmax 1700, 1610, 1495, 1300 cm^{-1}. ^1H NMR (DMSO, 200 MHz) δ 7.86 (2H, d, *J* = 1.7 Hz, ArH), 7.82 (2H, d, *J* = 1.9 Hz, ArH), 7.66 (2H, ddd, *J* = 8.6, 7.1, 1.7 Hz, ArH), 7.39 (4H, m, ArH), 7.27 (2H, m, ArH), 7.11 (1H, m, ArH), 5.61 (2H, s, NH_2), 2.24 (1H, m, CH of keto 38%), 2.22 (1H, m, CH, steric center), 2.0 (1H, m, OH of enol 62%), 1.25 (1H, d, CH2, *J* = 7.2 Hz), 1.09 (1H, m, CH_2); ^{13}C NMR (DMSO, 50 MHz) δ 207.0 (C=O), 165.6 (C=O), 161.9 (C-OH), 153.5 (ArC-NH_2), 132.7 (ArC-OCH_3), 123.9 (ArC), 123.2 (ArC), 116.3 (ArC), 115.7 (ArC-OH), 90.1 (C-H), 70.0 (CH_2), 32.0 (OCH_3), 30.0 (CH); EI MS M.I peak m/z 290.1 [$C_{19}H_{16}O_2N^+$] (16%), 223.1 [$C_{14}H_{511}O_2N^+$] (14%),162 [$C_9H_6O_3^+$] (43%) 121 [$C_7H_5O_2^+$] (12%), 120.0 [$C_7H_4O_2^+$] (100%), 77.1 [$C_6H_5^+$] (15%), 51.0 [$C_4H_3^+$] (14%), 26.9 [$C_2H_3^+$] (6%).

3-[3-(4-chlorophenyl)-3-oxo-1-phenylpropyl]-4-hydroxy-2H-chromen-2-one (7)

Compound 7 was obtained as brownish sticky solid (Yield 76%); m.p.166 ºC; ee 24% *[S]*; IR υmax 1720, 1625, 1500, 1300 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 9.52 (1H, s, OH of enol 43%), 7.97 (1H, t, *J* = 1.9 Hz, ArH), 7.91 (2H, m, ArH), 7.66 (1H, t, ArH, *J* = 3.4 Hz), 7.62 (1H, d, ArH, *J* = 3.4 Hz), 7.49 (1H, d, ArH, *J* = 4.0 Hz), 7.45 (1H, d, ArH, *J* = 2.4 Hz), 7.42 (2H, t, *J* = 1.9 Hz, ArH), 7.38 (1H, d, *J* = 1.6 Hz, ArH), 7.26 (2H, dd, ArH), 7.14 (1H, m, ArH), 4.86 (1H, dd, *J* = 9.9, 2.5 Hz, CH_2), 4.37 (1H, m, CH, steric center), 3.68 (1H, dd, *J* = 19.1, 2.6 Hz, CH_2), 2.28 (1H, s, OH), 1.60 (1H, d, *J* = 6 Hz, CH of keto 53%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 167.7 (C=O), 132.5 (C-OH), 131.8 (ArC), 130.8 (ArC), 130.0 (ArC), 129.2 (ArC), 128.8 (ArC), 128.2 (ArC), 128.5 (ArC), 116.0 (C-H), 68.0 (CH_2), 38.8 (CH); EI MS M.I peak m/z 404.2 [$C_{24}H_{17}O_4Cl^+$] (5%).

3-(3-(4-chlorophenyl)-1-(3,4,5-trimethoxyphenyl)-3-oxopropyl)-4-hydroxy-2H-chromen-2-one (8)

Compound 8 was obtained as medium brown solid (Yield 65%); m.p. 110ºC; ee 41% *[S]*; IR υmax 1725, 1600, 1510, 1330 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.95 (1H, m, ArH), 7.65 (1H, t, *J* = 3.0 Hz, ArH), 7.61 (1H, d, *J* = 2.5 Hz, ArH), 7.46 (1H, m, ArH), 7.37 (1H, dd, *J* = 8.6, 3.8 Hz, ArH), 7.29 (1H, s, ArH), 7.13 (1H, m, ArH), 7.03 (1H, m, ArH), 6.29 (1H, s, ArH), 6.12 (1H, s, ArH), 4.16 (1H, d, *J* = 1.6 Hz, CH_2), 4.14 (1H, d, *J* = 1.8 Hz, CH_2), 3.85 (3H, s, OCH_3), 3.69 (1H, m, OH, enol 49%), 3.63 (1H, m, OCH_3), 3.58 (1H, s, CH, steric center),

3.45 (1H, d, CH of keto 51%), 3.44 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 206.9 (C=O), 153.0 (C=O), 152.9 (C-OH), 134.3 (ArC-Cl), 132.5 (ArC), 131.5 (ArC), 130.9 (ArC), 129.4 (ArC), 128.8 (ArC), 128.7 (ArC), 128.50 (ArC), 126.55 (ArC), 107.4 (ArC), 106.2 (CH), 105.2 (ArC), 68.2 (CH_2), 56.26 (CH), 56.16 (OCH_3), 55.82 (OCH_3), 38.76 (OCH_3), 30.90 (OCH_3), 29.69 (OCH_3). EI MS M.I peak m/z 383.5 [$C_{21}H_{16}O_5Cl^+$] (2%), 279.2 [$C_{18}H1_5O_3^+$] (6%), 212.1 [$C_{14}H_{12}O_2^+$] (18%), 167.1 [$C_{12}H_7O^+$] (28%), 149.0 [$C_9H_9O_2^+$] (100%), 121.1 [$C_7H_5O^+$] (10%), 71[$C_4H_7O^+$] (21%), 57 [$C_3H_5O^+$] (35%), 43[$C_2H_3O^+$] (24%).

3-(1,3-bis(4-chlorophenyl)-3-oxopropyl)-4-hydroxy-2H-chromen-2-one (9)

Compound 9 was obtained as offwhite solid (Yield 62%); m.p. 122 ºC. ee 23% *[S]*; IR υmax 1710, 1625, 1500, 1360 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) *δ* 9.64 (1H, s, OH of labile proton of enol 60%), 7.96 (1H, d, *J* = 1.9 Hz, ArH), 7.93 (2H, d, *J* = 2.0 Hz, ArH), 7.89 (1H, d, *J* = 1.7 Hz, ArH), 7.42 (2H, d, *J* = 2.1 Hz, ArH), 7.29 (1H, d, *J* = 2.2 Hz, ArH), 7.26 (2H, d, *J* = 2.6 Hz, ArH), 7.22 (1H, s, ArH), 7.12 (1H, m, ArH), 5.19 (1H, dd, *J* = 10.2, 1.6 Hz, CH_2), 4.80 (1H, dd, *J*= 10.2, 2.3 Hz, CH_2), 4.27 (1H, m, CH, steric center), 3.67 (1H, d, *J* = 2.3 Hz, CH of keto 40%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.28 (C=O), 163.0 (C-OH), 130.0 (ArC), 129.5 (ArC), 129.2 (ArC), 128.2 (ArC), 34.0 (CH), 30.0 (CH_2), 29.12 (CH_2), 14.10 (CH); EI MS M.I peak m/z 429.1 [$C_{23}H_{18}O_4Cl_2^+$] (3%), 383.4 [$C_{22}H_{20}O_4Cl^+$] (3%), 183 [$C_{13}H_{11}O^+$] (10%), 167 [$C_9H_{11}O_3^+$] (30%), 149.0 [$C_9H_9O_2^+$] (100%), 71 [$C_4H_7O^+$] (33%), 57 [$C_3H_5O^+$] (60%).

3-(3-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-oxopropyl)-4-hydroxy-2H-chromen-2-one (10)

Compound 10 was obtained as yellowish white solid (Yield 60%); As product was sticky so melting point cannot be determined; ee 98% *[S]*; IR υmax 1745, 1600, 1480, 1300 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) *δ* 7.71 (1H, s, ArH,), 7.65 (2H, d, *J* = 3.3 Hz, ArH), 7.64 (1H, s, ArH), 7.62 (2H, d, *J* = 3.3 Hz, ArH), 7.55 (1H, d, *J* = 19.5 Hz, ArH), 7.48 (2H, s, ArH), 7.46 (2H, s, ArH), 7.45 (1H, s, ArH), 7.43 (1H, s, ArH), 5.26 (1H, d, *J* = 5.3 Hz, CH_2), 5.22 (1H, d, *J* = 1.5 Hz, CH_2), 5.19 (1H, d, *J* = 1.6 Hz, CH of enol 46%), 5.16 (1H, m, CH, steric center), 4.16 (3H, s, OCH_3), 4.03 (1H, bs, OH of enol 54%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 200.04 (C=O), 173.3 (C=O), 150.55 (C-OH), 135.12 (ArC), 132.21 (ArC), 131.01 (ArC-Cl), 128.8 (ArC-OCH_3), 128.67 (ArC), 127.87 (ArC), 125.44 (ArC), 125.23 (ArC), 123.22 (ArC), 122.57 (ArC), 122.23 (ArC), 121.56 (ArC), 110.21 (CH), 68.12 (CH_2), 38.76 (CH), 22.98 (OCH_3); EI MS M.I peak m/z 439.4 [$C_{24}H_{23}O_5Cl^+$] (22%), 383.5 [$C_{21}H_{16}O_5Cl^+$] (23%), 257 [$C_{18}H_9O_2^+$] (28%), 183 [$C_{13}H_{11}O^+$] (100%), 71[$C_4H_7O^+$] (33%), 57 [$C_3H_5O^+$] (80%), 43 [$C_3H_3O^+$] (54%).

3-[3-(4-chlorophenyl)-1-(4-fluorophenyl)-3-oxopropyl]-4-hydroxy-2H-chromen-2-one (11)

Compound 11 was obtained as brownish solid (Yield 72%); m.p. 134^{o}C; ee 96% *[S]*; IR υmax 1720, 1610, 1490, 1300 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 9.62 (1H, s, OH, labile proton of enol 44%), 7.96 (1H, d, *J* = 1.9 Hz, ArH), 7.93 (2H, d, *J* = 1.9 Hz, ArH), 7.63 (1H, m, ArH), 7.46 (1H, m, ArH), 7.43 (2H, d, *J* = 1.9 Hz, ArH), 7.39 (1H, d, *J* = 2.1 Hz, ArH), 7.29 (1H, m, ArH), 7.15 (1H, dd, *J* = 8.1, 1.3 Hz, Ar H), 6.94 (2H, d, ArH, *J* = 8.6

Hz), 4.82 (1H, d, *J* = 10.0 Hz, CH_2), 4.37 (1H, dd, *J* = 19.1, 10.2 Hz, CH_2), 4.16 (1H, d, *J* = 1.6 Hz, CH of keto 51%), 3.68 (1H, s, CH of stereogenic center); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 206.91 (C=O), 117.27 (O-C=O), 150.25 (C-OH), 131.89 (ArC-Cl), 130.87 (ArC-F), 130.03 (ArC), 129.81 (ArC), 129.66 (ArC), 129.23 (ArC), 128.80 (ArC), 123.95 (ArC), 116.26 (ArC), 115.18 (ArC), 114.76 (ArC), 68.17 (CH_2), 38.76 (CH), 30.90 (CH); EI MS M.I peak m/z 422.2 [$C_{24}H_{16}O_4FCl^+$] (10%).

Bilogical Experiments

Plasma recalcification time (PRT) method.

Anticoagulant potential of test compounds was determined by *PRT* method [24]. The blood samples were obtained from healthy volunteers in tubes containing 3.8% sodium citrate (9:1) in order to prevent the clotting process. Centrifugation (15 min. at rate 3000 rpm) was carried out to obtain platelet poor plasma. 0.2 ml plasma, 0.1 ml of different concentration of test compounds (100, 300 and 1000 µM) and 0.3 ml of $CaCl_2$ (25 mM) were added together in a clean fusion tube and incubated at 37^oC in a water bath. Warfarin was used as positive control. The clotting time was recorded with a stopwatch by tilting the test tubes every 5 sec and results are shown in form of IC_{50} values (Fig. 6 and Fig. 7).

Microplate alamar blue assay (maba) method.

Different derivatives of 4-hydroxycoumarin including warfarin were screened for their antibacterial activities against *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative), *Bacillus subtillis, Staphyloccus aureus*, and *Salmonella typhi* (gram-positive) by microplate alamar blue assay (maba) [25] using DCM as solvent. The ofloxacin was used as a standard drug. The applied concentration of compounds was 200µg/ml. The zone of inhibition was measured in mm (millimeters) and then %inhibition was calculated (Table 2).

Agar tube dilution method.

The antifungal activities of all synthesized derivatives of 4-hydroxycoumarin were evaluated with the help of agar tube dilution method [26]. Concentration of samples was 400µg/ml of DMSO Incubated at 37^oC and incubation period was 7days. The tested fungal strains were Inhibition *Trichphyton rubrum, Candida albicans, Aspergillus niger, Microsporum canis, Fusarium lini and Canadida glabrata*. The Amphotericin B was used as standard drug. The antifungal activities of the compounds were measured in % inhibition (Table 3).

In silico molecular docking studies

Molecular docking studies were carried out by using Discovery studio 2016, Chemsketch, AutoDock tools-1.5.6 and PyRx. First acquired crystal structure of VKOR1 (PDB ID:3kp9) in PDB format from RCSB Protein data bank, [15] then already attached ligand was removed. Ligands were drawn in chemsketch

and assigned smile notation and then open babel was used to add hydrogens and 3D coordinates to convert structures in PDB format. AutoDock tools were used to add polar hydrogens, kollman charges, compute gasteiger charges and set grid box in protein structure and saved it as PDBQT format. Then opened ligand in it and chose torsion for AutoDock using upto 12 torsional degree of freedom (DOF) and then saved it too in PDBQT format. Finally, docking of ligands with VKOR1 was carried out using AutoDock Vina [27].

Warfarin is a vitamin K antagonist drug. In the present study the binding affinity of warfarin was compared with the series of newly synthesized substituted warfarin analogues. The amino acids of 3kp9 pocket surrounded by the ligand are given in Table 5. Most of synthesized compounds have open chain form predominantly due to addition of bulky group instead of methyl group of warfarin.

Table 5
Amino acids of 3kp9's pocket surrounded by ligand

Names of amino acids	Type	Three letter code
Valine	Hydrophobic	VAL: 59, 75, 140
Isoleucine	Hydrophobic	ILE: 137
Glutamic acid	Acidic	GLU: 115
Threonine	Nucleophilic	THR: 34, 72
Alanine	Small	ALA: 65,73,136
Glycine	Small	GLY: 76
Phenylalanine	Aromatic	PHE: 114
Leucine	Hydrophobic	LEU: 33, 37, 121, 126
Methionine	Hydrophobic	MET: 111, 118, 122
Cysteine	Nucleophilic	CYS: 133
Tyrosine	Aromatic	TYR: 132
Glutamine	Amide	GLN: 213
Arginine	Basic	ARG: 63
Tryptophan	Aromatic	TRP: 64
Lysine	Hydrophilic	LYS: 41, 133

Conclusions

Overall, 11 warfarin analogues were successfully synthesized, characterized and screened for anticoagulant, antibacterial and antifungal activities in this work. Enantioselectivity ranged from 23-98% having *(S)* enantiomer as major product. The configurations were assigned by using CD spectroscopy and comparison of reported warfarin spectrum. Moreover docking studies clearly showed the better binding affinity of the keto form. So structure of warfarin analogues was modified in such a way that keto form is formed predominantly and chance of its hemiketal form is reduced. This target was successfully achieved, as clearly reflected by proton NMR results. Moreover, almost all the synthesized compounds have shown improved anticoagulant activity. These results are very encouraging as a clear correlation between docking studies and *in-vitro* anticoagulant studies are seen. IC_{50} value of synthesized compounds showed improved IC_{50} values (249.88-376.61μM) than standard drug warfarin (408.70μM) except compound 9 (419.22 μM). Compound 4 and 7 showed 68-68.25% inhibition against gram positive strains of bacteria. Compound 6 shows 70% inhibation against fungal strain *candida albicans*. This research work showed that in most of the cases, by limiting the tautomeric forms in warfarin analogues, the anticoagulant activity was improved. These compounds appear to be good candidates for further biological and clinical evaluation.

Declarations

Acknowledgements. NR is grateful to Prof. Dr. Tibor Kurtan, University of Debrecen, Hungary for provision of the catalyst. ZA is thankful to Higher Education Commission Pakistan for IRSIP award and Ruhr University Bochum Germany for hosting.

Conflict of interest

The authors have declared that there is no conflict of interest.

References

1. Kresge N, Simoni RD, Hill RL The finding that prothrombin contains γ-carboxyglutamic acid: the work of Johan Stenflo. Journal of Biological Chemistry. 2009 May 1;284(18):e1-. **ISSN**: 0021-9258
2. Aguilar MI, Hart R Oral anticoagulants for preventing stroke in patients with non-valvular atrial fibrillation and no previous history of stroke or transient ischemic attacks.Cochrane Database of Systematic Reviews. 2005(3). https://doi.org/10.1002/14651858.CD001927.pub2
3. Porter WR (2010 Jun) Warfarin: history, tautomerism and activity. Journal of computer-aided molecular design. 24:553–573. https://doi.org/10.1007/s10822-010-9335-7. 6
4. Fang MC, Chang Y, Hylek EM, Rosand J, Greenberg SM, Go AS, Singer DE Advanced age, anticoagulation intensity, and risk for intracranial hemorrhage among patients taking warfarin for atrial fibrillation.Annals of Internal Medicine. 2004 Nov16;141(10):745–52. https://doi.org/10.7326/0003-4819-141-10-200411160-00005

5. Pirmohamed M (ed) (2006) Br J Clin Pharmacol. ;62(5):509-11.https://doi.org/10.1111/j.1365-2125.2006.02806.x
6. Wells PS, Holbrook AM, Crowther NR, Hirsh J Interactions of warfarin with drugs and food. Annals of internal medicine. 1994 Nov 1;121(9):676-83. https://doi.org/10.7326/0003-4819-121-9-199411010-00009
7. Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M (2005) Interactions of warfarin with drugs and food. Arch Intern Med. ;165(1095):106. https://doi.10.1001/archinte.165.10.1095
8. Heimark LD, Trager WF The preferred solution conformation of warfarin at the active site of cytochrome P-450 based on the CD spectra in octanol/water model system.Journal of medicinal chemistry. 1984Aug; 27(8):1092–5. https://doi.org/10.1021/jm00374a027
9. Guasch L, Peach ML, Nicklaus MC Tautomerism of warfarin: combined chemoinformatics, quantum chemical, and NMR investigation. The Journal of organic chemistry. 2015 Oct 16;80(20):9900-9. https://doi.org/10.1021/acs.joc.5b01370
10. Halland N, Hansen T, Jørgensen KA Organocatalytic asymmetric Michael reaction of cyclic 1, 3-dicarbonyl compounds and α, β-unsaturated ketones—a highly atom-economic catalytic one-step formation of optically active Warfarin anticoagulant. Angewandte Chemie. 2003 Oct 20;115(40):5105-7. https://doi.org/10.1002/ange.200352136
11. Rezaee E, Shadzad HR, Nazari M, Tabatabai SA (2021 Aug) Design, synthesis, and biological evaluation of some 1, 2, 3-triazole derivatives as novel amide-based inhibitors of soluble epoxide hydrolase. Med Chem Res 2:1–9. https://doi.org/10.1007/s00044-021-02752-3
12. D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, Brancaccio V, Grandone E, Margaglione M A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin.Blood. 2005 Jan15;105(2):645–9. https://doi.org/10.1182/blood-2004-06-2111
13. Kasperkiewicz K, Małecka M, Ponczek MB, Nowak P, Budzisz E Design, synthesis, X-ray structures of the new coumarin derivatives and perspectives of binding them to albumin and vitamin K epoxide reductase complex subunit 1. Crystal Growth & Design. 2016 Jan 6;16(1):456-66. https://doi.org/10.1021/acs.cgd.5b01456
14. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ et al (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47(7). https://doi.org/10.1021/jm0306430
15. Li W, Schulman S, Dutton RJ, Boyd D, Beckwith J, Rapoport TA Structure of a bacterial homologue of vitamin K epoxide reductase. Nature. 2010 Jan;463(7280):507-12. https://doi.10.1038/nature08720
16. Goodstadt L, Ponting CP Vitamin K epoxide reductase: homology, active site and catalytic mechanism. Trends in biochemical sciences. 2004 Jun 1;29(6):289-92. https://doi.org/10.1016/j.tibs.2004.04.004

17. Singh AK, Bhattacharyya-Pakrasi M, Pakrasi HB Identification of an atypical membrane protein involved in the formation of protein disulfide bonds in oxygenic photosynthetic organisms. Journal of biological chemistry. 2008 Jun 6;283(23):15762-70. https://doi.org/10.1074/jbc.M800982200
18. Dutton RJ, Boyd D, Berkmen M, Beckwith J Bacterial species exhibit diversity in their mechanisms and capacity for protein disulfide bond formation. Proceedings of the National Academy of Sciences. 2008 Aug 19;105(33):11933-8. https://doi.org/10.1073/pnas.0804621105
19. Khan KM, Saify ZS, Khan MZ, Zia-Ullah. Hayat S1381–7. https://doi.org/10.1080/14756360409162453
20. Céspedes CL, Avila JG, Martínez A, Serrato B, Calderón-Mugica JC, Salgado-Garciglia R Antifungal and antibacterial activities of Mexican tarragon (Tagetes lucida).Journal of agricultural and food chemistry. 2006 May17;54(10):3521–7. https://doi.org/10.1021/jf053071w
21. Xie JW, Yue L, Chen W, Du W, Zhu J, Deng JG, Chen YC (2007) Highly enantioselective Michael addition of cyclic 1, 3-dicarbonyl compounds to α, β-unsaturated ketones. Organic letters. Feb 1;9(3):413-5. https://doi.org/10.1021/ol062718a
22. Nielsen AT, Houlihan WJ (2004) The aldol condensation. Organic reactions. Apr 15:16:1–438. https://doi.org/10.1002/0471264180.or016.01
23. Climent MJ, Corma A, Iborra S, Primo J Base catalysis for fine chemicals production: Claisen-Schmidt condensation on zeolites and hydrotalcites for the production of chalcones and flavanones of pharmaceutical interest. Journal of Catalysis. 1995 Jan 1;151(1):60-6. https://doi.org/10.1006/jcat.1995.1008
24. Kang IK, Kwon OH, Kim MK, Lee YM, Sung YK In vitro blood compatibility of functional group-grafted and heparin-immobilized polyurethanes prepared by plasma glow discharge. Biomaterials. 1997 Aug 1;18(16):1099-107. https://doi.org/10.1016/S0142-9612(97)00035-5
25. Reis RS, Neves I Jr, Lourenço SL, Fonseca LS, Lourenço MC (2004 May) Comparison of flow cytometric and Alamar Blue tests with the proportional method for testing susceptibility of Mycobacterium tuberculosis to rifampin and isoniazid. Journal of clinical microbiology. 42:2247–8. https://doi.org/10.1128/JCM.42.5.2247-2248.2004. 5
26. Bauernfeind A, Petermüller C (1983 Apr) In vitro activity of ciprofloxacin, norfloxacin and nalidixic acid. European Journal of Clinical Microbiology 2(2):111–5. https://doi.org/10.1007/BF02001575
27. Ewing TJ, Makino S, Skillman AG, Kuntz ID (2001 May) DOCK 4.0: search strategies for automated molecular docking of flexible molecule databases. J Comput Aided Mol Des 15(5):411–428. https://doi.org/10.1023/A:1011115820450

Schemes 1

Schemes 1 is available in the Supplementary Files section

Figures

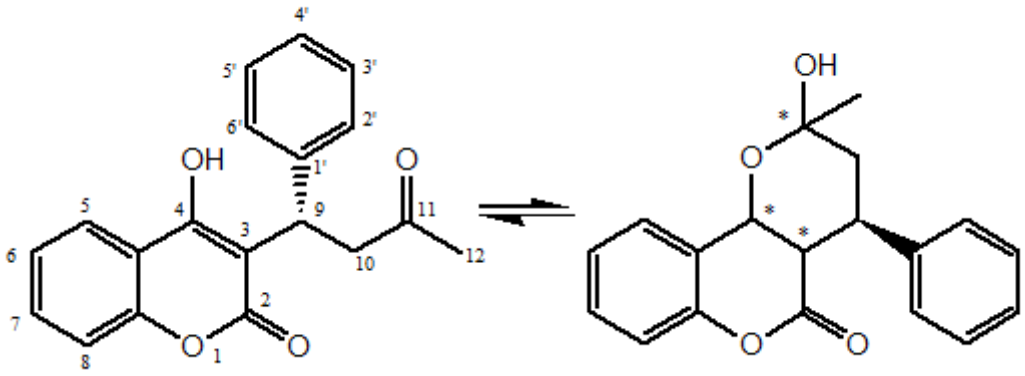

Figure 1

Major tautomeric forms, keto and hemiketal, of (S) warfarin

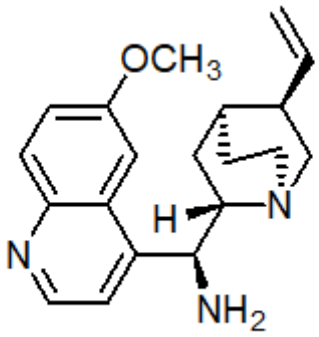

Figure 2

Organocatalyst (9-amino-9-deoxyepiquinine)

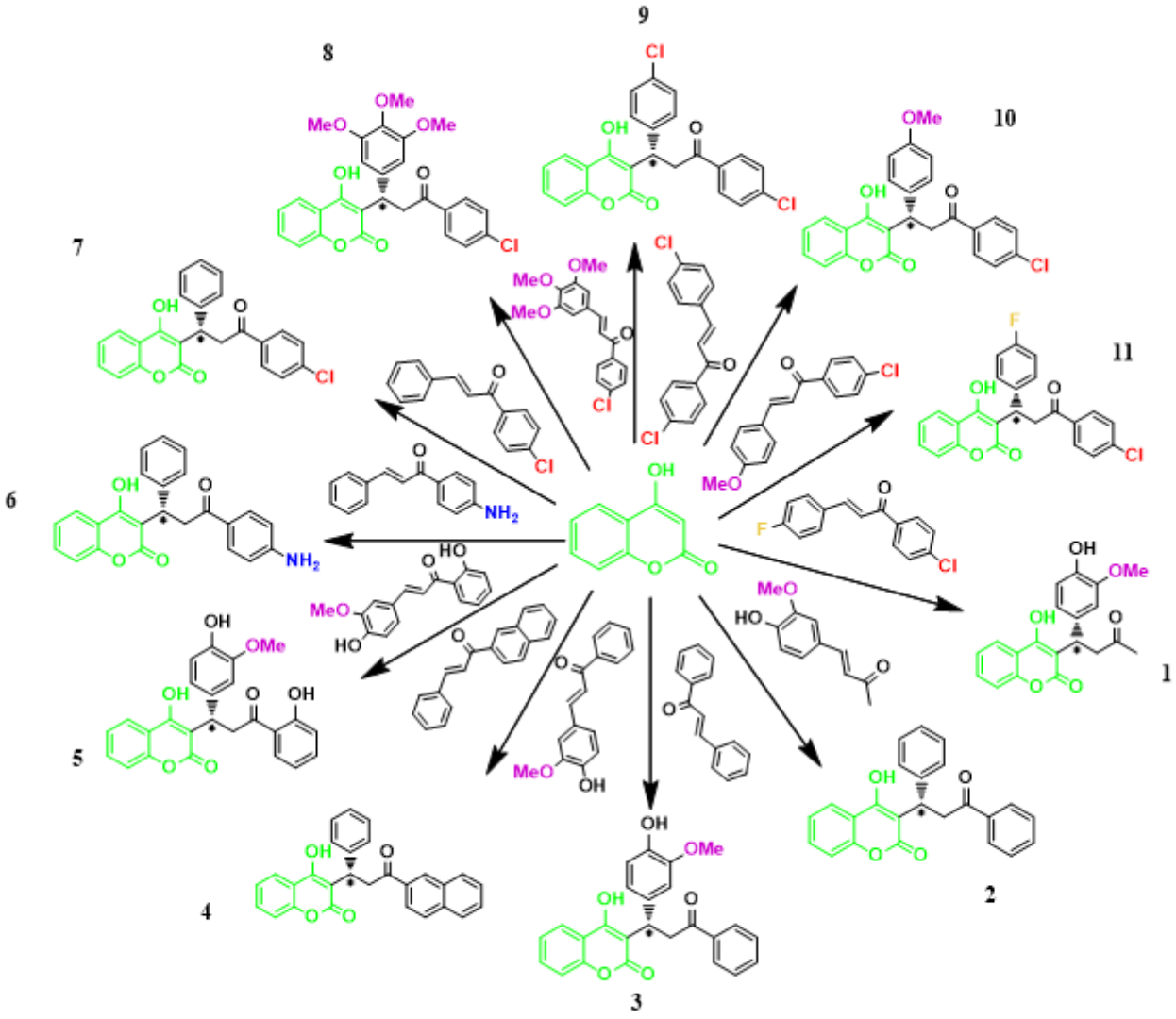

Figure 3

The synthesized analogues of warfarin and their precursors

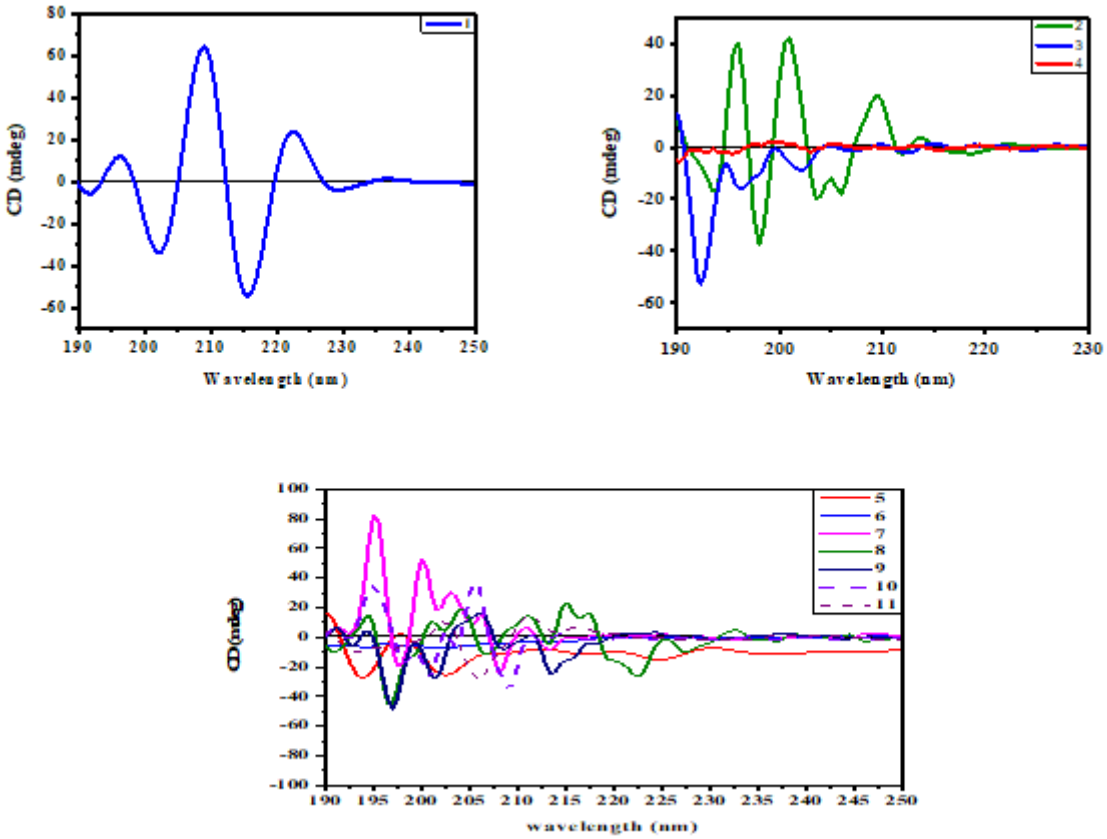

Figure 4

CD spectra of synthesized warfarin analogues

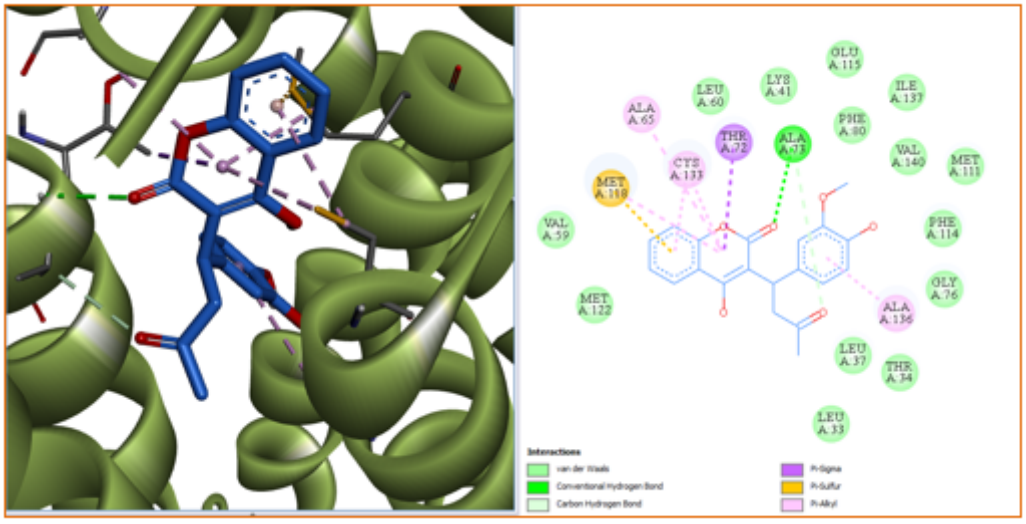

Figure 5

2D interaction of crystal structure of VKOR1 with compound 1

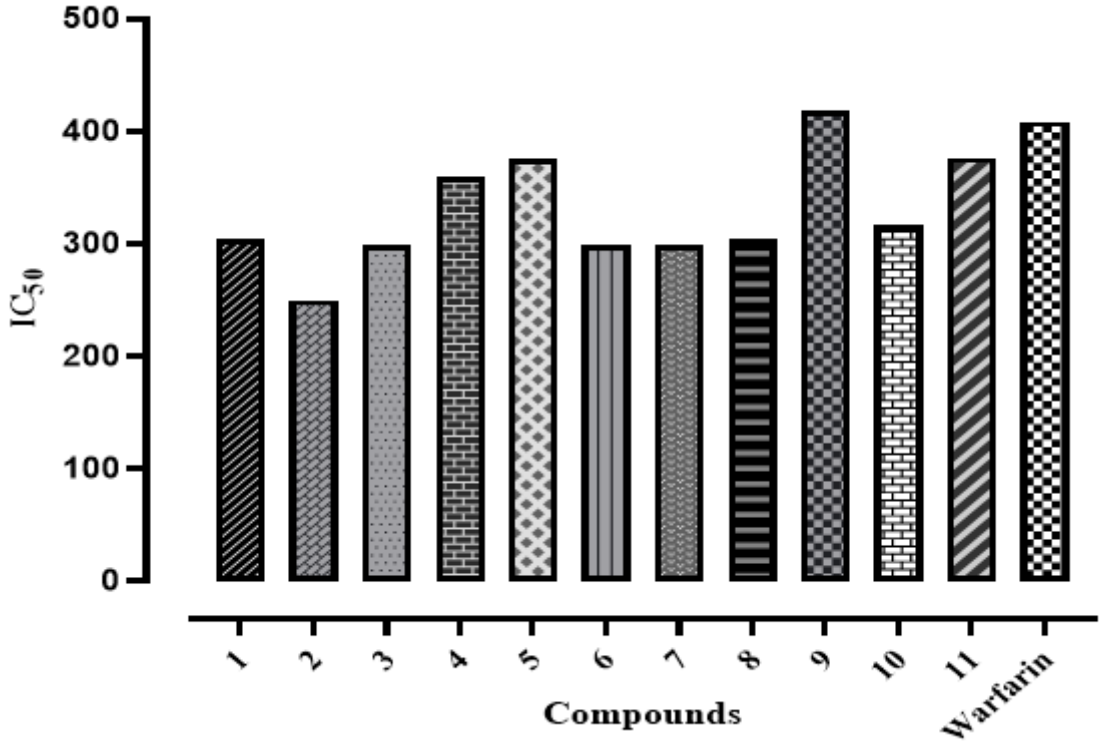

Figure 6

IC50 values of synthesized compounds in comparison of standard drug warfarin

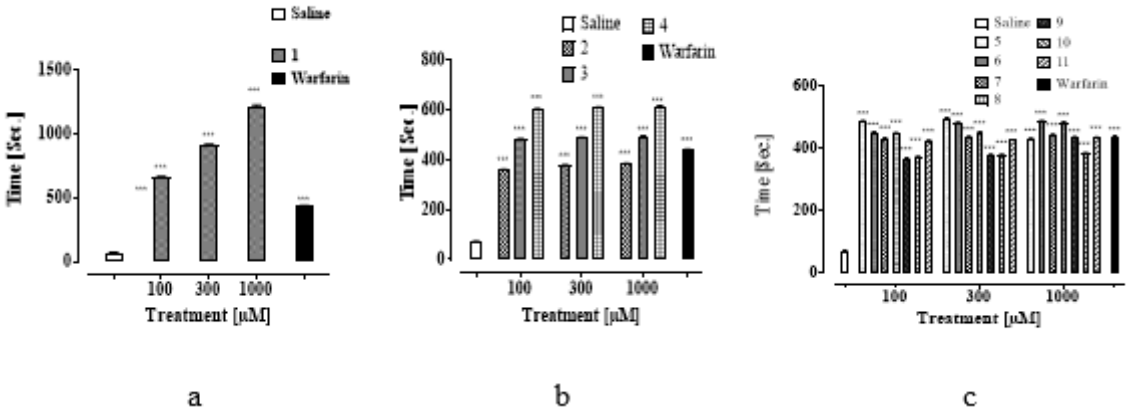

Figure 7

Bar chart showing increase/decrease in plasma recalcification time (PRT) caused by different concentrations of a) compound 1, and warfarin, b) compound 2, 3, 4 and warfarin and c) compound 5, 6, 7, 8, 9, 10, 11 and warfarin. Data expressed as mean ± SEM, n=5, ***P< 0.001 vs. saline group, one way ANOVA with post-hoc Tukey's test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg
- supplimentrymaterial.pdf
- Scheme1.png