Enantiomeric separation of thiourea derivatives of naringenin on amylose and cellulose polymeric chromatographic chiral columns

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Abstract: Due to a great demand for amylose and cellulose polymeric chromatographic chiral columns, the enantiomeric separation of thiourea derivatives of naringenin was achieved on the different amylose (Chiralpak-IB) and cellulose chiral (Chiralcel-OJ and Chiralcel-OD-3R) columns with varied chromatographic conditions. The isocratic mobile phases used were ethanol and methanol, where ethanol/hexane and methanol/hexane were used as gradient mode and were prepared in volume/volume relation. The separation and resolution factors for all the enantiomers were in the range of, 1.25 to 3.47 and 0.48 to 1.75, respectively. The enantiomeric resolution was obtained within 12 minutes making fast separation. The docking studies confirmed the chiral recognition mechanisms with binding affinities in the range of -4.7 to -5.7 Kcal/mol. The reported compounds have good anti- coagulant activities and may be used as anti-coagulants in the future. Besides, chiral separation is fast and is useful for enantiomeric separation in any laboratory in the world.

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1. Introduction

The role of anti-coagulant agents is very important in controlling blood pressure, and arteries blockage. Therefore, blood coagulation must be carefully regulated because inappropriate clot formation can lead to thrombotic complications, such as heart attack, cerebrovascular accident (stroke) and pulmonary embolism. [1] The anti-coagulant drugs work to prevent blood clots (thrombosis) from forming in veins, arteries, or the heart by slowing blood clotting. Generally, phytochemicals, especially flavonoids, are known to prevent and treat these types of diseases, while fruits and vegetables are their natural sources. Naringenin has been used to inhibit the activation of platelet and arterial thrombosis [2], inhibition of rivaroxaban [3], and change ranolazine pharmacokinetics [4]. Also, naringenin is used in perfumes, and cosmetics and have a bioactive effect on human health such as hepatoprotective, anti-inflammatory, anti-mutagenic, anticancer, and anti-microbial agent [5-10]. Naringenin ($C_{15}H_{12}O_5$) is a natural organic compound belonging to the flavanone family, a subclass of flavonoids. It is mainly found in fruits (grapefruit and oranges) and vegetables. While reading the above papers, it was realized that the anti-coagulant activity of naringenin is not quite good and needs modification in its structure. Thiourea is an important class of substances containing sulfur. The synthesis and biological activities evaluation of thiourea compounds has been reported in the literature [11-15]. Hu et al. [16] described biological evaluation and phase transfer catalysts stimulating the one-pot synthesis of N-Aryl-N'-(4-ethyloxy benzoyl)-thiourea derivatives. The other authors reported analgesic and antioxidant [17], anti-tumor agents and antimicrobial [18-21], cytotoxic effects [21,22] and anticancer activity [23, 24] of the thiourea compounds. Therefore, an idea was generated to make a new compound using naringenin and thiourea. Naringenin is a chiral molecule and its enantiomeric separation has been reported in the literature [25-27] but its derivatives have not been synthesized along with their chiral separations.

For many years, our research laboratory was directed toward organic synthesis, the study of the biological activities of the synthesized compounds and their chiral separation using different chromatographic methods [28-43]. Finally, a new bioactive compound was synthesized. The synthesis was carried out in two steps *i.e.* by producing an intermediate compound (I); followed by adding isothiocyanate as a key element. A new desired thiourea derivative (II) was obtained. Both I and II compounds were characterized using spectroscopic methods. The anticoagulant activities of both compounds were tested using activated partial thromboplastin time (APTT) and prothrombin time (PT) assays with human plasma. The synthesized compounds were chiral in nature and, hence, it was necessary to separate the enantiomers due to the importance of enantiomeric resolution in biological and pharmaceutical fields. The enantio-separation was performed by chiral high-performance liquid chromatography as it is an excellent method for chiral separation [44-52]. A variety of polysaccharides-based chiral stationary phases (CSPs) with various mobile phases were used in this study.

2. Experimental:

2.1 Chemicals and reagents

For the synthesis of proposed compounds, phenylisothiocyanate had been purchased from FlukaChemika. Naringenin and secondary amine had been purchased from Sigma-Aldrich. All solvents used in the experiment were HPLC or analytical grade and purchased from Sigma-Aldrich (Seelze, Germany).

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2.2 Instruments used

The following instruments were used in this research work.

2.2.1 Characterization instruments

The melting points were determined using the Büchi® B-545 melting point apparatus equipped with capillary tubes, capable of reaching temperatures up to 400°C. Spectroscopic analyses were conducted employing UV-Vis, FT-IR, and NMR techniques. UV spectra were acquired in various solvents utilizing a UNI-CAM UV300 spectrophotometer. FT-IR spectra were recorded using an Agilent Cary 630 FTIR spectrophotometer equipped with a diamond ATR accessory. NMR analysis was recorded with a super-conducting electromagnet made by the brand Bruker actively armored of 400 MHz frequency monitored by Avance III HD console, corresponding to 9.4 Tesla magnetic field, and an exploitation software Topspin 3.5 PL6.

2.2.2 Chromatographic instruments

The HPLC system utilized was a Shimadzu Scientific Instruments LC-20A (Shimadzu, Kyoto, Japan), comprising a 20 µL Rheodyne 1907 sample loop injector, an LC-20A pump, a vacuum degasser DGU-20A5, and a Shimadzu SPD-20 UV detector with variable wavelength capability. Experiments were conducted at a temperature of 27 ± 1°C. Chromatographic

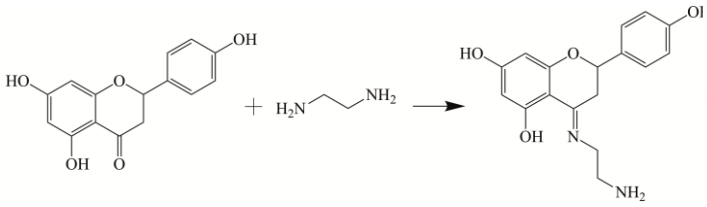
data were collected, stored, and analyzed using LC Lab solution software (Shimadzu, Tokyo, Japan).

2.2.3 Anticoagulant study instruments

PT and APTT assays were measured using the Sysmex CA-50 Semi-automated Coagulation Analyzer (Sysmex Corporation, Hyogo, Japan). The other instruments were centrifuge machine, automatic pipettes, etc.

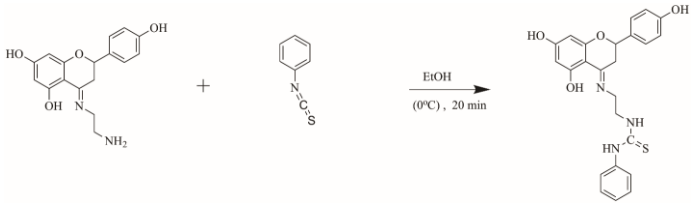
2.3 Synthesis of new thiourea derivatives

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one; 8 mM) and ethylenediamine (8 mM) were refluxed in toluene solvent (5 mL). Phenyl isothiocyanate (5 mg) was added to the mixture at 110°C. After 8 hours a yellow product was obtained and was left for 2 hrs at 25°C. Hexane was added to the product and left for 20 minutes at 25°C. After that, the mixture was passed to the Rota vapor in order to eliminate the solvents (toluene and hexane). In a reflux mounting, the product was mixed with toluene which was added drop by drop through the refrigerant in order to have the total solubility of the product. The mixture was left for 4 hours at 25°C, then put in the freezer for another 4 hrs (Scheme 1). A crystallized product (I) was noticed and filtered using a filter pump. Phenylisothiocyanate was added to the filtered product and dissolved using ethanol in an ice-water bath (0°C) for 20 minutes. The mixture was evaporated at 25°C for 48 hrs. These steps resulted from a crystallized product (II) (Scheme 2). The products were confirmed by UV-Visb, FT-IR, and NMR studies.



(I)

Scheme 1. Synthesis of (E)-4-[(2-aminoethyl) imino)-2-(4-hydroxyphenyl)] chromane-5,7-diol.



(II)

Scheme 2. Synthesis of (E)-1-(2-((5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-ylidene) amino) ethyl)-3-phenylthiourea.

2.4 Sample preparation

Both compounds were prepared in methanol. The concentration of the solution prepared was 1.0 mg/mL. The subsequent dilutions were made as per the requirements.

2.5 Chromatographic conditions

The isocratic mobile phases used were ethanol and methanol, where ethanol/hexane and methanol/hexane were used as gradient mode and were prepared in volume/volume relation. The injection volume was 10 µL with UV detection at 290 nm. Chromatographic separations were performed at room temperature using both isocratic and gradient modes, with flow rates ranging from 0.2 to 0.5 mL/min, subject to adjustment based on specific experimental requirements. A total of eight columns (1 achiral + 7 chiral) were used in this study. C-$_{18}$ ODS stationary phase (250 × 4.6 mm ID, particle size 5 µm) as achiral stationary phase. The chiral polysaccharide-based CSPs used were Chiralpak-IB, Chiralcel-OD, Chiralcel-OD-H, Chiralcel-OJ, Chiralcel-OD-3R, Chiralpak-AD and Chiralpak-AS-3R purchased from Chiral Technologies Europe (Illkirch, France).

2.6 Anticoagulant activity

Activated partial thromboplastin time (APTT) and prothrombin time (PT) were determined according to the established protocol in most cases and according to the publication [53]. Whereas, the anticoagulant activity was expressed as clotting time (unit in seconds). APTT and PT were performed using normal human plasma and four increasing concentrations of synthesized products:

(C_1 = 20 µg/mL, C_2 = 40 µg/mL, C_3= 80 µg/mL, C_4 = 100 µg/mL).

For the PT assay, 10 µL of sample extract was mixed with 90 µL of citrated serum and incubated in a water bath for 3 minutes. Then 200 µL of PT reagent (which was placed in a dry tube and incubated in a 37°C water bath to activate it) was added and mixed in the reactor. The timer was stopped when fibrin (coagulation) was obtained. The recorded time corresponded to the appropriate percentage of coagulation. Whereas for the APTT test, 90 µL of the citrated human plasma was mixed with 10 µL of the sample extract. Then 100 µL of the APTT reagent (incubated for 3 minutes at ambient temperature) was added to the reactor. The coagulation time was recorded by a timer. All the measurements were performed in quadruplets and the results were analyzed using the one-way ANOVA test on Microsoft Excel Software. The whole procedure is shown in Figure 1.

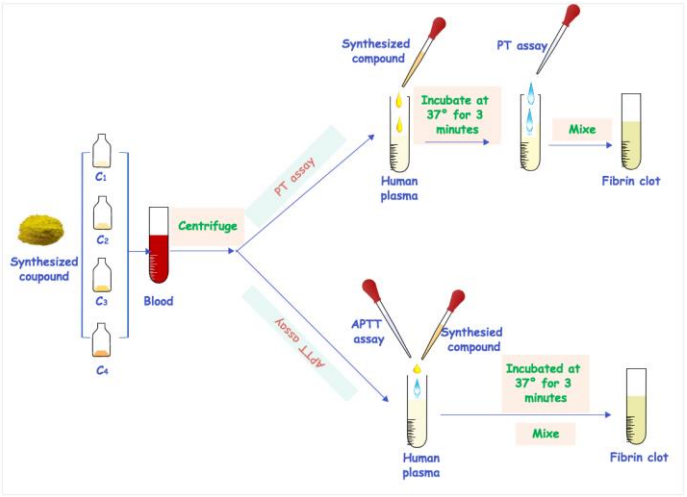


Figure 1. Operating mode of the anticoagulant activity.

2.6 Docking studies

The structures of four tautomers (stereoisomers) were prepared in Marvin Sketch and saved in pdb files. Like receptor preparation, all the pdb files of four tautomers (stereoisomers) were converted into pdbqt format using AutoDock Tools (ADT) 4.2. All the pdbqt formatted files of tautomers (stereoisomers) were docked with pdb file of the chiral selector (cellulose tris (3,5 dimethyl phenyl carbamate) using AutoDock vina. Before the docking method using AutoDock vina, all the coordinates were set (x = 30.054, y = 22.75, and z = 4.171). Numerous docking runs were utilized for each tautomer with a chiral selector for the small free energy of binding confirmation from the large bunch. The analysis of the modes of the interactions and the number of hydrogen bonds were studied by PyMOL. On the other hand, hydrophobic interactions between the tautomers and chiral selectors were also studied using LigPlot 1.4.5 [54].

3. Results and discussion:

3.1 Synthesis of new thiourea derivative

The synthesis of a new thiourea derivative was carried out by refluxing naringenin and ethylenediamine in toluene by adding phenyl isothiocyanate. The obtained product (I) was mixed with phenylisothiocyanate at 0°C to obtain a crystallized yellow product (II). The reaction yields were 89-95%. The structures of the synthesized compounds were confirmed by the analysis of their spectral data (UV, IR, and NMR ^{1}H, ^{13}C).

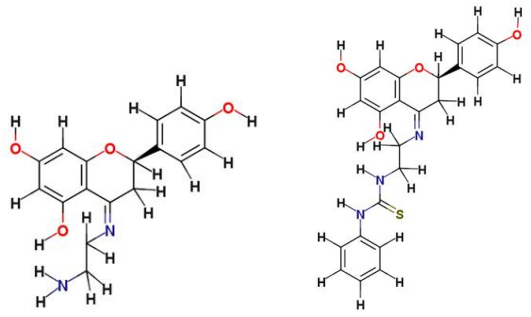
3.2 Spectroscopy analysis

(E)-4-((2-aminoethyl)imino)-2-(4-hydroxyphenyl)chromane-5,7-diol, $C_{17}H_{18}N_2O_4$ (I): This compound was obtained as a yellow powder, yield 90%, MP : 150 - 151 °C, UV_{max} (MeOH, nm): 275 (band I); 320 (band II), IR (KBr, cm^{-1}): 1147 (C-O), 3340 (O-H), 1547- 1498 (C=C), 3081 (=CH_{arom}), 1,610 (C=N), 1210 (C-N), 2950 (C–H), 3430 (N-H). ^{1}H NMR (400 MHz, DMSO, ppm): 12,03 (s, 1H, C-7-OH); 9,58 (s, 1H, C-5-OH); 7,32 (s, 1H, C-4'-OH); 6,78 – 6,81 (m, 2H, H-6', H-2'); 6,08 – 6,12 (m, 2H, H-5', H-3'); 5,08 -5,15 (dd, 2H, H-6, H-8); 3,63 -3,69 (t, 1H, H-2); 3,30 – 3,48 (m, 2H, H-1"^{a},H-1"^{b}); 3,15 – 3,21 (m, 2H, H-2"^{a}, H-2"^{b}); 2,70 (dd, 1H, H-3^{b}); 2,29 (dd, 1H, H-3^{a}); 1,13 – 1,16 (t, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO, ppm): 168, 77 (C-4); 165,45 (C-7); 163,40 (C-9); 158,30 (C-5); 128,98 (C-4'); 128,88 (C-1'); 115,68 (C-6', C-2'); 115,62 (C-5', C-3'); 72,32

(C-10); 70,97 (C-6); 70,87 (C-8); 70,08 (C-2); 68,74 (C-1"); 60,94 (C-2"); 18,50 (C-3).

(E)-1-(2-((5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-ylidene)amino)ethyl)-3-phenylthiourea, $C_{24}H_{23}N_3O_4S$ (2): This compound was obtained as a crystallized yellow product, yield 85%, MP: 147 - 150 °C, UV_{max} (DMF, nm): 279 (band I); 325 (band II), IR (KBr, cm^{-1}): 1149 (C-O), 3342 (O-H), 1546- 1499 (C=C), 3083 (=CH_{arom}), 1,612 (C=N), 1218 (C-N), 2948 (C–H), 3428 (N-H), 1120 (C=S). ^{1}H NMR (400 MHz, DMSO, ppm): 12,02 (s, 1H, NH-C-1'"); 9,58 (s, 1H, C-4'-OH); 7,40 (s, 1H, C-7-OH); 7,38 (s, 1H, C-5-OH); 7,34 (t, 1H, NH-C-3"); 7,27 – 7,30 (m, 2H, H-2"',H-6'"); 7,11 (m, 2H, H-3'", H-5'"); 6,78 (dd, 2H, H-6',H-2'); 6,10 (m, 1H, H-4'"); 5,88 (m, 2H, H-5', H-3'); 5,47 (m, 2H, H-6, H-8); 5,09 (t, 1H, H-2); 3,67 (m, 2H, H-1"^{a}, H-1"^{b}); 3,43 (m, 2H, H-2"^{a}, H-2"^{b}); 3,17 (dd, 1H, H-3^b); 2,69 (dd, 1H, H-3^a). ^{13}C NMR (100 MHz, DMSO, ppm): 178,78 (C-3"); 168,13 (C-4); 165,45 (C-7); 164,23 (C-9); 162,03 (C-5); 158,24 (C-4'); 136,55 (C-1'"); 131,40 (C-1'); 128,47 (C-6', C-2'); 128,41 (C-3'", C-5'"); 124,85 (C-2'", C-6'"); 123,91 (C-4'"); 104,94 (C-10); 97,29 (C-6); 94,88 (C-8); 77,10 (C-2); 50,63 (C-1"); 42,27 (C-2"); 36,76 (C-3).

After analyzing the data the structures were determined as given in Figure 2.



I II

Figure 2: The synthesized thiourea derivatives.

3.3 Chiral separation

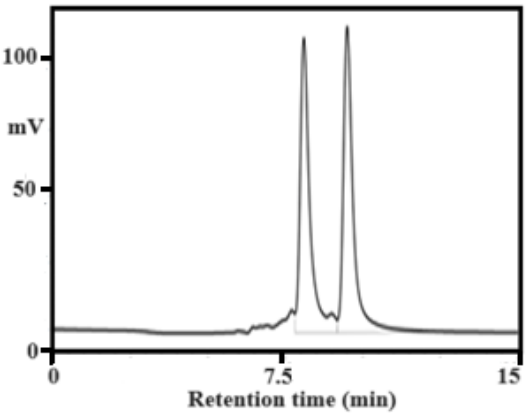
To have good results of separation, optimize HPLC conditions were optimized by modifying the chromatographic parameters. Mobile phases used were methanol and ethanol as isocratic processes; over time ethanol/hexane and methanol/hexane were applied like gradient mode by changing every time the volume/volume relation under a flow rate ranged from 0.2 to 0.5 mL/min at ambient temperature. The purity of the synthesized compounds (I and II) was ascertained by recording HPLC chromatograms on an achiral column. The preliminary separation with C_{18} bonded achiral stationary phase gave us definitive proof of the purity of synthesized compounds because a single peak was obtained for both compounds. Table 1 below summarizes the best results that were obtained on different CSPs.

It is clear from this Table that retention times for first enantiomers varied from 1.69 to 9.59 minutes while these values varied from 2.15 to 9.62 for second enantiomers for both compounds I and II. The values of the retention factors ranged from 0.08 to 2.39 for the first enantiomer while these values were from 0.16 to 2.98 for the second enantiomer. The values of separation factors ranged from 1.25 to 3.47 for both compounds; indicating a good separation of the enantiomers of both the compounds. The resolution factor ranged from 0.48 to 1.75 for both compounds. However, the enantiomers of both compounds separated on all three columns reported (Table 1) but under different HPLC conditions. For example, Chiralpak-IB column could resolve the enantiomers of compound I (Rs = 1.75) using 50% MeOH/ 50% hexane as a mobile phase with 0.5 mL/min flow rate. Chiralcel-OD could resolve all four enantiomers of both compounds using mobile phase 70% EtOH/ 30% Hexane (Rs = 1.55) and 90% EtOH/ 10% Hexane mobile phase (Rs = 1.35) at 05 mL/min flow rate. Also, Chiralcel-OD 3R could resolve the enantiomers of II compound (Rs = 1.35) with 60% EtOH/ 40% Hexane as mobile phase at 0.2 mL/min flow rate. By considering these results, it was decided that Chiralpak-IB column was the best for the separation of the enantiomer of compound I and Chiralcel-OD for compound II. The enantiomers separated chromatograms of compounds I and II are given in Figure 3.

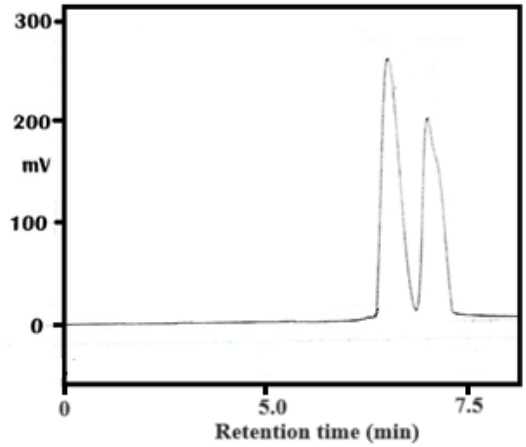
Table 1. Chromatographic parameters of the chiral separation of new thiourea derivatives.

Chiral stationary phases	Compounds	Mobile phases	Flow rate (mL/min)	tr_1 (min)	tr_2 (min)	k_1	k_2	α	Rs
Chiralpak-IB	I	50% MeOH/ 50% Hexane	0.5	8.09	9.49	2.39	2.98	1.25	1.75
	II	80% MeOH/ 20% Hexane	0.5	7.01	7.52	0.08	0.16	1.98	0.87
Chiralcel-OD	I	70% EtOH/ 30% Hexane	0.5	9.59	11.14	0.62	0.88	1.42	1.55
	II	90% EtOH/ 10% Hexane	0.5	7.82	8.43	0.23	0.33	1.42	1.35
Chiralcel-OD 3R	I	60% EtOH/ 40% Hexane	0.2	6.66	9.62	0.73	1.49	2.06	1.44
	II	60% EtOH/ 40% Hexane	0.5	1.69	2.15	0.13	0.43	3.47	0.48

Chiralpak-IB column for I and Chiralcel-OD for II compounds.



(a)



(b)

Figure 3. Chiral separation of (a): compound I with Chiralpak-IB and (b): compound II with Chiralcel-OD columns.

The tailing factors were calculated for the resolved enantiomers. The values of the tailing factors are given in Table 2 and were in the range of 1.0 to 1.15. These values clearly indicated a good symmetry of the peaks. Also, the values of theoretical plates were calculated and given in Table 2. The values of the theoretical plate were in the range of 100 to 1440. The poor values of the theoretical plates resulted in partial resolution while high values of theoretical plates were associated with good base-lined resolution. These values indicated a good performance of the Chiralpak-IB column for I and Chiralcel-OD for II compounds.

Table 2: Theoretical plate and tailing factors of the resolved enantiomers.

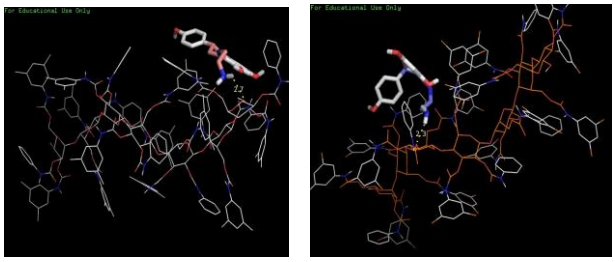
Columns	Compds.	Mobile phases	Number of theoretical plates		Tailing factors	
			Ist Enan.	2nd Enan.	Ist Enan.	2nd Enan.
Chiralpak-IB	I	50% MeOH/ 50% Hexane	1097	1440	1.0	1.0
	II	80% MeOH/ 20% Hexane	786	900	1.10	1.0
Chiralcel-OD	I	70% EtOH/ 30% Hexane	1045	1127	1.0	1.0
	II	90% EtOH/ 10% Hexane	755	947	1.0	1.0
Chiralcel-OD 3R	I	60% EtOH/ 40% Hexane	709	1139	1.0	1.0
	II	60% EtOH/ 40% Hexane	100	151	1.15	1.14

3.4 Simulation study

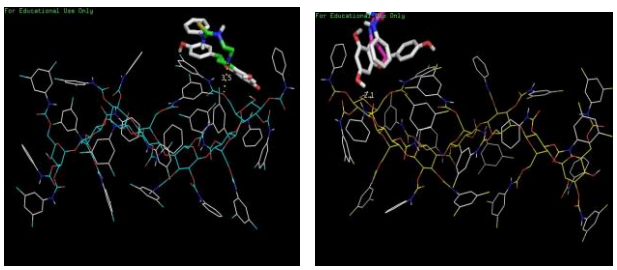
The simulation study is one of the important tools to determine the mechanism of chiral separation. The chiral separation was studied by modeling the enantiomers of first compound with cellulose tris-(3,5-dimethylphenylcarbamate) chiral selector present in Chiralpak-IB column. Similarly, the enantiomers of II compound were modeled with cellulose tris-(3,5-dimethylphenylcarbamate chiral selector present in Chiralcel-OD column. The results of the modeling in terms of binding affinities, number of hydrogen bonds and residues involved, and the bond length (A°) are given in Table 3. It is apparent from this table that binding affinities of R- and S-enantiomers of compound I were -5.7 and -4.9 kcal/mol, showing strong bindings of R-enantiomer than S-enantiomer. The number of hydrogen bond was one in both the enantiomers. The lengths of hydrogen bonds were 2.3 and 2.5 A° in R- and S-enantiomers. The residues involved in binding for R-enantiomers were .368//UNK`0/O & O of -OH group while these were .171//UNK`0/HN & H of NH_2 group in the S-enantiomer. In the case of compound II, the binding affinities were -5.4 and -4.7 kcal/mol, showing strong binding of R-enantiomers than S-enantiomers. The number of hydrogen bonds was one in both the enantiomers. The lengths of hydrogen bond were 2.1 and 3.5 A° in R- and S-enantiomers. The residues involved in binding for R-enantiomers were .301//UNK`0/O & N of =N- group while these were .238//UNK`0/O & H of -OH group in S-enantiomer. The models of the interactions of all four enantiomers i.e. two enantiomers of compound I and two enantiomers of compound II are given in Figure 4. A perusal of this Figure indicates the number of hydrogen bonds and the pattern of the interactions of the enantiomers with the chiral selector. It very interesting to note that the enantiomers of II compound have lower binding affinity than the enantiomers of compound I. This indicates that the enantiomers of I compounds interacted more strongly than the enantiomers of compound II. The poor interactions of enantiomers of II compound may be because of their large size; creating a steric effect and leading to poor separation. These data are supported by the chiral separation retention times.

Table 3: The modeling results of the enantiomers of compounds I and II.

Enantiomers	Binding affinities (Kcal/mol)	No. of hydrogen bonds	Residues involved in H bond (Bond length in A°)
Compound I			
R	-5.7	1	.368//UNK`0/O & O of -OH group (2.3)
S	-4.9	1	.171//UNK`0/HN & H of NH_2 group (2.5)
Compound II			
R	-5.4	1	.301//UNK`0/O & N of =N- group (2.1)
S	-4.7	1	.238//UNK`0/O & H of -OH group (3.5)



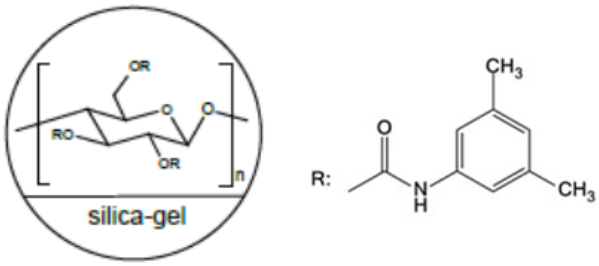
I-R I-S



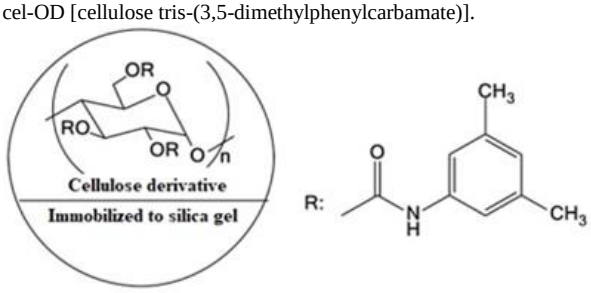
II-R II-S

Figure 4: The models of the interactions of all four enantiomers with chiral selectors.

The mechanism is a very important part of the chiral separation. The chiral selectors i.e. cellulose tris-(3,5-dimethylphenylcarbamate) [Chiralpak-IB column] and cellulose tris-(3,5-dimethylphenylcarbamate [Chiralcel-OD column] have chiral grooves of cellulose along with various functionalities such as phenyl, amide, carbonyl groups. The structures of Chiralpak-IB and Chiralcel-OD columns are given in Figure 5, which confirm these functionalities. The compound I has phenyl, hydroxyl, ether and primary amine groups. The compound II has phenyl, hydroxyl, ether and secondary and tertiary amine groups. Lets make a picture of all these groups in our mind and try to establish the chiral mechanism. The groups of the enantiomers interacted with the groups of the chiral sectors. The chiral groove provided the chiral surface to the enantiomers and these are bonded different by the interactions such as π-π, hydrogen bonding, dipole-induced dipole interactions, van der Waals interactions and steric effect. Briefly, the enantiomers fit on chiral selector grooves in the different fashions and get separated by mobile phase flow.



Chiral cel-OD [cellulose tris-(3,5-dimethylphenylcarbamate)].



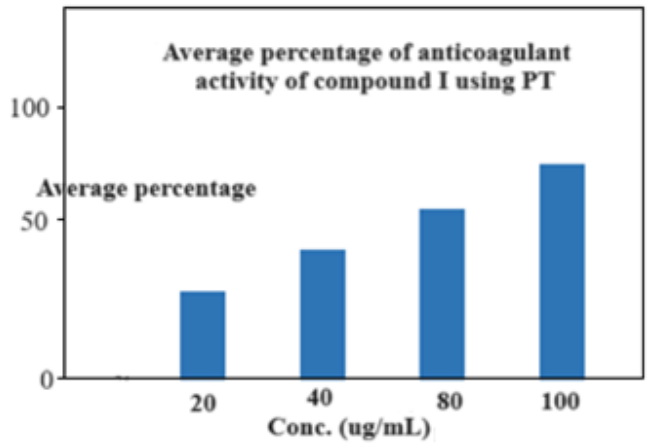
Chiralpak-IB [cellulose tris-(3,5-dimethylphenylcarbamate)].

Figure 5: Structure of chiral selectors used.

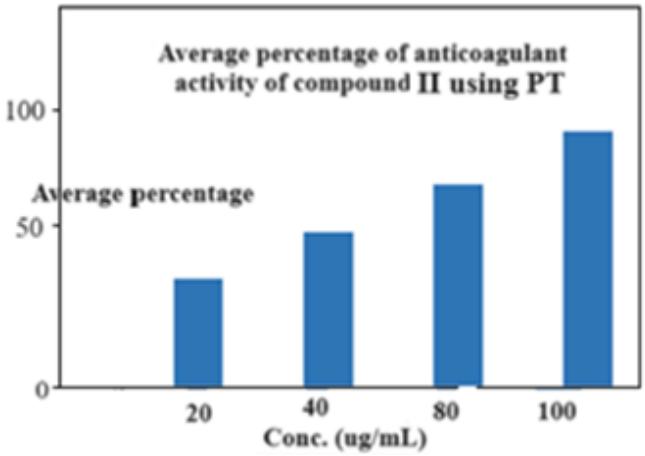
3.5 Anticoagulant activity

The synthesis of novel thiourea derivatives aimed at inhibiting components of the intrinsic coagulation pathway while minimizing bleeding risks has garnered significant research attention. In vitro, the anticoagulant activity of compounds is often assessed using APTT and PT, which serve as crucial indicators [55]. APTT reflects endogenous coagulation sensitivity, while PT serves as a reliable measure of extrinsic coagulation [56]. Both parameters are closely linked to the levels and functionality of coagulation factors, demonstrating robust sensitivity and stability.

The two compounds were tested for their anticoagulant activity, applying prothrombin time (PT) and activated partial thromboplastin time (APTT) techniques using human plasma. The synthesized compounds at four different concentrations (20, 40, 60 80 and 100 µg/L). ANOVA results showed that the anticoagulant effect of the two compounds I and II using PT and APTT tests was significant ($p < 0.05$). These were importantly prolonged by the two compounds synthesized at concentrations equal to or greater than 20 µg/mL, and a maximum prolongation of the coagulation percentages was obtained with a concentration of 100 µg/mL. As shown in Figures 6 and 7. The average percentage of the anticoagulant activity was noticed to be increasing when the concentrations of the synthesized products were high, which is a very good and desired correlation. Compound I showed 78 to 84% PT anti-coagulant activity while compound II showed 83 to 91% PT anti-coagulant activity at 20 to 100 µg/L concentrations. On the other hand, compound II showed 77.5 to 84.5% APTT anti-coagulant activity while compound II showed 82.5 to 90.5% APTT anti-coagulant activity at 20 to 100 µg/L concentrations. Overall, compound II prolonged the PT and APTT more efficiently than compound I. The higher activity of compound II may be due to the presence of -HN-SC-NH-. It is due to the fact that this moiety exerts the mechanism of action of vitamin K antagonists (VKA), which consists in the inhibition of the enzyme VKORC1; resulting in a decreased activity of the coagulation factors [57]. The standard used was naringenin and the values of PT and APTT of the prepared compounds were much higher. These results indicated better performance of the synthesized compound in comparison to the standard drug heparin.

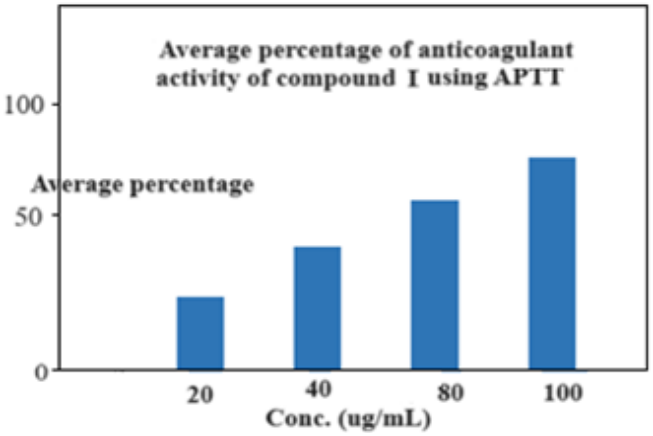


(a)

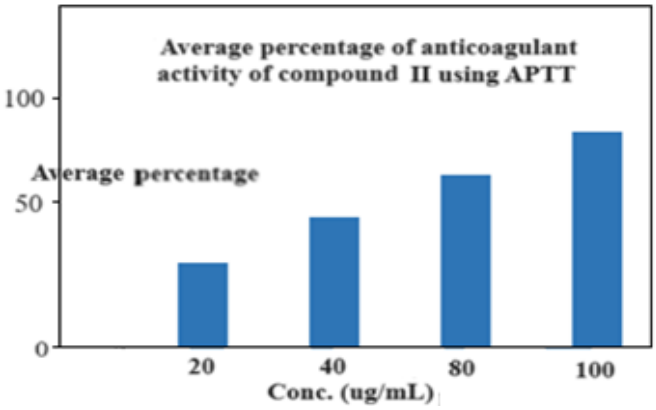


(b)

Figure 6. Average percentage (%) of the anticoagulant activity of compounds (a): I and (b): II using PT.



(a)



(b)

Figure 7. Average percentage (%) of the anticoagulant activity of compounds (a): I and (b): II using APTT.

2. Conclusion:

Due to the negative symptoms caused by blood clotting, the anticoagulant activity of newly synthesized chiral compounds has become an interesting study. The new chiral compounds (I and II) were synthesized and characterized by spectroscopic studies. The synthesized compounds were screened as anticoagulants using PT and APTT assays. These compounds were found to be effective with a slightly high value of compound II. The higher activity of the II compound in comparison to the I compound was attributed due the presence of thiourea function -HN-SC-NH- which may inhibit the enzyme VKORC1; resulting in a decreased activity of the coagulation factors. The enantiomers of these compounds were separated on three polysaccharides chiral columns in HPLC. The enantiomers of the two compounds could resolve successfully on Chiralpak-IB, Chiralcel-OD and Chiralcel-OD 3R columns under varied HPLC conditions. This study provided evidence that two compounds including thiourea derivative possess anticoagulant activities. The crystalized products have been obtained with yields of 90 and 85%. The structures of these compounds were proved using spectral data analysis UV, IR, and NMR H, NMR C. While the anticoagulant activities of the two compounds I and II have been realized using PT and APTT tests. The obtained results using the ANOVA test showed that compounds I and II have very significant anticoagulant activity.

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