Synthesis and docking studies of thiophene scaffolds in COX-2

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DOI: http://dx.doi.org/10.3998/ark.5550190.0012.a05

Abstract
A series of new thiophene compounds **6a-6l** was synthesized from the reactions of *in situ* generated cross-conjugated enaminothiones **2** and α-bromoketones/ethyl bromoacetate. Moreover, the synthesis of 1,3-thiazine **12** and functionalized thiopyran **18** heterocycles from the cycloaddition reactions of *N,N'*-bis[(dimethylamino)methylene]thiourea **9**, is also described. Additionally, the binding conformations of these compounds **6a-6l** and some anti-inflammatory drugs (NSAIDs) were determined by their docking in the active site of the Cyclooxygenase-2 (COX-2) enzyme.

Keywords: Thiophene, COX-2, docking, cycloaddition, QUANTUM

Introduction

Sulphur compounds are of great chemical and pharmaceutical significance and display diverse properties such as antifungal,[1] anti-HIV,[2] antipsoratic,[3] and antimicrobial activities.[4] Some imidazo[2,1-*b*]-[1,3]thiazines and pyrimido[2,1-*b*]-[1,3]thiazines are well known anti-inflammatory agents.[5,6] Likewise, thiophene compounds are well known to exhibit various biological and medicinal activities such as BACE1 inhibitors,[7] antitubercular,[8] anti-depressant,[9] anti-inflammatory,[10] anti-HIV PR inhibitors,[11] and anti-breast cancer activities.[12] It is known that the biological half-life of antidepressant drugs such as viloxazine became longer when the benzene ring is replaced by thiophene.[13] It is also observed that the thienyl ring mimics the phenyl group of phenylalanine in peptidomimetics,[14] in many drugs.[15,16] Currently, a number of non-steroidal anti-inflammatory drugs (NSAIDs) containing thiophene ring such as Tiaprofenic acid (**a**) and Tenidap (**b**) **(**Figure 1) are available for the treatment of pain and inflammatory disorders. These conventional NSAIDs are known to inhibit cyclooxygenase COX enzymes (COX-1, COX-2) which catalyze the formation of prostaglandins (PGs) from arachidonic acid.

Literature study reveals that although a number of reactions involving acyclic enaminothiones (**c**, Figure 1) as a precursor have been reported,[17] the synthetic potential of cross-conjugated enaminothiones **d** (Figure 1) remained almost unexplored. Recently, we reported successful cycloaddition reactions of some *in situ* generated enaminothiones with acrylates,[18] and this prompted us to explore their synthetic utility with other dienophiles. In this paper, we wish to report the cycloaddition reactions of some cross-conjugated enaminothiones with α-bromo-ketones/ethyl bromoacetate explored for the synthesis of thiophene scaffolds. Although, few thiophene derivatives,[19] with different synthetic routes have already been reported, this is a first report in which cross-conjugated enaminothiones have been used for the synthesis of thiophene scaffolds. Additionally, two new functionalized sulfur heterocycles are also synthesized by the cycloaddition reactions of N,N'-*bis*[(dimethylamino)methylene]thiourea with chloro ketene and dimethyl acetylenedicarboxylate (DMAD). Since, the NSAIDs block the COX-2 enzyme for their biological action, it was thought worthwhile to screen these drugs and the currently synthesized molecules for their docking in the binding site of COX-2 receptor. The main objective of docking study was to get a better understanding of three dimensional (3D) structural adjustments of these molecules inside the binding pocket of COX-2.

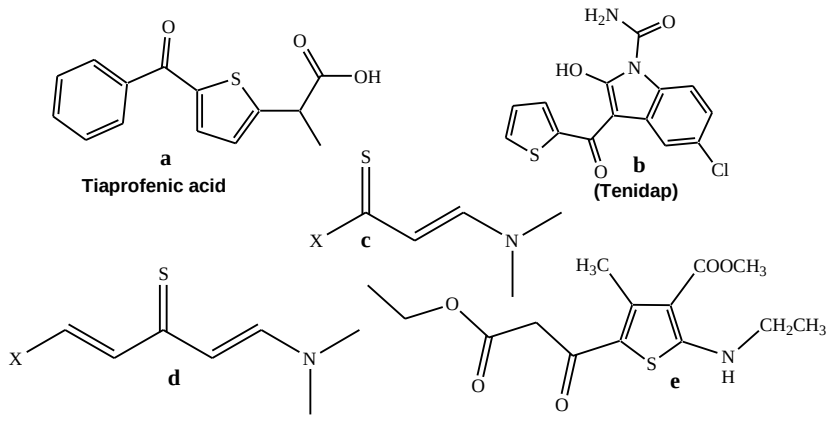


Figure 1. Showing two NSAIDs (**a**, **b**), conjugated acyclic enaminothione (**c**), cross-conjugated enaminothione (**d**) and functionlized thiophene compound (**e**).

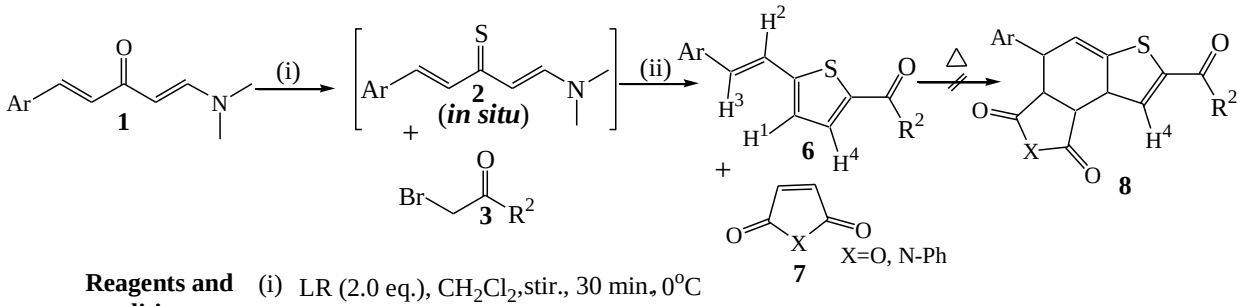
Hence, this work is divided mainly into two sections; the first section comprises experimental procedures for preparation of some sulfur heterocycles **6a-6l** (Scheme 1) from enaminothiones **2** and *N,N'*-bis[(dimethylamino)methylene]thiourea **6** systems, followed by docking studies of these newly synthesized molecules in the active site (Arg 513, Val 523, Phe 51, Asn 382, His 386, Gln 454, Trp 387, His 90) of COX-2 enzyme using the Quantum 3.3.0 docking program.[20]

Results and Discussion

Reactions of enaminothiones 2 with α-bromo-ketones/ethyl bromoacetate

Initially, the synthesis of cross-conjugated enaminones **1** was carried out using reported procedures.[18] The subsequent treatment of **1** with the Lawesson's reagent,[21] (LR) in dichloromethane at 0 °C (Scheme 1) led to the generation of enaminothiones **2** (*in situ*), quenching of which with the α-bromo-ketones/ethyl bromoacetate **3** led to the formation of thiophene scaffolds **6** in good yields (59-79%, Table 1).

The plausible reaction mechanism followed for the formation of **6** involved the initial nucleophilic attack of the sulfur atom of **2** at the methylenebromide carbon of **3** leading to the formation of the intermediate **4**. This intermediate was deprotonated *in situ* by the presence of triethylamine. Ring closure proceeded spontaneously by the loss of a dimethylamine [$(CH_3)NH_2$] molecule from the intermediate **5** to provide thiophene derivatives **6** (Scheme 1).



Scheme 1

Table 1. Reactions of enaminothiones **2** with α-bromo-ketones/ethyl bromoacetate **3**

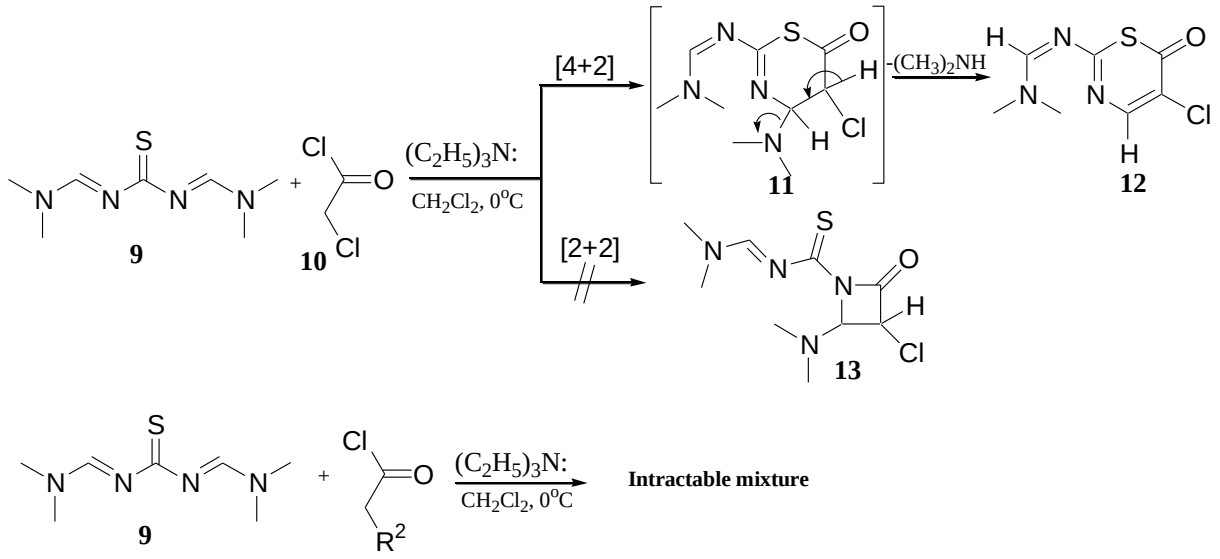
S.No.	Compound	R^2	Ar	Yield (%)
1	6a	$-OCH_2CH_3$	phenyl	64
2	6b	phenyl	phenyl	72
3	6c	4-methoxyphenyl	phenyl	76
4	6d	4-methylphenyl	phenyl	68
5	6e	$-OCH_2CH_3$	4-methoxyphenyl	70

Table 1. Continued

S.No.	Compound	R2	Ar	Yield (%)
6	6f	phenyl	4-methoxyphenyl	61
7	6g	4-methoxyphenyl	4-methoxyphenyl	79
8	6h	4-methylphenyl	4-methoxyphenyl	73
9	6i	$-OCH_2CH_3$	4-chlorophenyl	56
10	6j	phenyl	4-chlorophenyl	64
11	6k	4-methoxyphenyl	4-chlorophenyl	72
12	6l	4-methylphenyl	4-chlorophenyl	69

The isolated compounds were characterized on the basis of their analytical data and spectral evidence. For example, the ^{1}H-NMR spectrum of Ethyl-5-styryl-thiophene-2-carboxylate **6a** showed a doublet (J = 3.9 Hz) at δ 7.03 ppm for H^1, two doublets (J = 15.9 Hz) for two *trans* olefinic protons (H^2 & H^3) and a doublet (J = 3.9 Hz) at δ 7.68 ppm for proton H^4. The detailed,[13] C spectrum signals, as given in experimental section, also further substantiated the assigned structure. Attempts to utilize the butadiene segment of **6** in further cycloadditions with highly reactive dienophiles **7** viz. N-phenylmaleimide (NPM) and maleic anhydride (MA) under xylene reflux for **4** days led to the recovery of starting materials without yielding a trace of expected bicycloadducts **8** (Scheme 1). The observed non-reactivity of **6** was thought probably owing to the strong aromatic character of the thiophene ring

On the other hand, a literature survey,[22] revealed that *N,N'*-bis[(dimethylamino)methylene thiourea **9** (Scheme 2), despite of being an excellent synthon, yet its synthetic potential has not been explored to a considerable extent. The first cycloaddition of its thiazadiene unit was restricted to only few dienophiles, and prompted us to explore its synthetic utility with other dienophiles viz. ketenes and dimethyl acetylenedicarboxylate (DMAD).



Scheme 2

Accordingly, the cycloadditions of **9** with different monosubstituted (chloro and phenyl ketenes) and conjugated ketenes (vinyl, isopropenyl and butadienly ketenes), generated in situ from the corresponding acid chlorides and triethyl amine (Scheme 2), were investigated. The reaction in case of chloroketene formed from **10**, was found to be highly regioselective affording an exclusive [4+2] adduct **12** without any trace of corresponding [2+2] product **13** (Scheme 2). The compound obtained was characterized as *N*'-(5-Chloro-6-oxo-6H-[1,3]thiazin-2-yl)-*N*,*N*-dimethylformamidine **12** on the basis of its spectroscopic and analytical data. In its ^{1}H-NMR spectrum, compound **12** displayed two characteristic singlets at δ 3.15 ppm and δ 3.22 ppm for single dimethylamine protons confirming the formation of compound 12 over 13. Moreover, the single characteristic peak at ~ 1660 cm^{-1}, in its IR spectrum, due to conjugated carbonyl group also corroborated the assigned structure for 12. Compound 12 was presumably formed by elimination of dimethylamine [$NH(CH_3)_2$] molecule from the initially formed [4+2] cycloadduct intermediate **11** (Scheme 2). On the other hand, the cycloadditions of **9** to phenyl ketene and conjugated ketenes were found to be very sluggish and all attempts, by varying the reaction conditions, invariably resulted in an intractable mixture, from which no pure product could be isolated (Scheme 2) even after careful column chromatography.

The second cycloadditions of **12** to different ketenes, sulfene, NPM and MA, under varying reaction conditions, were unsuccessful leading to the isolation of starting material without any transformations (TLC based). The lower nucleophilicity of the thiazine nitrogen of **12** is probably due to extended conjugation of the π-electrons across the carbonyl group as depicted in Figure 2. The electron pull due to electronegative chloro (I effect) substituent could also be another reason for the lower reactivity of thiazine nitrogen.

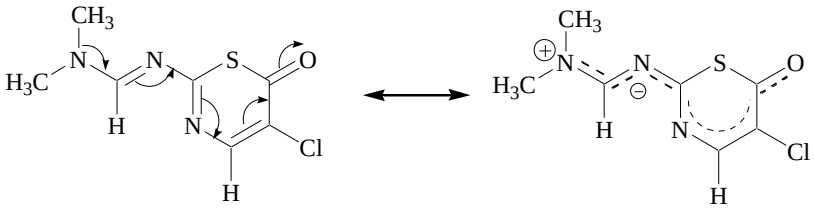
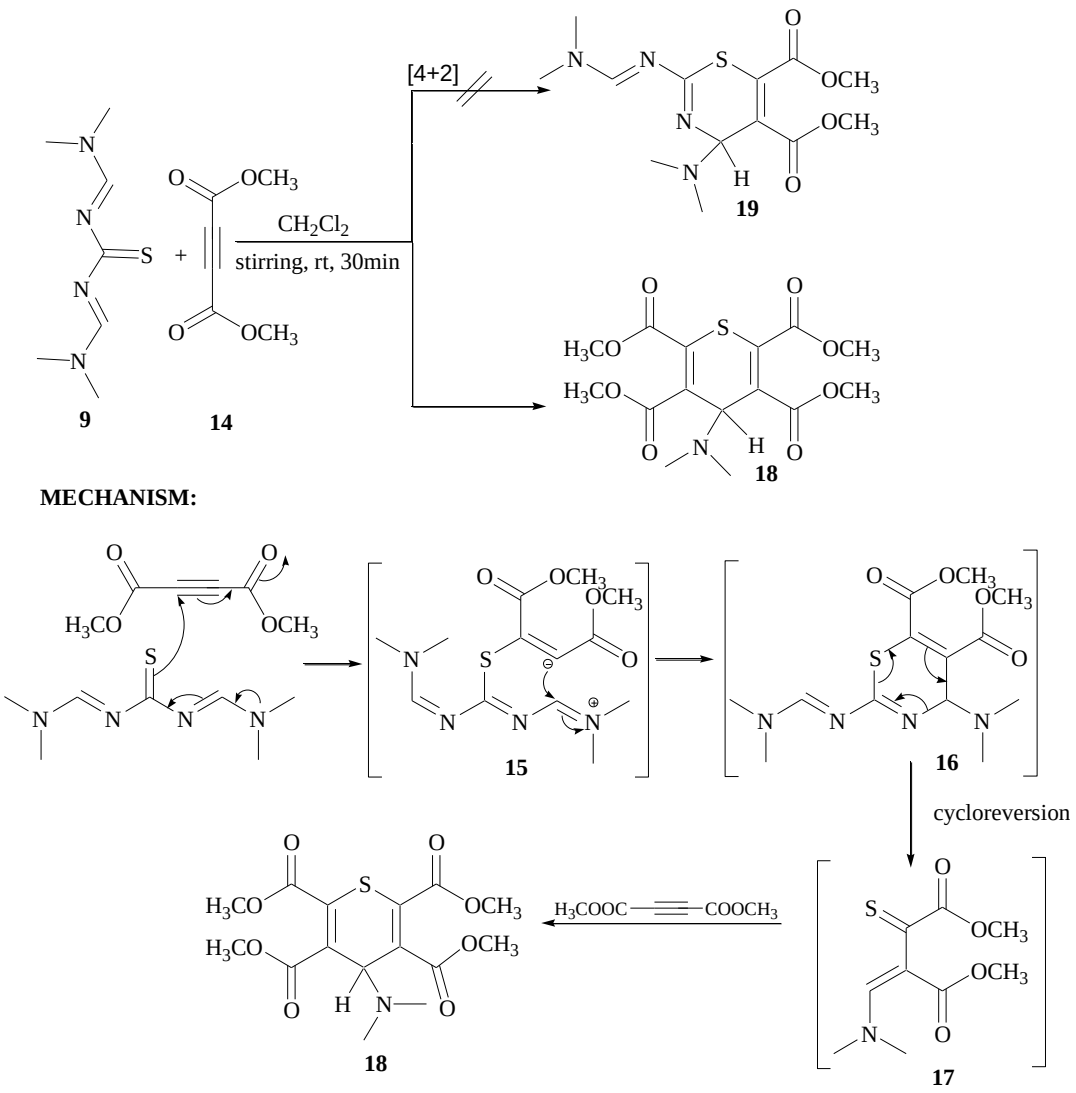


Figure 2

Stirring a mixture of **9** (1eq.) and DMAD **14** (2 eq.) for 30 min in dichloromethane led to an exclusive formation of product **18** without any trace (TLC based) of the expected [4+2] cycloadduct **19**, as depicted in Scheme 3. The probable mechanism for this reaction (Scheme 3) showed the formation of a [4+2] cycloadduct **16** through an intermediate **15**, which subsequently underwent cycloreversion to yield another intermediate **17**. The second [4+2] cycloaddition of thiabutadiene of intermediate **17** with another DMAD molecule resulted into cycloadduct **18**. The equimolar reflux of **9** and **14** in toluene for 4-5h led to the generation of compound **18** (TLC based) with the incomplete reaction, clearly ruling out any possibility of product **19** (Scheme 3) formed in the reaction.



Scheme 3

Docking results

All compounds were flexibly docked with COX-2 using QUANTUM 3.3.0 software.[20] Figure 3 represents the ribbon representation of COX-2 protein with all docked compounds **6a-6l** (NSAIDs and **e**) clearly suggesting their preferred binding in the active site of protein. The results of docking, for instance, no. of H bonds, bond distance, donor-acceptor H bond, binding energy etc. for each compound are summarized in Table S1 (see supplementary data). In order to get a deeper insight into the nature and type of interactions, the complexes between each compound and COX-2 receptor were visualized using QUANTUM,[20] and are depicted in Figures 4-6 (Figures S1, S2 and S3 are provided in supplementary data).

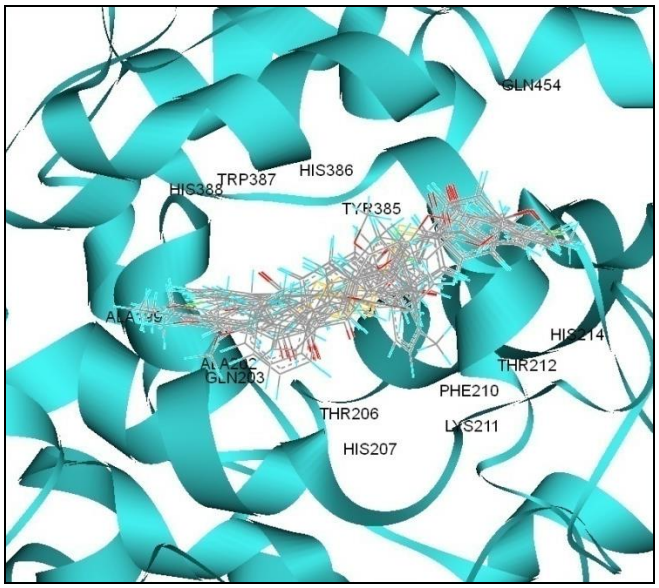


Figure 3. Ribbon representation of COX-2 protein with compounds **6a-6l** (NSAIDs and **e**) docked into the cavity. All docked compounds are shown in line form and are coloured according to their atoms name.

Since H bonds play a significant role in the structure and function of biological molecules, the current ligand-receptor interactions were analyzed on the basis of H bonding, as depicted in Tables **2** and **3**. In order to reduce the complexity, hydrophobic and π-cation interactions (>4Å) are not shown in the figures. The ethoxycarobonyl (-$COOCH_2CH_3$) groups of compounds **1** and **3** interact with Trp387 and His386, respectively, through their carbonyl oxygen atoms (Figure 4a). Moreover, compound **1** stabilize itself in the binding pocket of COX-2 by adjusting its thiophene ring perpendicular to the imidazole rings of His207 and His388 amino acid residues through cation-π interactions. Compound **2** (Figure 4b), on other hand, interact with His386 through sulfur atom of thiophene ring, while compound **5** form H bond with His 386 through oxygen of ketone group (Figure 4b). In addition, the phenyl ring of compound **2** interact with the imidazole ring of His207 via single cation-π interaction, while compound **5** display two parallel cation-π interactions with imidazole rings of His207 and His214 of COX-2 protein (Figure 4b). The docking results of rest of the compounds **4**, **6-12** are provided in the supplementary data.

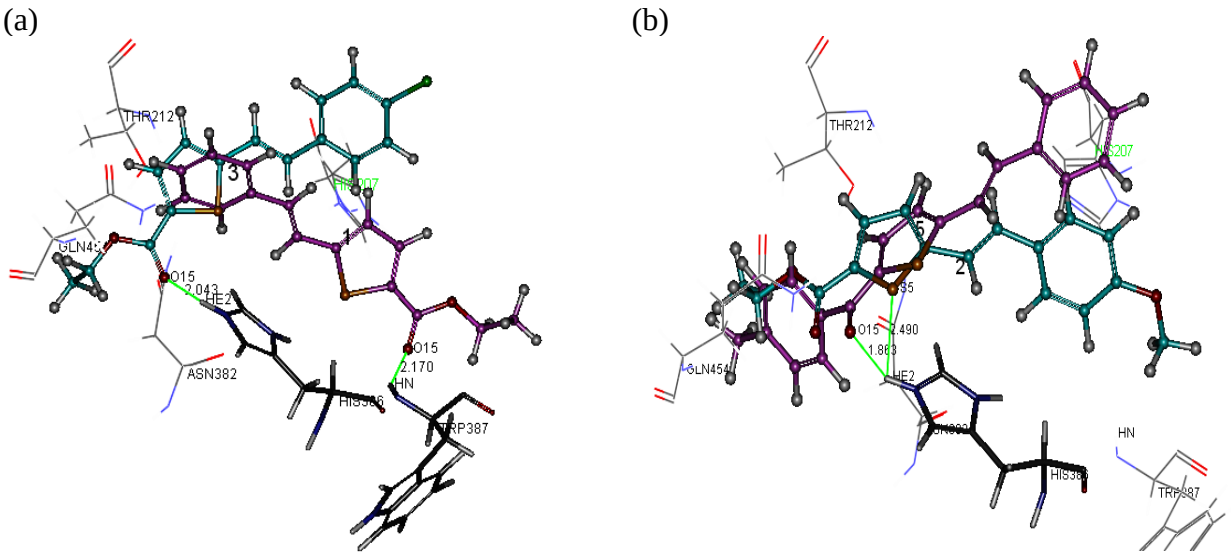


Figure 4. Docked conformations of compounds **1,3** (a) and **2,5** (b) showing important amino acid residues of COX-2. Ligands are shown as ball and stick, and the interacting amino acids are shown as sticks. H Bonds shown in green.

In order to get a deeper insight into the 3D-binding orientations of NSAIDs into the catalytic site of COX-2, we performed docking of three NSAIDs viz. aspirin, ibuprofen, TA **a** (Figure 1), and compound **e** (Figure 1) which is reported to be potent anti-inflammatory agent experimentally,[23] using similar default parameters in the QUANTUM.[19] Docking results are pictorially depicted in the Figure S3 (see supplementary data) while the drug-receptor interactions in terms of number of H bonds, bond distance, donor-acceptor H bond, binding energy etc. for each compound are summarized in Table S2 (see supplementary data). The results obtained clearly reveals that that the amino acid residues close to the reference molecules (NSAIDS and **e**) are mostly the same as those observed in the currently synthesized thiophene-protein complexes. However, a significant improvement in the binding energies was observed in case of compounds **6a-6l** and **e** compared to the NSAIDs (Table S1 and S2). This could probably be due to the presence of thiophene and aromatic rings in the synthesized compounds. The higher binding energy value of TA (Table S2) amongst other NSAIDs also further corroborates the role of thiophene ring in the binding process.

Finally, docking result of predicted the binding conformation of a selective inhibitor (SC-558) for COX-2 isoenzyme with a root mean square deviation (all atoms) of 0.99, with respect to its X-ray structure (1CX2, http://www.pdb.org), clearly validated the proposed binding conformations of the synthesized compounds and NSAIDs in COX-2 (Figure 5).

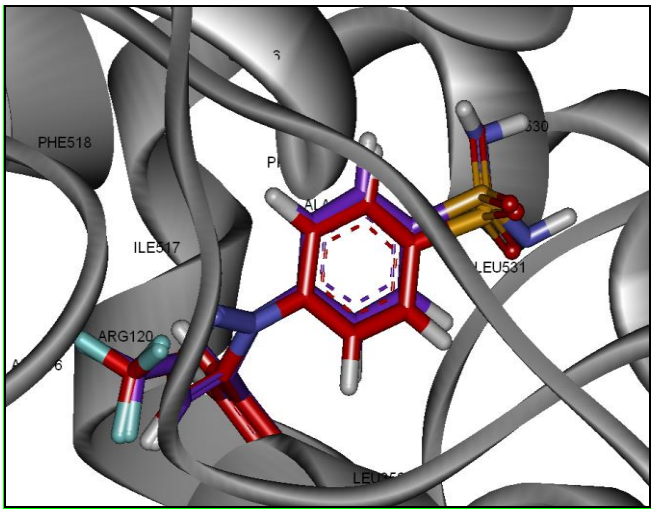


Figure 5. Overlapped conformations of SC-558 obtained from docking (in red) and X-ray complex (in blue). COX-2 protein shown in ribbon form.

Conclusion

Synthesis of thiphene scaffolds **6a-6l** from cross-conjugated enaminothiones **2** is described. Morever, the cycloaddition reactions of *N,N'*-bis[(dimethylamino) methylene]thiourea **9** with chloroketene and DMAD were also investigated. Additionally, the docking experiments performed on the compounds **6** and some NSAIDs indicate that His 386, His388, His214, His207 and Gln4 amino acids are important residues that stabilize the conformations of the compounds in the binding pocket of COX-2. Docking results also reveal that the perpendicular orientation of thiophene and/or phenyl rings to the plane of imidazole rings of Histine residues (His386, His388, and His207) is significant for locking the geometries of **6** in the COX-2.

Experimental Section

General. The cross-conjugated enaminones **1** were prepared according to the reported procedures.[18,24] Dichloromethane dried over *di*-phosphorus pentoxide and stored over molecular sieves (4Å). Melting points were determined by open capillary method using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform ($CDCl_3$) with Bruker AC-E 300 (300 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift values are expressed as ppm downfield from TMS and *J* values are in Hz. Splitting patterns are indicated as s: singlet, d: doublet, dd: doublet of doublet, t: triplet, m: multiplet and q: quartet, bs: broad singlet. ^{13}C-NMR spectra were also recorded on

AC-E 300 (75.0 MHz) spectrometer in a deuterochloroform using TMS as an internal standard. Mass spectra were recorded on Shimadzu GCMS-QP-2000 mass spectrometer. Elemental analyses were performed on Heraus CHN-O-Rapid Elemental Analyzer.

Docking methodology

The crystal structure of the COX-2 complex with SC-558 (1CX2) was obtained from RCSB protein data bank (http://www.pdb.org). 3D structures of all compounds **6a-6l**, **12**, **18** (NSAIDs and compound **e**) were drawn using the Visualizer module of Materials Studio (MS).[25] and were geometrically optimized using Forcite module of MS. For COX-2 preparation, the whole enzyme was selected and hydrogen atoms were added to it. Ligand conversion and minimization of all the compounds was performed using small molecules/ion module of the QUANTUM 3.3.0 program (http://www.q-pharm.com).[20] The protein was energetically minimized separately using Bio-macromolecules module of the QUANTUM. After minimization, COX-2 was selected as the receptor and the active sites were generated covering all the binding site residues. Docking studies were performed using the QUANTUM considering the default parameters. Of the 10 best poses, one (conformation) having the lowest free binding energy was selected for further analysis.

General procedure for the reaction of cross-conjugated enaminothiones (2) with α-bromo-ketones/ethyl bromoacetate

To a well stirred solution of enaminothiones **2**, prepared by stirring of Lawesson's Reagent (20 mmol) with corresponding enaminones **1** (10 mmol) in dry CH_2Cl_2 at 0 ^oC for 30 minutes, was added α-bromo-ketones/ethyl bromoacetate **3** (10 mmol). After 15 min of stirring at the same temperature, the triethylamine (22 mmol) was added. The reaction mixture was further stirred for 1h. The solvent was then evaporated under reduced pressure and the residue, thus obtained, was purified by silica gel chromatography (ethylacetate:hexane; 1:10). The solid compounds obtained: were recrystallized using ethylacetate: hexane mixture (1:6, v/v).

Ethyl-5-styryl-thiophene-2-carboxylate (6a). Colorless solid, yield 64%, mp 110-111 ^oC, IR (KBr) 1701, 1442, 1278 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.38 (t, *J* = 7.2 Hz, 3H, -O-C-CH_3), 4.35 (q, *J* = 7.2 Hz, 2H, -OCH_2), 7.03 (d, *J* = 3.9 Hz, 1H, H^1), 7.05 (d, *J* = 15.9 Hz, 1H, H^2), 7.18 (d, *J* = 15.9 Hz, 1H, H^3), 7.25-7.49 (m, 5H, ArH), 7.68 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 13.3, 60.1, 120.1, 125.1, 125.7, 127.4, 127.8, 130.2, 130.7, 132.9, 135.2, 148.3, 161.2 ppm; MS (EI) *m/z*: 258 (M^+). Anal. Calcd. for $C_{15}H_{14}O_2S$: C, 69.74; H, 5.46%. Found: C, 69.82; H, 5.39%.

Phenyl-(5-styryl-thien-2-yl)-methanone (6b). Light yellow solid, yield 72%, mp 133-134 ^oC. IR (KBr) 1614, 1448 cm^{-1}, ^1H NMR (300MHz, $CDCl_3$): δ 7.10 (d, *J* = 3.9 Hz, 1H, H^1), 7.13 (d, *J* = 15.9 Hz, 1H, H^2), 7.22 (d, *J* = 15.9 Hz, 1H, H^3), 7.27-7.61 (m, 10H, ArH), 7.85 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 120.0, 125.5, 125.8, 127.4, 127.6, 127.8, 128.1, 131.1, 131.3, 134.7, 135.1, 137.2, 140.4, 150.5, 186.8 ppm; MS (EI) *m/z*: 290 (M^+). Anal. Calcd. for $C_{19}H_{14}OS$: C, 78.59; H, 4.86%. Found: C, 78.55; H, 4.94%.

(4-Methoxyphenyl)-(5-styryl-thien-2-yl)-methanone (6c). Yellow crystalline solid, yield 76%; mp 121-122 ºC; IR (KBr) 1615, 1447 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 3.83 (s, 3H, -OCH_3), 6.88 (d, *J*= 8.7 Hz, 2H, ArH), 6.97 (d, *J* = 3.9 Hz, H^1), 7.04 (d, *J* = 15.9 Hz, 1H, H^2), 7.11-7.43 (m, 6Hz, 5ArH & H^3), 7.44 (d, *J* = 8.7 Hz, 2H, ArH), 7.81 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 55.3, 114.4, 119.9, 125.4, 126.3, 128.2, 131.2, 132.4, 133.2, 134.6, 135.8, 138.9, 139.1, 150.4, 160.1 186.8 ppm; MS (EI) *m/z*: 320 (M^+). Anal. Calcd. for $C_{20}H_{16}O_2S$: C, 74.97; H, 5.03%. Found: C, 75.07; H, 4.97%.

(5-Styrylthien-2-yl)-4-methylphenyl-methanone (6d). Yellow solid, yield 68%; mp 125-126 ºC; IR (KBr) 1615, 1446 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 2.31 (s, 3H, -CH_3), 6.97 (d, *J* = 3.9 Hz, 1H, H^1), 7.09 (d, *J* = 8.5 Hz, 2H, ArH), 7.11 (d, *J* = 15.9 Hz, 1H, H^2), 7.17-7.60 (m, 8H, 7ArH & H^3), 7.80 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 21.0, 119.8, 124.7, 125.6, 127.3, 127.9, 128.2, 128.8, 130.9, 132.4, 134.4, 134.9, 138.2, 140.5, 151.3, 186.6 ppm; MS (EI) *m/z*: 304 (M^+). Anal. Calcd. for $C_{20}H_{16}OS$: C, 78.91; H, 5.30%. Found: C, 79.02; H, 5.38%.

5-[2-(4-Methoxyphenyl)-vinyl]-thienyl-2-carboxylic acid ethyl ester (6e). Pale white solid, yield 70%; mp 143-144 ºC; IR (KBr) 1697, 1602, 1510 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.37 (t, *J* = 7.2 Hz, 3H, -O-C-CH_3), 3.82 (s, 3H, -OCH_3), 4.34 (q, *J* = 7.2 Hz, 2H, -OCH_2), 6.87 (d, *J* = 8.7 Hz, 2H, ArH), 6.99 (d, *J* = 3.9 Hz, H^1), 7.02 (d, *J* = 15.9 Hz, 1H, H^2), 7.08 (d, *J* = 15.9 Hz, 1H, H^3), 7.41 (d, *J* = 8.7 Hz, 2H, ArH), 7.67 (d, *J* =3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 14.3, 55.2, 61.0, 114.2, 118.9, 125.4, 127.9, 128.9, 129.5, 130.8, 133.9, 149.8, 159.8, 162.2 ppm; MS (EI) *m/z*: 288 (M^+). Anal. Calcd. for $C_{16}H_{16}O_3S$: C, 66.64; H, 5.59%. Found: C, 66.51; H, 5.48%.

5-[2-(4-Methoxyphenyl)-vinyl]-thien-2-yl-phenyl-methanone (6f). Yellow solid, yield 61%; mp 115-116 ºC; IR (KBr) 1618, 1446 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 3.84 (s, 3H, -OCH_3), 6.84 (d, *J* = 8.7 Hz, 2H, ArH), 6.93 (d, *J* = 4.1 Hz, H^1), 7.09 (d, *J* = 15.9 Hz, 1H, H^2), 7.11-7.49 (m, 8Hz, 7ArH & H^3), 7.84 (d, *J* = 4.1 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 55.4, 114.6, 120.1, 124.8, 126.2, 128.1, 129.5, 131.8, 133.9, 135.2, 135.9, 138.2, 139.3, 150.6, 160.4, 186.4 ppm; MS (EI) *m/z*: 320 (M^+). Anal. Calcd. for $C_{20}H_{16}O_2S$: C, 74.97; H, 5.03%. Found: C, 75.02; H, 5.13%.

(4-Methoxyphenyl)-5-[2-(4-methoxyphenyl)-vinyl]-thien-2-yl-methanone (6g). Yellow solid, yield 79%; mp 139-140 ºC; IR (KBr) 1617, 1445 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 3.83 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 6.82 (d, *J* = 8.6 Hz, 2H, ArH), 6.87 (d, *J* = 8.6 Hz, 2H, ArH), 6.96 (d, *J* = 3.9 Hz, H^1), 7.06 (d, *J* = 15.9 Hz, 1H, H^2), 7.16-7.70 (m, 5H, 4ArH & H^3), 7.83 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 55.3, 55.4, 114.5, 119.4, 123.7, 125.7, 128.5, 129.9, 132.4, 134.6, 135.5, 136.7, 146.5, 150.5, 164.7, 166.8, 186.7 ppm; MS (EI) *m/z*: 350 (M^+). Anal. Calcd. for $C_{21}H_{18}O_3S$: C, 71.98. H, 5.18%. Found: 72.09; H, 5.26%.

5-[2-(4-Methoxyphenyl)-vinyl]-thien-2-yl-4-methylphenyl-methanone (6h). Light yellow solid, yield 73%; mp 105-106 ºC; IR (KBr) 1617, 1446 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 2.34 (s, 3H, -CH_3), 3.81 (s, 3H, -OCH_3), 6.85 (d, *J* = 8.7Hz, 2H, ArH), 6.91 (d, *J* = 3.9 Hz, H^1), 7.11 (d, *J* = 15.9 Hz, 1H, H^2), 7.13 (d, *J* = 8.6 Hz, 2H, ArH), 7.18 (d, *J* = 15.9 Hz, 1H, H^3), 7.20 (d, *J* = 8.7 Hz, 2H, ArH), 7.46 (d, *J* = 8.6 Hz, 2H, ArH), 7.85 (d, *J*=3.9 Hz, 1H, H^4) ppm; ^{13}C NMR

($CDCl_3$): δ 20.9, 55.3, 114.2, 124.1, 127.2, 127.3, 128.5, 129.3, 129.6, 130.5, 130.8, 137.2, 142.0, 145.8, 150.4, 163.9, 186.2 ppm; MS (EI) *m/z*: 334 (M^+). Anal. Calcd. for $C_{21}H_{18}O_2S$: C, 75.42; H, 5.43%. Found: C, 75.33; H, 5.36%.

5-[2-(4-Chlorophenyl)-vinyl]-thienyl-2-carboxylic acid ethyl ester (6i). Yellow solid, yield 56%; mp 127-128 ºC; IR (KBr) 1696, 1602, 1510 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.38 (t, *J* = 7.2 Hz, 3H, -O-C-CH_3), 4.29 (q, *J* = 7.2 Hz, 2H, -OCH_2), 6.99 (d, *J* = 3.88 Hz, H^1), 7.04 (d, *J* = 15.9 Hz, 1H, H^2), 7.07 (d, *J* = 15.9 Hz, 1H, H^3), 7.25 (d, *J* = 8.7 Hz, 2H, ArH), 7.41 (d, *J* = 8.7 Hz, 2H, ArH), 7.67 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 14.3, 59.4, 114.3, 117.9, 125.3, 127.6, 128.5, 130.1, 131.2, 133.7, 149.5, 158.7, 161.9 ppm; MS (EI) *m/z*: 292 (M^+). Anal. Calcd. for $C_{15}H_{13}ClO_2S$: C, 61.53%; H, 4.48. Found: C, 61.70; H, 4.53%.

5-[2-(4-Chlorophenyl)-vinyl]-thien-2-yl-phenyl-methanone (6j). Yellow solid, yield 64; mp 148-149 ºC; IR (KBr) 1617, 1446 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 6.95 (d, *J* = 4.0 Hz, H^1), 7.07 (d, *J* = 15.9 Hz, 1H, H^2), 7.05-7.58 (m, 10Hz, 9ArH & H^3), 7.83 (d, *J* = 4.1 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 114.5, 119.1, 123.9, 125.8, 128.0, 129.2, 131.8, 133.9, 134.2, 135.9, 138.2, 139.5, 151.5, 161.2, 185.2 ppm; MS (EI) *m/z*: 324 (M^+). Anal. Calcd. for $C_{19}H_{13}ClOS$: C, 70.25; H, 4.03%. Found: C, 70.12; H, 4.09%.

(4-Methoxyphenyl)-5-[2-(4-chlorophenyl)-vinyl]-thien-2-yl-methanone (6k). Light yellow solid, yield 72%; mp 150-151 ºC; IR (KBr) 1616, 1440 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 3.85 (s, 3H, -OCH_3), 6.80 (d, *J* = 8.5 Hz, 2H, ArH), 6.88 (d, *J* = 8.5 Hz, 2H, ArH), 6.93 (d, *J* = 3.9 Hz, H^1), 7.04 (d, *J* = 15.9 Hz, 1H, H^2), 7.07 (d, *J* = 15.9 Hz, 1H, H^3), 7.20 (d, *J* = 8.6 Hz, 2H, ArH), 7.45 (d, *J* = 8.6 Hz, 2H, ArH), 7.83 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 55.4, 114.2, 118.2, 122.6, 125.7, 128.5, 130.0, 132.3, 134.7, 135.2, 136.7, 146.4, 149.2, 156.7, 165.8, 185.2 ppm. MS (EI) *m/z*: 354 (M^+). Anal. Calcd. for $C_{20}H_{15}ClO_2S$: C, 67.69; H, 4.26%. Found: 67.58; H, 4.20%.

5-[2-(4-Chlorophenyl)-vinyl]-thien-2-yl-4-methylphenyl-methanone (6l). Light yellow solid, yield 69%; mp 108-109 ºC; IR (KBr) 1617, 1446 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 2.31 (s, 3H, -CH_3), 6.89 (d, *J* = 3.9 Hz, H^1), 7.08 (d, *J* = 15.9 Hz, 1H, H^2), 7.18 (d, *J* = 8.6 Hz, 2H, ArH), 7.20 (d, *J* = 15.9 Hz, 1H, H^3), 7.21 (d, *J* = 8.7 Hz, 2H, ArH), 7.39 (d, *J* = 8.6 Hz, 2H, ArH), 7.44 (d, *J* = 8.7 Hz, 2H, ArH), 7.81 (d, *J* = 3.9 Hz, 1H, H^4) ppm; ^{13}C NMR ($CDCl_3$): δ 20.8, 114.1, 122.3, 126.1, 128.0, 128.3, 129.7, 129.6, 130.8, 131.8, 137.1, 142.6, 145.7, 150.6, 157.0, 186.0 ppm; MS (EI) *m/z*: 338 (M^+). Anal. Calcd. for $C_{20}H_{15}ClOS$: C, 70.89; H, 4.46%. Found: C, 70.80; H, 4.42%.

Procedure for the reaction of *N,N'*-bis[(dimethylamino)methylene]thiourea (9) with chloro ketene

To a well-stirred solution of **9** (10 mmol) and triethylamine (40 mmol) in dry methylene chloride (30 mL) was added drop wise a solution of chloroacetyl chloride (20 mmol) in dry methylene chloride (30 mL) over a period of one hour at 0 ºC. After completion of the reaction (tlc), the reaction mixture was first washed with saturated sodium bicarbonate solution (2x25 mL) and water (2x50 mL) and the organic layer dried over anhydrous sodium sulfate. Removal of solvent

under reduced pressure yielded the crude product, which was purified by silica gel column chromatography using a mixture of ethyl acetate and hexane (20:80, v/v).

***N'*-(5-Chloro-6-oxo-6H-[1,3]thiazin-2-yl)-*N,N*-dimethyl-formamidine (12).** Yellow crystals, yield 52%; mp 110-111 ^{o}C; IR (KBr) 1660, 1537 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$): δ 3.15, 3.22 [2s, 6H, $N(CH_3)_2$], 7.94 (s, 1H, olefinic), 8.52 (s, 1H, olefinic) ppm; ^{13}C NMR ($CDCl_3$): δ 35.6, 41.6, 116.7, 151.9, 155.4, 175.2, 177.4 ppm; MS (EI) *m/z*: 217 (M^+). Anal. Calcd. for $C_7H_8ClN_3OS$: C, 38.62; H, 3.70; N, 19.30%. Found: C, 38.73; H, 3.63; N, 19.35%.

Procedure for the reaction of *N,N'-bis*[(dimethylamino)methylene]thiourea (6) with DMAD

A solution of **32** (10 mmol) and DMAD (20 mmol) was stirred for 30 min in dry dichloromethane. After completion (tlc), solvent was evaporated under reduced pressure and the resultant crude mixture was purified through silica gel chromatography. The solid compound was recrystallised using ethyl acetate: hexane mixture (1:5 v/v).

4-Dimethylamino-4*H*-thiopyran-2,3,5,6-tetracarboxylic acid tetramethyl ester (18). Pale white solid, yield 48%; mp 149-150 ^{o}C; IR (KBr) 1735, 1730 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$): δ 2.28 [s, 6H, N $(CH_3)_2$], 3.82 (s, 6H, 2x-OCH_3), 3.85 (s, 6H, 2x-OCH_3), 4.98 (s, 1H, -CH) ppm; ^{13}C NMR ($CDCl_3$): δ 40.7, 52.8, 53.4, 59.6, 125.7, 133.1, 163.6, 166.6 ppm; MS (EI) *m/z*: 373 (M^+). Anal. Calcd. for $C_{15}H_{19}NO_8S$: C, 48.25; H, 5.13; N, 3.75%. Found: C, 48.33; H, 5.07; N, 3.68 %.

Acknowledgements

Dr P Singh gratefully acknowledges the Durban University of Technology (DUT) and National Research Foundation (NRF) for the financial assistance. Spectral assistance from the Department of Chemistry, GNDU, Amritsar, is gratefully acknowledged.

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