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Synthesis, Molecular Modelling and Biological Evaluation of Novel Pyrimidine Derivatives as Anti-inflammatory Agents

Naglaa Mohamed Ahmed[1*], Shahira Nofal[2] and Samir Mohamed Awad[1]

[1]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Helwan University, Ein- Helwan, 11795, Cairo, Egypt.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Ein - Helwan, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. Authors NMA and SMA designed, synthesized, analyzed the data of all compounds, managed the literature searches and contributed to finalizing the manuscript and its supplementary materials in their final version. Author SN performed the biology part. Authors NMA, SMA and SN wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aim: As part of ongoing studies in developing new anti-inflammatory agents, 2-thioxo-1,2,3,4-tetrahydropyrimidine derivative **1** was synthesized by direct Biginelli condensation and used for the synthesis of novel series of pyrimidin-2-thione derivatives **(2a-d** to **7a-b)**.
Materials and Methods: All compounds were examined for their anti-inflammatory activity using the carrageenan-induced rat paw edema assay in comparison to ibuprofen, as a reference drug. Molecular docking studies were carried out using SYBLYL-X v.2.1 software.
Study Design: A series of pyrimidine derivatives were synthesized by a simple and available method leads to a molecule of promising anti-inflammatory activity, the docking studies show good agreement with anti-inflammatory results. Future researches are recommended to assure the importance of these new derivatives for various applications.

**Corresponding author: E-mail: nogamoon2005@yahoo.com;*

Place and Duration of Study: Pharmaceutical Organic Chemistry Department and Pharmacology and Toxicology Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt, between February 2018 and March 2019.

Results: Compounds showed 61 to 86% anti-inflammatory activity where-as ibuprofen showed 69% activity. Compounds **2a, 2b**, **2c**, **2d**, **3a**, **3b**, **3c**, **3d**, **7a**, **7b** induced strong anti-inflammatory activity, comparable with that of ibuprofen, they showed significantly difference at 4h post-carrageenan. Compound **3c** (86%) showed the best result of edema inhibition in rats. Moreover, compounds **1, 2c** and **3c** were subjected to *in vitro* enzyme assay investigations against COX-1 and COX-2. All tested compounds showed higher potency towards COX-2 over COX-1. Compound **3c** realized higher potency towards COX-2 (IC_{50}= 0.046 µM) than compounds **1**(IC_{50}= 0.21 µM) and **2c** (IC_{50}=0.11 µM) as well as ibuprofen (IC_{50}= 43.628 µM). Structure-activity relationship (SAR) has been discussed.

Conclusion: A series of pyrimidine derivatives were synthesized by a simple and available method gave a molecule of promising anti-inflammatory activity, the docking studies showed good agreement with anti-inflammatory results.

Keywords: Anti-inflammatory; biginelli; cyclooxygenase inhibition; molecular docking; pyrimidine; SAR.

1. INTRODUCTION

The pyrimidine ring represents one of the most important medicinal chemistry scaffolds. The biological activities of pyrimidine derivatives are multiple and include antibacterial, antimicrobial [1-3], antitubercular [4-6], anticancer [7], anti-HIV [8,9], antioxidant [10,11], anti-leishmanial [12], antiviral [13], anti-diabetic [14-16], antithyroid [17,18], anticonvulsant [19] and anti-Alzheimer activities [20]. Pyrimidine ring [21] is also an integral part of DNA nucleic acid composition which explains the fact that pyrimidine derivatives display diverse medicinal activities. Acetiamine **I**, Afloqualone **II**, Proquazone **III** exhibited good NSAID potential, and Epirazole **IV**, another NSAID, is reported as a COX-2 inhibitor, are examples for drugs containing pyrimidine moiety that was synthesized and used as Analgesics and NSAID [22] [Fig. 1].

Various compounds based on the pyrimidine scaffolds are known to exhibit analgesic and anti-inflammatory activities [23-25,1] in addition to COX-2 inhibitory activity [26,27]. Literature Survey revealed that pyrimidine scaffold benzamide derivatives [28] and pyrimidine carboxylic acids [29] **V** possess good anti-inflammatory activity, pyrimidine-pyridine hybrids [30] showed better COX-2 inhibitory activity than celecoxib and 3,6-disubstituted 1,2,3,4-tetrahydro pyrimidine derivatives [31] **VI** are reported as selective COX-2 inhibitors. Moreover, thieno [2,3-d]pyrimidines [32] **VII** and curcumin-derived pyrimidines [33] operate as potent anti-inflammatory agent, COX-2 inhibitors [Fig. 2].

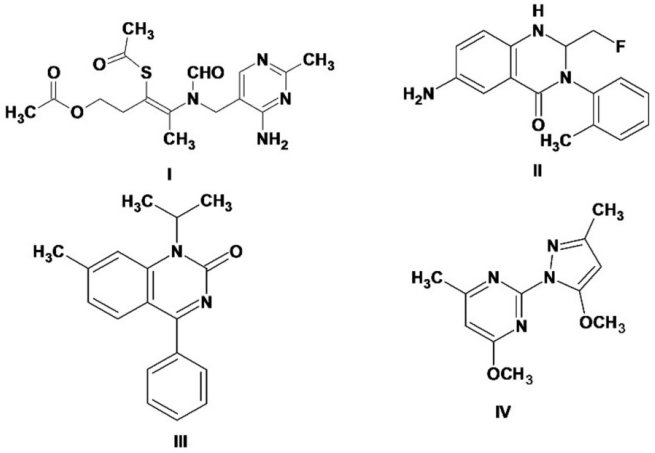


Fig. 1. Anti-inflammatory drugs (NSAIDs)

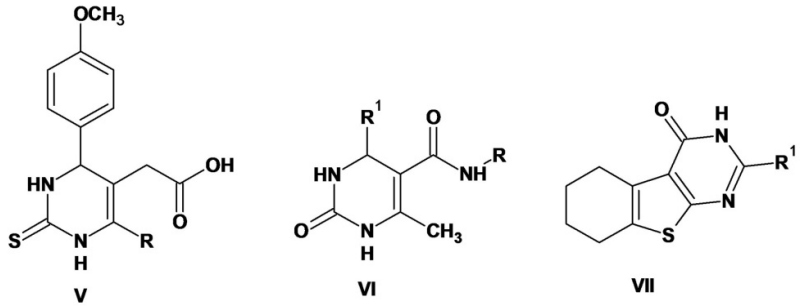


Fig. 2. Some reported pyrimidine containing anti-inflammatory agents

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most successful and widely prescribed drugs known to alleviate pain, fever and inflammation [34]. Their anti-inflammatory efficacy has been attributed to their inhibition of cyclooxygenase (COX) enzymes (COX-1 and COX-2), which lead to suppression of prostaglandin H2 (PGH2) biosynthesis from arachidonic acid (AA) [35]. Present NSAIDs drugs inhibit both COX-1 and COX-2 with minimal specificity [36] and possess serious side effects, mainly gastric ulcers, cardiovascular and renal toxicities [37]. Therefore, the development of new compounds having anti-inflammatory activity with an improved selectivity against the COX-2 enzyme and safety profile is still a necessity.

Motivated by these facts, herein we report the synthesis, *in vivo* anti-inflammatory activity of novel pyrimidin-2-thione derivatives by the carrageenan-induced rat paw edema assay. Moreover, the most active compounds were evaluated for their *in vitro* COX-1/COX-2 inhibition. Structure and activity relationship (SAR) and molecular modeling study were also investigated.

2. MATERIALS AND METHODS

2.1 Chemistry

All melting points were determined in capillary tube on a Boetius melting point microscope and were uncorrected. FT-IR spectra were recorded as KBr pellets on a Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt. ^{1}HNMR and ^{13}C-NMR spectra(Supplementary data)were recorded in DMSO-d6 on a Varian Mercury (300 MHz) spectrometer (Varian UK) and chemical shifts were given as ppm from TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan), Faculty of Science, Cairo University, and Cairo, Egypt. Microanalyses were performed using Vario, Elementar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt and the results were within the accepted range (0.40) of the calculated values.

2.2 Methods

1-{4-[4-(Dimethylamino)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl} ethanone (1)

The titled compound was synthesized by direct Biginelli condensation method [38].

General procedure for preparation of compound (2)

The appropriate primary amine was added to a solution of the pyrimidine derivative **1** (0.01 mol) in absolute ethanol (50 ml) containing 1% conc. H_2SO_4, The reaction mixture was heated under reflux for 3-5 h; the resulted product was cooled, poured onto ice-water (100 ml) and neutralized with 25% ammonia solution (0.5 ml). The produced precipitate was filtered and recrystallized from ethanol to give compounds **2a-d**.

4-(4-Dimethylamino-phenyl)-6-methyl-5-(1-phenylimino-ethyl)-3,4-dihydro-1H-pyrim-idine-2-thione (2a)

Yield: 80%. m.p. 155-160°C. IR (KBr) υ (cm^{-1}): 3382 (NH), 1584 (C=N), 1245 (C=S). MS (EI) m/z: 364(M+,20%). ^{1}HNMR(DMSO-d6, 300 MHz) δ(ppm):2.23(s,3H,CH_3),2.34(s,3H,CH_3, pyrimidi-

ne), 2.50 (s,6H, $2CH_3$), 5.00 (s,1H, pyrimidine), 7.19-8.65 (m,9H,Ar-H),10.04, 10.64 (s,2H,2NH, D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 178.7(C=S) ,164.8 (C=N) , 99.8(C, C-5) ,146.4 (C, C-6) ,128.0 (CH_3),118.6, 125.9, 126.8 ,127.1,128.5, 130.0, 140.0, 150.7 (Ar/olefinic carbon), 55.8 (C, C-4),40.6 (NCH_3) ,18.3 (CH_3). Anal. Calcd. for $C_{21}H_{24}N_4S$(364.507): C, 69.20 ; H, 6.64 ; N, 15. 37; S, 8.80%. Found: C, 69.21; H, 6.66;N, 15.39; S,8.87%.

4-(4-Dimethylamino-phenyl)-6-methyl-5-(1-o-tolylimino-ethyl)-3,4-dihydro-1H-pyrim-idine-2-thione (2b)

Yield: 86%. m.p. 168-170°C. IR (KBr) υ (cm^{-1}): 3385 (NH), 1600 (C=N), 1220 (C=S). MS (EI) m/z: 378(M+,30%). ^{1}HNMR(DMSO-d6,300MHz)δ (ppm):2.01(s,3H,CH_3),2.25(s,3H,CH_3, pyrimidine) ,2.3(s,3H,CH_3),2.5(s,6H,CH_3),5.00(s,1H, pyrimid-ine), 6.68-7.55 (m, 8H,Ar-H),9.60, 9.70 (s,2H, 2NH, D_2O exchangeable) . ^{13}C NMR (300 MHz, DMSO-d6):178.3(C=S) ,164.2(C=N) , 99.5(C, C-5) ,145 (C, C-6) ,128 (CH_3) ,149.7,142.3,132, 131,130,128.5,126.9,126.8,121.9,112.5 Ar/ olefi-nic carbon), 54(C, C-4),40(NCH_3) ,12,18.5 (CH_3). Anal. Calcd. for $C_{22}H_{26}N_4S$ (378.533): C,69.80; H,6.92; N,14.80; S, 8.47%. Found: C, 69.89; H,6.98; N,14.87; S,8.49%.

4-(4-Dimethylamino-phenyl)-6-methyl-5-(1-(4-acetophenylimino)ethyl)-3,4-dihydro-1H-pyrimidine- 2-thione (2c)

Yield: 83%. m.p. 140-142°C. IR(KBr) υ (cm-1): 3380 (NH), 1590 (C=N), 1690(C=O), 1215(C=S). MS (EI)m/z:406.(M+,35%). ^{1}HNMR(DMSO-d6, 300MHz)δ (ppm): 2.20(s,3H,CH_3),2.26(s,3H, CH_3 , pyrimidine), 2.40 (s,3H, CH_3,$COCH_3$), 2.5 (s, 6H, CH_3), 5.16(s,1H, pyrimidine),6.60-7.76(m, 8H,Ar-H), 9.60, 9.78 (s,2H,2NH, D_2O exchang-eable). ^{13}CNMR (300 MHz,DMSO-d6):196 (C=O) ,179 (C=S) ,165(C=N) , 98(C, C-5), 146 (C, C-6) ,128.5 (CH_3), 112,121,121.9,128.2, 129, 131, 142.2, 135, 153 (Ar/ olefinic carbon),52(C, C-4) , 40.3(NCH_3) ,22.8 (CH_3), 18.5 (CH_3). Anal. Calcd.$C_{23}H_{26}N_4OS$ (406. 543): C,7.95 ;H, 6.45;N, 13.78; S,7.89%. Found: C,7.97; H,6.43 ;N,13.70; S,7.80%.

5-[1-(3-Chloro-phenylimino)-ethyl]-4-(4-dimethylamino-phenyl)-6-methyl-3,4-dihydro-1H-pyrimidine-2-thione (2d)

Yield: 80%. m.p.170-175°C. IR (KBr) υ ($cm^{-1)}$: 3390 (NH), 1600 (C=N), 1210 (C=S). MS (EI) m/z: 398 (M+, 3.8 %), 400 (M+2, 1.2 %).^{1}H NMR (DMSO-d6, 300 MHz) δ (ppm): 2.08 (s,3H,CH_3), 2.25 (s, 3H, CH_3, pyrimidine), 2.5 (s,6H, CH_3),5.2 (s, 1H, pyrimidine), 6.90-7.45 (m,8H,Ar-H), 9.98,10.2 (s,2H,2NH, D_2O exchan-geable). ^{13}C NMR (300 MHz, DMSO-d6): 178(C=S) , 162(C=N) , 97(C, C-5) ,144(C, C-6) ,128 (CH_3) ,112.2,120,122, 127,128, 131, 131.5, 135,142.5 ,150(Ar/olefinic carbon), 53(C, C-4) , 43.3(NCH_3),18.1(CH_3). Anal.Calcd.for $C_{21}H_{23}ClN_4S$ (398.952): C,63.22; H,5.81;N,14.04; S, 8.04%. Found: C, 63. 20; H,5.83; N,14.09; S, 8.08%.

General procedure for preparation of compound 3

To a hot solution of paraformaldehyde (0.9 g, 0.01 mol) and the appropriate amine (0.01 mol) in absolute ethanol (25 ml), methyl ketone **1** in 10 ml ethanol was added. The reaction mixture was heated for 3-6 h, Then it was cooled, and the precipitate was filtered off, dried, and crystallized from methanol to afford compounds **3a-d.**

1-[4-(4-Dimethylamino-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl]-3-phenyl amino- propan-1-one (3a)

Yield:79%. m.p. 250-252°C. IR(KBr)ν(cm^{-1}):3345 (NH),2986(CH-aliphatic),3167(CH-aromatic), 684 (C=O). MS (E1) m/z: 394(M^+, 64%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm):2.24 (s,3H, CH_3, pyrimidine), 2.50 (s,6H,$2CH_3$),3.40 (t,2H,CH_2), 3.73 (t,2H,CH_2), 5.15 (s,1H, Pyrimidine),5.90 (s, 1H,NH,D_2O exchangeable), 7.16-8.89(m,9H, Ar-H),10.84,11.64(s,2H,NH,pyrimidine D_2O exchan-geable). ^{13}C NMR (300 MHz, DMSO-d6): 197.0 (C=O), 178.0(C=S), 103.0(C, C-5), 150.0(C, C-6), 111.0, 112.1, 116.0,128.2,129.0, 131.1, 142.2 ,143.0(Ar/olefinic carbon),55.0(C, C-4), 43.0 (NCH_3), 40.0, 45.0 ($2CH_2$), 21.0 (CH_3). Anal. Calcd for $C_{22}H_{26}N_4OS$ (394.533): C, 66.97;H, 6.64;N,14.20;S, 8.13%. Found: C, 66.95; H, 6.70;N,14.17;S, 8.03%.

1-[4-(4-Dimethylamino-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl]-3-o-tolyl amino - propan-1-one (3b).

Yield:81%. m.p. 245-47°C. IR(KBr)ν(cm^{-1}):3344 (NH), 2959 (CH-aliphatic), 3124(CH-aromatic), 1685(C=O). MS (E1) m/z: 408(M^+, 65%). ^{1}H NMR(DMSO-d_6, 300MHz) δ (ppm): 2.49 (s,3H, CH_3, pyrimidine), 2.63 (s,6H, $2CH_3$),2.83 (s,3H, CH_3),3.03 (t,2H, CH_2), 3.34 (t,2H,CH_2), 5.23(s, 1H,pyrimidine), 5.90(1H, NH, D_2Oexchangeable) ,6.44-8.09(m,8H,Ar-H),9.04,10.09(s,2H,NH, pyr -imidine D_2O exchangeable). ^{13}C NMR (300

MHz,DMSO-d6): 197.1(C=O), 179.5(C=S), 103.3 (C,C-5),150.7(C, C-6) , 113.3,116.4,118.6, 126.8, 128.7 ,128. 9,130.0, 142.9,145.9 (Ar/olefinic carbon) , 55.8(C, C-4), 42.4 (NCH_3) , 40.2, 45.6 ($2CH_2$), 21.3 (CH_3), 13.3(CH_3) .Anal. Calcd for $C_{23}H_{28}$ N_4OS:(408.56): C,67.61;H, 6.91;N, 13.71;S, 7.85%. Found: C, 67.59;H, 6.79;N, 13.67;S, 7.90%.

3-(4-Acetylphenylamino)-1-[4-(4-dimethyl amino-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine- 5-yl]-propan-1-one (3c).

Yield:73%. m.p. 225-227°C. IR (KBr)v (cm^{-1}): 3366 (NH),2981(CH-aliphatic), 3179 (CH-aromatic), 1688 (C=O). MS (E1)m/z: 436(M^+, 52%). ^{1}H NMR (DMSO-d_6,300MHz) δ(ppm): 2.25(s,3H, CH_3, pyrimidine), 2.40(s,3H,CH_3, CH_3CO), 2.5 (s,6H, $2CH_3$),3.2 (t,2H, CH_2), 3.3(t,2H, CH_2), 5.2(s,1H, pyrimidine), 5.8(s,1H, NH,D_2O exchan- geable), 7.1-7.8 (m,8H, Ar-H),9.6,9.7 (2H,NH, pyrimidine D_2O exchangeable) . ^{13}C NMR (300 MHz, DMSO-d6): 196.5,197.1 (2C=O), 178.2 (C=S), 103.2(C, C-5),150.2(C, C-6) ,112.0, 112.4 ,125.0, 128.4, 129.0, 131.4, 142.4,147.0 (Ar/ olefinic carbon),55.0(C, C-4), 43.0(NCH_3), 40.1, 45.0 ($2CH_2$),22.8, 21.0($2CH_3$). Anal. Calcd for $C_{24}H_{28}N_4O_2S$ (436. 57): C,66. 03;H,6.46; N,12. 83; S,7.34%. Found: C,66.19;H, 6.39;N, 12.87;S, 7.45%.

3-(3-Chloro-phenylimino)-1-[4-(4-dimethyl amino-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-yl]-propan-1-one (3d).

Yield:86%.m.p.265-267°C. IR (KBr) v(cm^{-1}): 3360 (NH), 2977 (CH-aliphatic), 3198 (CH-aromatic) ,1689(C=O). MS(E1)m/z:428(M^+,67%),430 (M+2, 20.1%). ^{1}HNMR (DMSO-d_6,300MHz) δ(ppm), 2.26 (s,3H,CH_3,pyrimidine) ,2.5 (s, 6H, $2CH_3$),3.1 (t,2H, CH_2), 3.2 (t,2H,CH_2), 5.3 (s,1H, pyrimidine) ,5.8 (1H,NH, D_2O exchangeable) ,7.3-7.8 (m, 8H,Ar-H),9.6, 9.8(s,2H, NH, pyrimidine D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6):197.2(C=O),178.2(C=S),103.5(C, C-5) ,150.5 (C,C-6) ,110.0, 112.0, 112.6, 117.0, 128.5 ,130.0 ,131.5,134.0,142.6,144.0(Ar/olefinic carbon), 55.0 (C, C-4), 43.0 (NCH_3), 40.5 , 45.2 ($2CH_2$), 21.5 (CH_3). Anal. Calcd for $C_{22}H_{25}ClN_4OS$ (428.98) : C, 61.60;H, 5.87;N,13.06;S, 7.47%. Found C, 61.59;H, 5.79;N,13.10;S, 7.50%.

General procedure for preparation of compound 4

A mixture of methyl ketone **1** (0.01 mole) and the appropriate aromatic aldehyde (0.01 mole) in 10% ethanolic sodium hydroxide solution (60 ml) was stirred at room temperature for 24 h. The mixture was then heated for 1-3 h, cooled, poured into ice-water and acidified with conc. HCl. The precipitate was filtered off, dried and recrystallized from aqueous DMF to give compounds **4a-d**.

1-[4-(4-(Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-3-phenyl-prop-2-en-1-one (4a)

Yield: 87%. m.p. 210-212°C. IR(KBr)v(cm^{-1}):3442 (NH),2943(CH-aliphatic),3077(CH-aromatic). 685 (C=O). MS (E1) m/z: 377(M^+,67%). ^{1}HNMR (DMSO-d_6,300MHz)δ (ppm):2.25(s,3H, CH_3, pyrimidine),2.5(s,6H, $2CH_3$)5.1 (s,1H, pyrimidine) ,7.07,7.11 (dd,2CH, CH=CH),7.13-7.55(m,9H, Ar-H), 9.50, 9.88 (s, 2H , NH, pyrimidine D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 187.3(C=O) , 178.4(C=S) , 159.6(C, C-6) ,129.6, 136.6(2CH),118.6(C,C-5),113.9, 126.9, 127.3, 128.6, 126.2, 128.1, 130.0, 145.3 (Ar/olefinic carbon),53.3 (C, C-4), 43.2(NCH_3), 18.6(CH_3). Anal. Calcd for $C_{22}H_{23}N_3OS$(377.50): C,70.00;H, 6.14; N, 11.13 ; S, 8.49%.Found:C,70.17; H,6.24; N,11.17; S, 8.50%.

1-[4-(4-Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-3-(4-methoxy phenyl)- prop-2-en-1-one (4b)

Yield: 83%.m.p. 217-219°C. IR (KBr) v (cm^{-1}): 3387 (NH), 2978 (CH-aliphatic), 3191 (CH-aromatic). 1675 (C=O). MS (E1)m/z: 407 (M^+,77%). ^{1}HNMR (DMSO-d_6, 300MHz)δ(ppm): 2.25(s,3H, CH_3, pyrimidine), 2.55(s,6H,$2CH_3$), 3.8(s,3H,OCH_3),5.1(s,1H,pyrimidine),6.2,6.4(dd,2 CH,CH=CH),7.17-8.395 (m,8H, Ar-H), 10.0, 11.0 (s, 2H, NH, pyrimidineD_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 187(C=O) , 178 (C=S), 159.2(C, C-6) ,129 ,134(2CH), 116.2 (C, C-5),114,112.2, 120,127,128, 129.2, 131.2, 142.2 , 159(Ar/olefinic carbon),56(OCH_3),53.2(C, C-4), 43.2(NCH_3), 18(CH_3). Anal. Calcd for $C_{23}H_{25}N_3O_2S$ (407.53):C, 67.79;H, 6.18; N, 10.31;S, 7.87%. Found: C, 67.87;H, 6.20; N,10.27; S, 7.71%.

1-[4-[4-(Dimethyl amino phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-3-(3, 4, 5-trimethoxy phenyl) -prop-2-en-1-one (4c)

Yield: 85%. m.p. 227-229°C. IR(KBr)v(cm^{-1}):3369 (NH), 2986(CH-aliphatic),3195 (CH-aromatic). 1687 (C=O). MS (E1) m/z: 467(M^+, 63%). ^{1}H

NMR (DMSO-d_6, 300MHz) δ(ppm):2.28 (s,3H, CH_3, pyrimidine),2.5(s,6H, $2CH_3$),4.2 (s,9H, $3OCH_3$), 5.1(s,1H, Pyrimidine), 6.4,6.5 (dd,2CH, CH=CH),7.3-7.9(m,6H,Ar-H),9.7,10.1 (s,2H,NH, pyrimidine D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 187.2(C=O) , 178.2(C=S) , 159(C, C-6) ,129.2 ,134.2 (2CH), 116(C, C-5),105, 112.4, 129, 129.2, 131.4, 132,142.2, 148(Ar/olefinic carbon), 56.3,56.6 (OCH_3),53.2 (C, C-4), 43.2(NCH_3), 18(CH_3). Anal. Calcd for $C_{25}H_{29}N_3O_4S$ (467.58): C, 64.22;H, 6.25;N,8.99; S, 6.86%. Found :C, 64.31;H, 6.28; N,8.87;S, 6.79%.

3-(4-Dimethylamino phenyl)-1-[4-(4-dimethyl amino phenyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro pyrimidin-5-yl]-prop-2-en-1-one (4d)

Yield: 79%. m.p. 230-232°C. IR(KBr)v(cm^{-1}): 3388 (NH),2973 (CH-aliphatic), 3167 (CH-aromatic). 1679 (C=O). MS(E1)m/z: 420(M^+,77%). ^{1}HNMR (DMSO-d_6,300MHz)δ (ppm): 2.25 (s,3H, CH_3, pyrimidine), 2.35,2.6 (s,12H, $4CH_3$) ,5.2(s,1H, Pyrimidine), 6.3,6.4 (dd,2CH, CH=CH),7.2-7.9(m,8H, Ar-H),9.7,9.9 (s, 2H, NH, pyrimidine D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6):187.5(C=O), 178.5 (C=S), 159.5(C, C-6) ,129 ,134(2CH), 116.5(C, C-5),112.5, 113,124,127, 129.1,131.5,142.1, 143(Ar/ olefinic carbon),53(C, C-4), 43(NCH_3), 18(CH_3). Anal. Calcd for $C_{24}H_{28}N_4OS$ (420.57): C, 68.54;H, 6.71; N, 13.32;S, 7.62%. Found: C, 68.87; H, 6.66;N ,13.29;S, 7.61%.

General procedure for preparation of compound 5

A mixture of methyl ketone **1** (0.003 mole), the appropriate aromatic aldehyde(0.003 mole), ammonium acetate (1.89 g,8 mole) and ethyl cyanoacetate (0.003 mole) in 60 ml absolute ethanol was refluxed for about 5-8 h. The reaction mixture was concentrated to its half volume, cooled, filtered off and the filtrate was poured into ice/ water and the precipitate was filtered off, dried and recrystallized from aqueous DMF to give compounds **5a-d**.

6-[4-(4-Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-2-oxo-4-phenyl-1,2-dihydro-pyridine-3-carbonitrile (5a)

Yield: 84%. m.p. 260-262°C. IR (KBr) v (cm^{-1}): 3355 (NH), 2987(CH-aliphatic), 2220(CN), 3177 (CH-aromatic). 1686 (C=O). MS (E1) m/z: 441 (M^+,77%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm):2.26 (s,3H, CH_3, pyrimidine), 2.5 (s,6H, $2CH_3$), 5.2(s,1H, pyrimidine), 7-7.8 (m,9H , Ar-H)),8.3(s,1H ,pyridone), 9.6, 9.8,10.2 (s,3H, 3NH, D_2O exchangeable) . ^{13}CNMR (300 MHz, DMSO-d6):178.0(C=S) , 162.0 (C=O) , 138.0 (C, C-6) , 112.0(C,C-5),112.0,126.2, 127.2, 128.2, 128.0, 131.0 , 134.2, 142.0(Ar/ olefinic carbon), 95.0, 104.0, 137.0, 169.0 (CH pyridone) ,117.0 (CN),54.0(C, C-4), 40.6(NCH_3), 18.0(CH_3). Anal. Calcd for $C_{25}H_{23}N_5OS$ (441.55):C, 68.00;H, 5.25;N,15.86;S,7.26%. Found: C, 68.05; H,5.29; N,15.89;S,7.24%.

6-[4-(4-Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-4-(4-methoxy-phenyl)-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (5b)

Yield:87%. m.p. 251-252°C. IR(KBr) v(cm^{-1}): 3356(NH),2977(CH-aliphatic), 2225(CN), 3178 (CH-aromatic). 1688(C=O). MS (E1) m/z: 471 (M^+, 71%). ^{1}H NMR (DMSO-d_6, 300MHz)δ (ppm): 2.25 (s,3H,CH_3,pyrimidine), 2.5(s,6H, $2CH_3$), 4.1 (s,3H,OCH_3), 5.1 (s,1H,pyrimidine), 7-7.9 (m,8H , Ar-H),8.2(s,1H, pyridone), 9.7, 9.8, 10.2 (s,3H, 3NH, D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 178.0 (C=S), 162.0 (C=O),138.2(C, C-6),112.2 (C ,C-5), 112.1, 114.5, 120.5, 127.5, 128.5 , 128.0, 131.1, 142.0, 159.5(Ar/olefinic carbon), 95.0,104.0, 137.3 , 169.6(CH pyridone) ,117.0(CN), 56.2 (OCH_3), 54.0 (C, C-4), 40.0 (NCH_3), 18.2(CH_3).Anal. Calcd for $C_{26}H_{25}N_5O_2S$ (471.57): C, 66.22;H, 5.34;N, 14.85; S, 6.80%. Found: C, 66.29; H, 5.44;N,14.78;S, 6.82%.

6-[4-(4-Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-2-oxo-4-(3,4,5-trimethoxy-phenyl)-1,2-dihydro-pyridine-3-carbonitrile (5c)

Yield: 72%. m.p. 240-242°C. IR(KBr) v(cm^{-1}): 3366(NH),2959(CH-aliphatic), 2209 (CN), 3069 (CH-aromatic). 1689(C=O). MS (E1) m/z: 531(M^+, 83%). ^{1}H NMR (DMSO-d_6, 300MHz)δ (ppm): 2.21 (s,3H,CH_3,pyrimidine), 2.50(s,6H, $2CH_3$), 3.33, 3.82,3.86(s, 9H,$3OCH_3$),5.21(s,1H pyrimidine),6.99-7.88 (m, 6H ,Ar-H), 7.90(s,1H, pyridone) 9.0,10.0 (s,3H,3NH, D_2O exchan - geable) . ^{13}C NMR (300 MHz, DMSO-d6): 178.7 (C=S) , 163.3(C=O) , 139.9(C, C-6) , 114.8(C, C-5),102.6, 112.6,127.6, 126.8, 128.5, 142.9, 148.7,150.5(Ar/olefinic carbon),97.3, 107.6 , 137.6, 169.1(CH pyridone) ,118.6(CN), 56.4, 56.6 (OCH_3),55.9(C, C-4), 40.2(NCH_3), 18.5 (CH_3).Anal. Calcd for $C_{28}H_{29}N_5O_4S$ (531,62): C,

63.26;H, 5.50;N,13.17;S, 6.03%. Found C, 63.39;H, 5.43;N,13.28;S, 6.17%.

4-(4-Dimethylamino phenyl)-6-[4-(4-Dimethyl amino phenyl)- 6-methyl-2-thioxo-1,2,3,4-tetra hydropyrimidin-5-yl]-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (5d)

Yield: 74%. m.p. 270-272°C. IR(KBr)v(cm^{-1}):3376 (NH),2978(CH-aliphatic),2221 (CN), 3188(CH-aromatic) ,1680(C=O). MS (E1) m/z: 484(M^+, 80%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.23 (s, 3H, CH_3, pyrimidine), 2.4,2.53(s,12H, 4CH_3),5.1 (s,1H, pyrimidine), 7.2-7.8 (m,8H,Ar-H),8.2(s,1H ,pyridone) , 9.7,9.9,10.2 (s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d6): 178.8 (C=S) , 162.0 (C=O) , 138.0(C, C-6) , 112.0(C, C-5),112.0, 113.0,124.0, 127.0, 128.1, 131.1, 142.0 ,143.0 (Ar/olefinic carbon), 95.0, 104.0,137.3, 169.6(CH pyridone) ,117.0 (CN), 56.3, 56.6 (OCH_3),54.4(C, C-4), 40.6 (NCH_3), 18.0(CH_3). Anal. Calcd for $C_{27}H_{28}N_6$ OS(484.61): C, 66. 92;H, 5.82; N,17.34;S, 6.62%. Found C, 66.97;H, 5.77;N,17.45;S, 6.59%.

General procedure for preparation of compound 6

A mixture of **1** (0.001 mole),malononitrile or ethyl cyanoacetate (0.001 mole) in presence of catalytic amount of triethylamine (4drops) was refluxed for 6 -8 h, then cooled, poured on ice/water and neutralized with dil. HCl, the produced precipitate was filtered off, dried under suction and recrystallized from DMF/ water to give **6 a, b**.

2-{1-[4-(4-Dimethylaminopheny)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]ethyl idene}-malonitrile (6a)

Yield: 67%. m.p. 180-2°C. IR(KBr) v (cm^{-1}):3357 (NH), 2988(CH-aliphatic), 3176(CH-aromatic), 2226(CN). MS(E1) m/z: 377(M^+, 67%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm):2.24 (s, 3H,CH_3, pyrimidine), 2.4 (s,3H,CH_3), 2.5(s,6H, 2CH_3), 5.1(s, 1H Pyrimidine),7.2-7.6 (m,4H, Ar-H),9.6,9.8 (s,2H, NH, pyrimidine D_2O exchangeable. ^{13}C NMR (300 MHz, DMSO-d_6): 178.0 (C=S), 174.0(CH), 112.2(C, C-5) ,138.0(C, C-6), 112.0,128.0,131.0, 142.2(Ar/ olefinic carbon), 117.0(CN),74.0(CH), 55.0(C, C-4) , 40.6(NCH_3), 12.0, 18.0 (CH_3).Anal. Calcd for $C_{18}H_{19}$ N_5S (337.44): C,64.07;H,5.68;N, 20.75;S, 9.50%. Found C, 64.17;H, 5.74 ; N,20.77;S, 9.49%.

2-Cyano-3-[4-(4-(dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine -5-yl]-but-2-enoate (6b)

Yield:70%. m.p. 150-152°C. IR (KBr) v (cm^{-1}): 3390(NH),2926(CH-aliphatic),3063(CH-aromatic) . 2225 (CN) ,1704(C=O). MS (E1) m/z: 384(M^+, 66%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 2.0(t,3H, CH_3), 2.25(s,3H,CH_3,pyrimidine), 2.46 (s,3H,CH_3),2.50 (s,6H,2CH_3),3.86(q,2H, CH_2),5.2 (s,1H pyrimid ine), 7.13-7.52(md,4H,aromatic), 9.6,9.9(s, 2H, NH pyrimidine,D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d_6):180.0(C=S) , 169.3(CH),165.6(C=O), 111.9(C, C-5) ,138.0(C, C-6) ,113.9, 127.3,128.1.1, 145.3(Ar/ olefinic carbon),118.6(CN),94.3(CH), 56.0(CH_2),55.0 (C, C-4) , 40.4(NCH_3) , 12.6,14.9, 18.0 (CH_3). Anal. Calcd for $C_{20}H_{24}N_4$ O_2S(384.49): C, 62.48;H, 6.29;N,14.57;S, 8.34%. Found: C, 62.57;H, 6.34;N,14.61;S, 8.36%.

General procedure for preparation of compound 7

To a suspension of the methyl ketone **1** (0.1 mol), malononitrile or ethyl cyanoacetate (0.1 mol) and 3.2 g sulfur in 30 ml ethanol, diethylamine (0.1 mol) was added drop wise for 30 m and the reaction mixture was heated (70°C) under stirring in absolute ethanol for 4-6 h. mixture was left for 24 h at 0°C. The obtained 2-aminothiophene crystalized in the form of yellow powder. The precipitate was filtrated, washed with water and recrystallized from ethanol to give **7a, b**.

2-Amino-4-[4-(4-dimethylaminopheny)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine - 5- yl] -thiophene- 3- carbonitrile (7a)

Yield:67%. m.p. 130-132°C. IR(KBr)v(cm^{-1}): 3257 (NH),2980 (CH-aliphatic), 3176(CH-aromatic), 2222 (CN) . MS(E1)m/z:369(M^+,67%). ^{1}HNMR (DMSO-d_6, 300MHz) δ(ppm): 2.20 (s, 3H,CH_3, pyrimidine),2.5 (s,6H, 2CH_3),4.08(s,2H, NH_2,D_2O exchangeable), 5.1 (s,1H pyrimidine),7.4-7.54 (m, 5H,Ar-H), 9,9.5 (s, 2H, NH, pyrimidine D_2O exchangeable. ^{13}C NMR (300 MHz, DMSO-d_6):178.0(C=S) , 111.0(C, C-5) ,140.0(C, C-6) , 110.0,112.2,123.0, 128.2, 131.1, 137.0,138.0, 142.1 (Ar/olefinic carbon),116.0(CN), 61.0 (C, C-4) , 40.0(NCH_3) ,18.0 (CH_3).Anal. Calcd for $C_{18}H_{19}N_5S_2$ (369.51): C,58.51; H,5.18; N,18.95; S,17.36%. Found: C,58.58; H,5.17; N,18.90; S,17.32%.

2-Amino-4-[4-(4-dimethylaminopheny)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5- yl] -thiophene-3-ethanoate (7b)

Yield:60%. m.p. 150-152°C. IR(KBr)v(cm^{-1}): 3318 (NH),2936(CH-aliphatic), 3198 (CH- aromatic), 1736 (C=O). MS (E1) m/z: 416(M^+, 66%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 1.29(t, 3H, CH_3) ,2.48 (s,3H,CH_3,pyrimidine), 2.50(s,6H, $2CH_3$), 3.08(q,2H,CH_2), 3.36 (s,2H, NH_2,D_2O exchangeable), 5.2 (s,1H pyrimidine), 6.82-8.09(m,5H,aromatic),9.0,10.0(s,2H,NH pyrimidine , D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d_6):178.8(C=S) , 160.5 (C=O) ,114.8 (C, C-5) ,139.9(C, C-6) ,118.6, 127.6, 128.0, 128.7, 128.9,130.0, 148.7(Ar/ olefinic carbon), 63.3(C, C-4),55.9(CH_2),40.6(NCH_3),13.1,18.3 (CH_3).Anal. Calcd for $C_{20}H_{24}N_4O_2S_2$ (416.56): C,57.67; H,5.81; N,13.45; S,15.40% . Found: C,57.65; H, 5.87; N,13. 40; S,15.44%.

2.2.1 Biological assay

2.2.1.1 Anti-inflammatory activity

Chemicals: All chemicals required for assay were used as analytical grade, and were purchased from Sigma-Aldrich Chemicals Co., St. Louis, MO, USA.

Animals: Adult male Sprague-Dawley rats (5 rats per group), weighing 120-150 g, were housed in polyethylene cages in a temperature-controlled (25 ± 1°C) environment and provided free access to food and purified drinking water.

Carrageenan-induced mouse paw edema: The anti-inflammatory activity was performed according to Winter et al. [39] prepared compounds (equimolar to active dose of the reference drug), control and standard drug were dissolved in 1 ml DMSO and administrated subcutaneously. One hour later, paw oedema was induced by sub-plantar injection of 0.1 ml of 1% carrageenan (Sigma-Aldrich, St. Louis, USA) in distilled water into the right paw. The paw volumes were measured using a water plethysmometer (Basile, Comerio, Italy) before and after injection of 1% carrageenan at different time intervals (1, 2, 3 and 4 h). The difference between the right and left paw volume was measured at the above-mentioned time intervals after induction of inflammation. Control group (five rats per group) received 1 ml DMSO (as to evaluate the interference of DMSO itself in biological test) subcutaneously and carrageenan in sub-plantar region.

Results were expressed as percentage inhibition of inflammation. Ibuprofen (70 mg kg-1) was used as the reference drug (Table 1).

Statistical Analysis: Results are expressed as the mean ± SEM, and different groups were compared using one way analysis of variance (ANOVA) followed by Tukey–Kramer test for multiple comparisons, using Graph Pad Instant (version 3.05) as the statistical software.

Calculation: Equimolar doses of tested compounds were calculated in relation to these of reference drug:

Swell = mean difference in rat paw volume between right and left paw ± SE.

$$\% \text{ inhibition} = (1 - rt/rc) \times 100$$

[rt = swell of tested group; rc = swell of control group].

2.2.2 Biochemical assay

2.2.2.1 In vitro cyclooxygenase inhibition assay

Drugs capacities to inhibit COX-1 and COX-2 enzymes were assessed using ELISA kits; Cayman colorimetric COX (ovine) inhibitor screening assay kit [40] (Catalog No. 760111) supplied by Cayman chemicals, Ann Arbor, MI, USA. The used protocol was according to the manufacturer protocol guide and instructions using ELISA plate reader.

The inhibitory COX activity of the most active tested compounds and the reference was assayed using Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111, Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's instructions. Aliquots (170 ml) of the assay buffer (0.1N Tris–HCl, pH 8.0), heme, and enzyme ovine (COX- 1 or COX- 2) were placed in a 96-well plate. The compounds were added to the aliquots. The plate was shaken for a few seconds and then incubated for 5 m at 25°C. The colorimetric substrate N, N, N′, N′- tetramethyl-p-phenylene diamine (20 ml,TMPD) and arachidonic acid (20 ml) were added to the aliquots. The plate was carefully shaken for a few seconds and then incubated for 5 m at 25°C. The absorbance was measured at 590 nm using a 96- well Tecan Safire plate reader. The mixture of 160 ml of assay buffer and 10 ml of heme served as a back-ground control. The mixture of 150 ml of

assay buffer, 10 ml of heme, and 10 ml of ovine COX- 1 or COX- 2 showed 100% initial activity as a control experiment in the absence of inhibitor. Celecoxib, Ibuprofen were used as reference standards in the study. The assays were performed in triplicate, and the IC_{50} values were calculated from the concentration curves by means of Graph Pad software PRISM.

2.2.3 Molecular modeling procedure

All Molecular modelling work was performed using SYBYL-X package (www.certara.com). Protein co-crystal structure was downloaded from the protein databank (PDB) database (www.rcsb.org). The protein was first optimized for docking by deleting all but one monomer in the quaternary structures. Next, the protein was prepared using "Prepare Protein" tool embedded in the Sybyl-X program. Ligands were converted to 3D structures and prepared using Concord module embedded in Sybyl-X's prepare ligands tool.

The prepared protein and ligand structures were then used for molecular docking using Surflex docking engine. A hypothetical protomol was generated to define the shape and features of the binding site using the binding mode of the co-crystallized ligand. Docking results were analyzed using the analyze results tool in Sybyl-X.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic route utilized for the synthesis of the target compounds is outlined in Schemes 1 and 2. 2-Thioxo-1,2,3,4-tetrahydropyrimidine derivative **1** was achieved by direct Biginelli condensation using ethyl acetoacetate, thiourea and N,N-dimethyl benzaldehyde in ethanol and few drops of HCl [38]. Biginelli method remains one of the most synthetic methods for preparation of pyrimidine derivatives [41,42]. It is a multiple component chemical reaction involves an aldol condensation of acetyl acetone and the aryl aldehyde which is the rate limiting step leading to the carbenium ion. The nucleophilic addition of thiourea gives intermediate, which quickly dehydrates to give the desired compound. Pyrimidine-2- thione **1** was used as asynthon for other pyrimidine derivatives. New Schiff bases **2a-d** have been synthesized from the condensation of various aromatic amines with methyl ketone **1** in the presence of a catalytic amount of conc.H_2SO_4 [43-45]. An imine, a compound in which the C = O double bond is replaced by a C = N double bond. This type of compound is known as an imine or Schiff base (azomethines). The IR spectra of **2a-d** revealed the absence of the absorption bands corresponding to the C=O group and the detection of a strong C=N stretching band at 1580-1600 cm^{-1} evidenced the formation of the Schiff base. Their ^{1}H-NMR showed singlet at 8.8-11.6 ppm corresponding to the D_2O exchangeable protons of 2NH of the pyrimidine ring. Pyrimidine-2-thione **1** undergoes Mannich reaction [46,47] with paraformaldehyde and certain primary aromatic amines yielding compounds **3a-d**. The mechanism of Mannich reaction starts with the formation of an iminium ion from the amine and the formaldehyde. The ketone can tautomerize to the enol form, after which it can attack the iminium ion. The final product is a ß-amino-carbonyl compound also known as a Mannich base as exemplified by compounds **3a-d** [Fig. 3]. Their IR spectra showed the presence of absorption bands at 1681-1689 cm^{-1} characteristic for the C=O groups. Their ^{1}H-NMR spectra showed triplets of CH_2-CH_2 at the range 3.03-3.34 ppm.

α,ß-Unsaturated compounds **4a-d** (Chalcones) were prepared via Claisen-Schimidt condensation of methyl ketone **1** and substituted benzaldehyde in basic medium [48]. Their IR spectra showed absorption bands at the carbonyl region 1675-1687 cm^{-1}. ^{1}H-NMR spectra showed the doublet-doublet corresponding to CH=CH of the chalcones. Pyridones **5a-d** were also prepared through one pot multicomponent reaction of methyl ketone **1**, an aldehyde, ethyl cyanoacetate and excess ammonium acetate in basic medium [49]. Their IR spectra revealed the appearance of the bands corresponding to CN around 2200 cm^{-1}, NH around 3350 cm^{-1} and C=O around 1680 cm^{-1}. The ^{1}H-NMR spectra showed the disappearance of the singlet corresponding to $COCH_3$ (Schemes 1). On the other hand, when methyl ketone **1** was condensed with malononitrile [50] or ethyl cyanoacetate in presence of catalytic amount of triethylamine, they give derivatives **6a** and **b**, respectively. The IR spectrum of **6a** revealed the absence of C=O groups and the appearance of the bands corresponding to CN at 2226 cm^{-1}. While the ^{1}H-NMR spectrum of **6b** showed triplet signal at 2 ppm and quartet signals at 3.8 ppm of $COOC_2H$. Finally, 2-amino thiophene derivatives **7a** and **7b** were synthesized through Gewald thiophene [51] synthesis via a Knoevenagel

condensation between methyl ketone **1** and ethyl cyanoacetate or malononitrile **2** to produce the stable intermediate **3**. The mechanism of the addition of sulphur is unknown. It is postulated to proceed through intermediate **4**. Cyclization and tautomerism will produce the desired product **6** [Fig. 4]. The IR spectrum of **7a** revealed the absence of the absorption bands of the C=O group and the appearance of the bands of CN. The ^{1}H-NMR spectra for **7a** and **b** showed singlet of the NH_2 protons at the range 3.36-4.08 ppm (Schemes 2). The structures of new compounds were assigned by MS, IR, ^{1}H-NMR, ^{13}C-NMR, as well as elemental analysis.

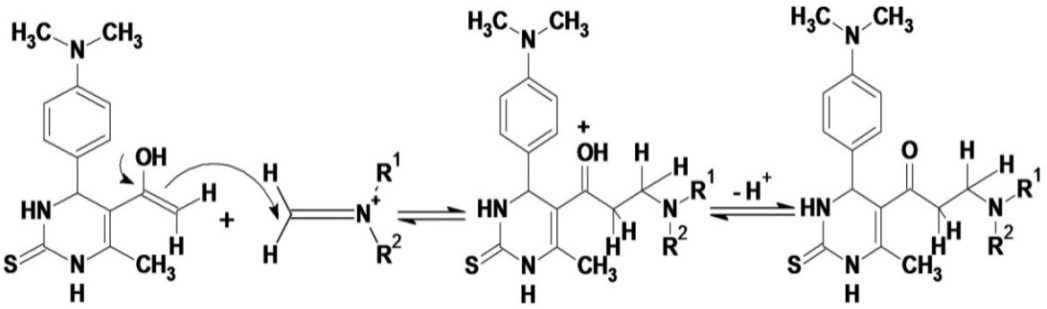


Fig. 3.Synthetic and mechanistic pathway for preparation of thiopyrimidines 3 a-d

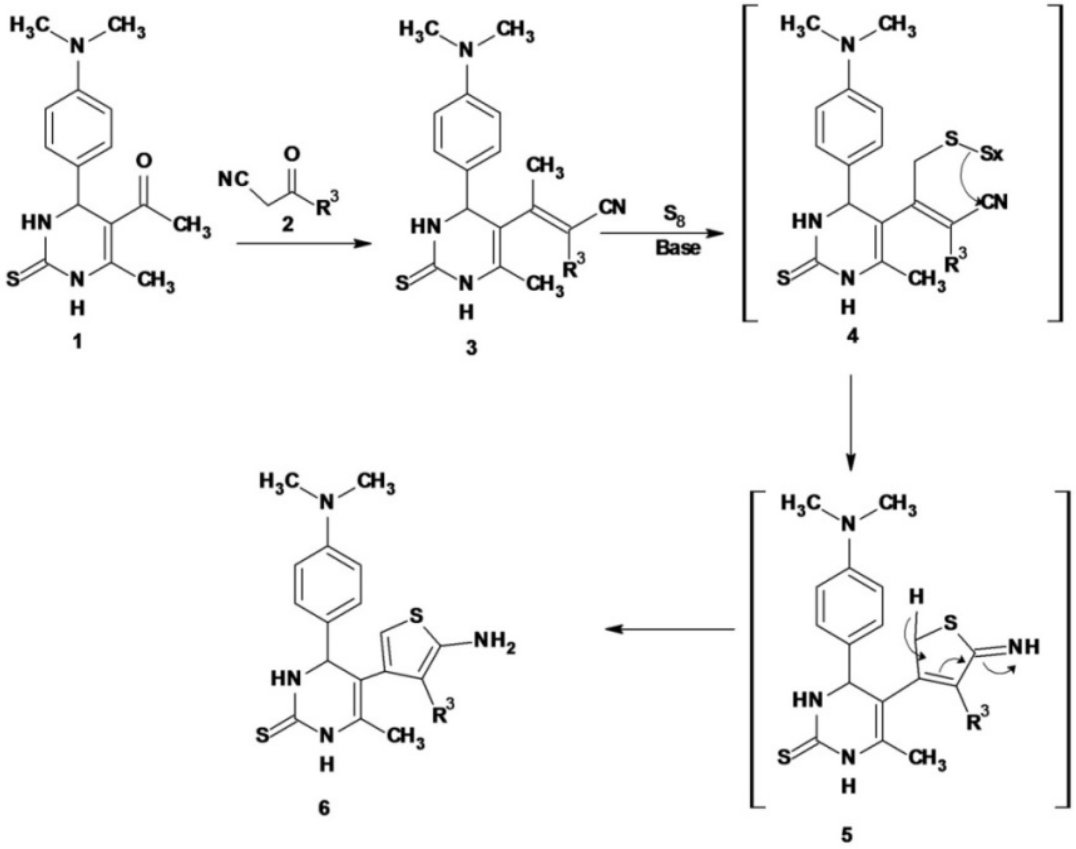
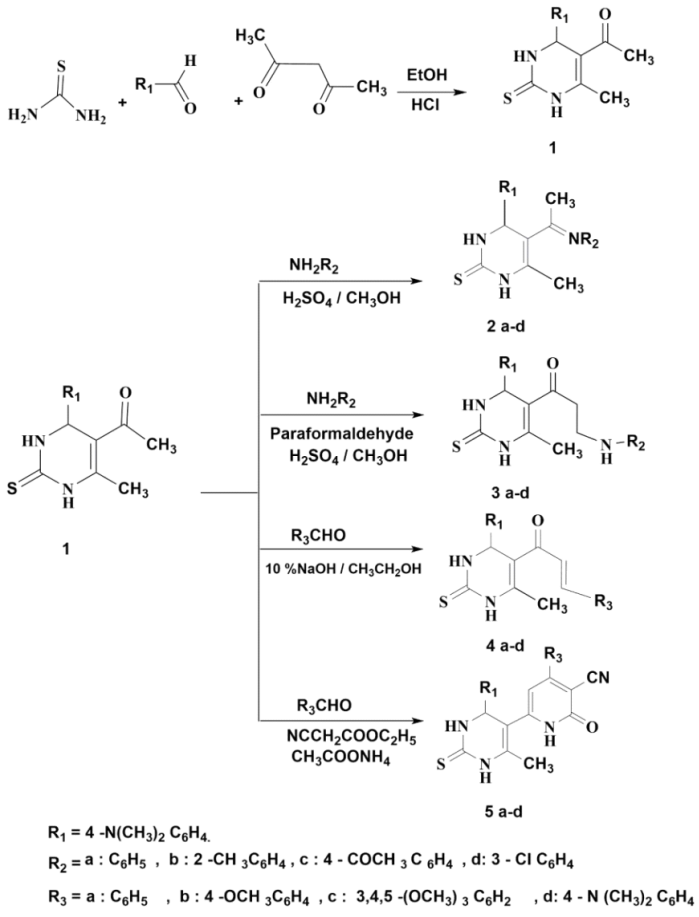


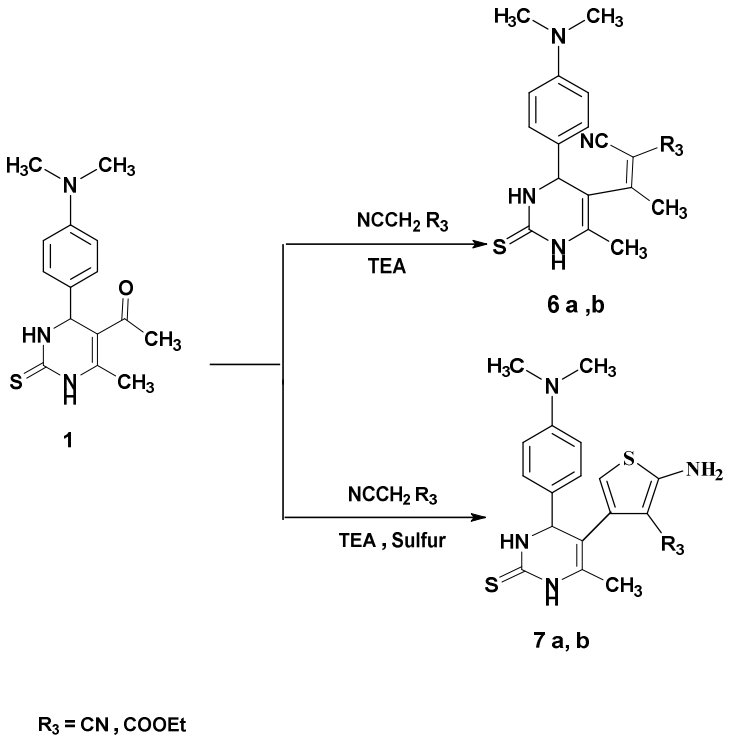
Fig. 4.Synthetic and mechanistic pathway for preparation of thiopyrimidines 7a,b



Scheme 1. Synthesis of the designed compounds (4a-t)

3.2 Anti-inflammatory Evaluation

All compounds were examined for their anti-inflammatory activity using the carrageenan-induced rat paw edema assay in comparison to ibuprofen, as a reference drug [Fig. 5]. According to the data results expressed in %protection; shown in Table 1, Compound **3c** (86%) showed the best % inhibition against Carrageenan-induced paw edema in rats at 4h to be more than that was shown by ibuprofen (69%). Interestingly, the results went aligned with what we got from the *in vitro* testing against COX-1 and COX-2 enzymatic assay (Table 2). Compounds **2a-d, 3a-d, 7a, 7b** induced strong anti-inflammatory activity, comparable with that of ibuprofen ,they showed significantly difference at 4h post-carrageenan and **2b**, **2c**, **3c** and **7a** have the same activity profile as ibuprofen (response increase by time). Compounds **2a**, **3d** and **7a** exerted good anti-inflammatory activity than ibuprofen at 3 h interval post-carrageenan. The compounds produced 76, 66, and 65% inhibition respectively, compared to 60% inhibition for ibuprofen. Compounds **2c**, **3b**, **3c**, **6b** and **7a** showed higher anti-inflammatory activities than ibuprofen at 1 hr interval post-carrageenan range from 22-74%. Compounds **1**, **2a**, **2d**, **3d**, **6a**, **7b** showed no anti-inflammatory activity at 1st and 2nd h interval post-carrageenan. Yet, they exerted from moderate to good anti-inflammatory activities at 4th h interval post-carrageenan. Compound **3a** had no anti-inflammatory activity at 1h post-carrageenan and showed moderate anti-inflammatory activity at 2nd and 3rd h and good activity at 4th h post-carrageenan. Compounds **4a-d** and **5a-d** were all inactive over all tested periods, showing % inhibition < 10, 18, 20 and 31 at 1st to 4th h, respectively, and were indicated as inactive in Table 1.



Scheme 2. Synthesis of the designed compounds (6,7a-b)

3.2.1 Structure-activity Relationships (SAR)

To analyze our structure-activity relationships, Regarding the nature of the aromatic nucleus in the side chain at C-5, the presence of acetyl group at para-position in the side chain at C-5 in compounds **2c**, **3c** has higher anti-inflammatory activity (78, 86%) during the 4th hour post-carrageenan than the methyl group at ortho-position in compounds **2b, 3b** and the chloro group at para -position in compounds **2d,3d** during the 4th hour post-carrageenan. The presence of this group may be making these compounds favorable stereochemically and electronically for interaction with the active site and thus showing good anti-inflammatory activity.

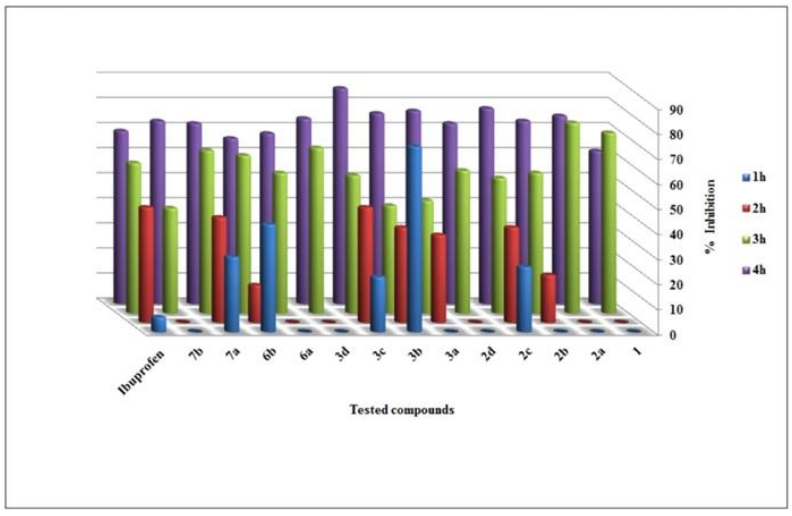


Fig. 5. *In vivo* anti-inflammatory effect (% Inhibition) of the tested compounds compared to the reference drug (ibuprofen)

Table 1. *In vivo* anti-inflammatory activity against carrageenan-induced paw edema in rats

	edema induced by carrageenan (% edema inhibition relative to control)[a]			
	1h	2 h	3 h	4 h
Compounds	mean ± SEM (% inh.)	mean ± SEM (% inh.)	mean ± SEM (% inh.)	mean ± SEM (% inh.)
1	0.24±0.005(-)	0.26±0.002 (-)	0.15±0.046 (72)	0.24±0.125 (61)
2a	0.22±0.003(-)	0.26±0.008 (-)	0.13±0.036 (76)	0.155±0.051(75**)
2b	0.23±0.005(-)	0.21±0.033(19)	0.24±0.057 (56)	0.17±0.137 (73**)
2c	0.17±0.003(26)	0.16±0.005(38)	0.25±0.005 (54)	0.136±0.03 (78***)
2d	0.23±0.001(-)	0.26±0.001 (-)	0.23±0.006 (57)	0.173±0.123(72**)
3a	0.24±0.007(-)	0.169±0.023(35)	0.297±0.021(45)	0.142±0.180(77***)
3b	0.06±0.31(74)	0.16±0.013 (38)	0.31±0.106 (43)	0.148±0.055(76***)
3c	0.18±0.036(22)	0.14±0.045(46)	0.24±0.124 (55)	0.086±0.124(86***)
3d	0.23±0.13 (-)	0.26±0.003 (-)	0.185±0.014(66)	0.16±0.035 (74**)
6a	0.24±0.003(-)	0.26±0.005 (-)	0.24±0.033 (56)	0.198±0.090(68**)
6b	0.13±0.032(43)	0.22±0.013(15)	0.2±0.081 (63)	0.201±0.03 (66**)
7a	0.16±0.020(30)	0.15±0.004(42)	0.19±0.080 (65)	0.173±0.01 (72***)
7b	0.23±0.003 (-)	0.26±0.005 (-)	0.31±0.081 (42)	0.167±0.013 (73**)
Ibuprofen	0.215±0.022(6)	0.14±0.054(46)	0.214±0.017(60)	0.193±0.015 (69*)
Control	0.23±0.048 (-)	0.26±0.038 (-)	0.54±0.08 (-)	0.62±0.03 (-)

[a] *solvent: 2.5 mL DMSO. Dose: 70 mg kg-1 ibuprofen and the equivalent amount of tested compounds.*
SEM: standard error of the mean.
%inhibition =(1-rt/rc)x100 [rt= mean of tested group; rc= mean of control group]
* *Significantly different compared to control at respective time point at p < 0.05*
** *Significantly different compared to ibuprofen at respective time point at p < 0.05*

3.3 *In vitro* Cyclooxygenase (COX) Inhibition Assay

The compounds **1**, **2c** and **3c** were subjected to enzyme assay investigations against COX-1, and COX-2. Results for the *in vitro* enzyme inhibition assays, displayed in Table **2**, revealed that compounds **1**, **2c** and **3c** showed higher potency towards COX-2 over COX-1. Compound **3c** realized higher potency towards COX-2 (IC_{50}= 0.046 µ M) than compounds **1**((IC_{50}= 0.21 µ M) and **2c** (IC_{50}= 0.11 µ M) as well as celecoxib and ibuprofen (IC_{50}=0.045, 43.628 µ M, respectively).

Further investigation of the *in vitro* results revealed that the highest *in vitro* anti-inflammatory activity of compound **3c** might be attributed to presence of acetyl group that would increase binding interaction to COX-2 enzyme through hydrogen bond interaction.

3.4 Molecular Modeling

Molecular docking studies were carried out using SYBLYL-X v.2.1 software. Crystal structures from the Protein Databank website (www.rcsb.) were downloaded. Protocol described in the "Experimental" section.

To propose an understanding of the anti-inflammatory and the enzyme inhibition assay results on a structural basis, docking of selected compounds into the active site of COX-2 was conducted. The flexible docking calculations give a prediction for correct binding geometry for each binder. The scoring functions and hydrogen bonds formed with the amino acids of the receptor. Three compounds which score significant anti-inflammatory activates **1,2c** and **3c** were docked into the active site of COX-2 enzyme. The Docking scores of the compounds were calculated from minimized ligand protein complexes, hydrogen bond distances are shown in Table 3. Results of their interaction energies with COX-2 enzyme revealed that tested compounds **2c, 3c** and **7a** showed proper fitting to the active site of COX-2 enzyme, scoring bond energies ranging from -3.6329 to 6.0459 Kcal/mol compared to 9.0092 for Ibuprofen reference drug. The crystal structure of compound **1** in complex with COX-2 shows no hydrogen bonding between the compound and any of the binding site residues. Binding however could be explained on the bases of extensive Van Der Waals contact with many hydrophobic residues which include Trp388, Tyr386, Phe519, Met523, Leu532, Val117, Leu360 and Val350

[Fig. 6]. Compound **2c** forms 2 hydrogen bonds with the guanido side chain of Arg514. Significant hydrophobic contact is found between the pyrimidine thione moiety of the compound with the side chains of Trp388, Tyr386, Phe519 and Met523. Furthermore, the side chains of Leu532, Val117, Leu360 and Val350 with the N,N-dimethylaniline moiety also show hydrophobic contact [Fig. 7]. The orientation of the most active compound **3c** is quite similar to that of **2c** [Fig. 8]. The Surflex Score values show good agreement with binding affinities obtained by docking studies as verified by pharmacological testing.

Table 2. *In vitro* COX-1 and COX-2 enzymes inhibitory activities, IC50 values of the tested compounds

Compound	COX-1 IC50 (μm) [a]	COX-2 IC50 (μm)
Celecoxib	14.7	0.045
Ibuprofen	31.954	43.628
1	5.41	0.21
2c	9.11	0.11
3c	12.6	0.046

[a] *Values are means of three determinations*

Table 3. Molecular docking results of active compounds into COX-2 enzyme using SYBLYL-X.2.1

Compounds	Surflex Score	N° of Hydrogen Bonds
Ibuprofen	9.0092	3
1	5.5331	0
2a	-0.2735	1
2b	2.9907	1
2c	-3.6329	2
2d	1.5165	0
3a	7.2142	0
3b	5.9312	1
3c	6.0459	2
3d	5.1953	1
6a	0.8744	1
6b	0.5471	1
7a	2.8091	2
7b	3.3581	1

Surflex score = Calculated–log K_d

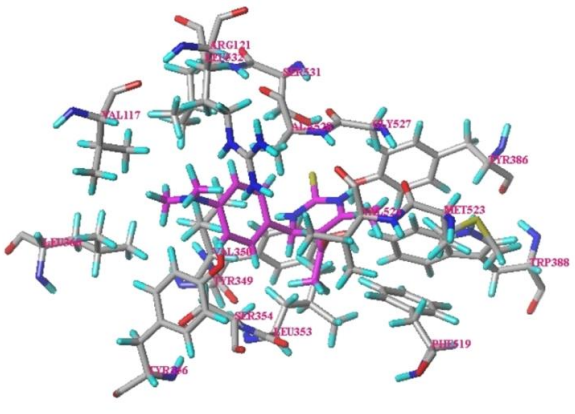


Fig. 6. Binding mode of compound 1 into binding site of COX-II, Showing extensive Vander Waals contact with many hydrophobic residues which include Trp388, Tyr386, Phe519, Met523, Leu532, Val117, Leu360 and Val350

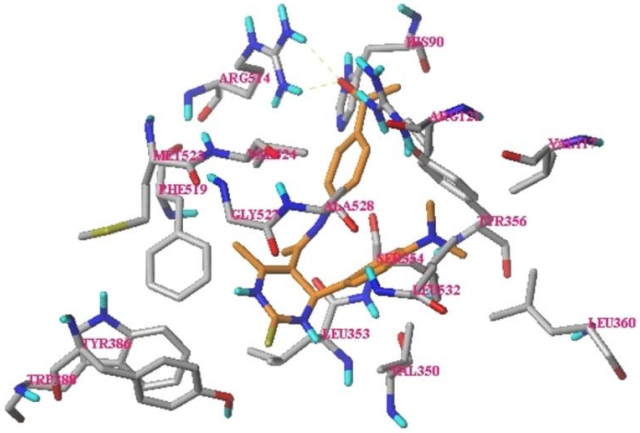


Fig. 7. Binding mode of compound 2c into binding site of COX-II, Showing two H-bonds with the guanido side chain of Arg514. Significant hydrophobic contact is found between the pyrimidine thione moiety of the compound with the side chains of Trp388, Tyr386, Phe519 and Met523. Furthermore, the side chains of Leu532, Val117, Leu360 and Val350 with the N ,N-dimethylaniline moiety also show hydrophobic contact

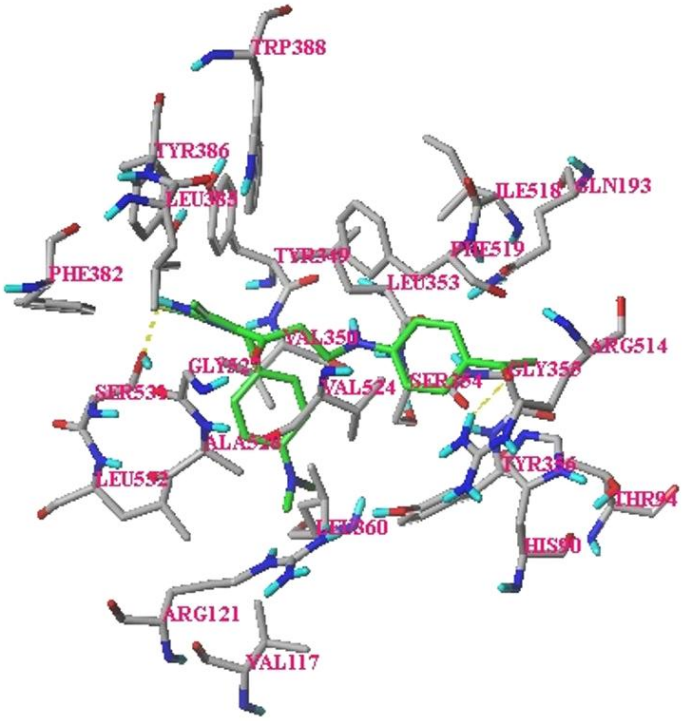


Fig. 8. Binding mode of compound 3c into binding site of COX-II, Showing two H-bonds with the guanido side chain of Arg514. Significant hydrophobic contact is found between the pyrimidine thione moiety of the compound with the side chains of Trp388, Tyr386, Phe519 and Met523. Hydrophobic contact is also found between the side chains of Leu532, Val117, Leu360 and Val350 with and the N,N-dimethylaniline moiety

4. CONCLUSIONS

In summary, we have synthesized a series of new pyrimidine derivatives **(2a-d-7a-b**) by a simple and available method leads to a molecule of promising anti-inflammatory activity. *In vivo* anti-inflammatory assay revealed that compounds **2a-d, 3a-d, 7a, 7b** induced strong anti-inflammatory activity, comparable with that of ibuprofen, were significantly difference at 4 h post-carrageenan and **2b**, **2c**, **3c and 7a** have the same activity profile as ibuprofen (response increase by time). Compound **3c** (86%) showed the best % inhibition against Carrageenan-induced paw edema in rats at 4 h to be more than that was shown by ibuprofen (69%). Interestingly, the results went aligned with what we got from the *in vitro* testing against COX-1 and COX-2 enzymatic assay. To analyze our structure-activity relationships, the presence of acetyl group at Para-position in the side chain at C-5 in compounds **2c, 3c** gives promising anti-inflammatory activity (78, 86%). Compound **3c** realized higher potency towards COX-2 (IC_{50}= 0.046 μM) than compounds **1**((IC_{50}= 0.21 μ M) and **2c** (IC_{50}=0.11 μM) as well as celecoxib and ibuprofen (IC_{50}=0.045 and 43.628 μM, respectively). The Surflex Score values show good agreement with binding affinities obtained by docking studies as verified by pharmacological testing. Further research is recommended to approve the importance of new derivatives for various applications.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH PUBLICATION NO. 85-23, REVISED IN 1996) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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