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

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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 SYBYLX 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.

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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, thiens [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)
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. 1H NMR and 13C-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 El 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. H2SO4. 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-pyrimidine-2-thione (2a)

Yield: 80%. m.p. 155-160°C. IR (KBr) u (cm⁻¹): 3382 (NH), 1584 (C=N), 1245 (C=S). MS (El) m/z: 364(M+,20%). 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 2.23(s,3H,CH₃), 2.34(s,3H,CH₃)
ne), 2.50 (s,6H, 2CH₃), 5.00 (s,1H, pyrimidine), 7.19-8.65 (m,9H,Ar-H),10.04, 10.64 (s,2H,2NH, D₂O exchangeable). ¹³C 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 (CH₃),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 (NCH₃), 18.3 (CH₃). Anal. Calcd. for C₂₂H₂₆NS₂ (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-(Dimethylamino-phenyl)-6-methyl-5-(1-oxotolylimino-ethyl)-3,4-dihydro-1H-pyrimidine-2-thione (2b)

Yield: 86%. m.p. 168-170°C. IR (KBr) v (cm⁻¹): 3385 (NH), 1600 (C≡N), 1435(22): 49 (NH). MS (EI) m/z: 385(M⁺,30%). ¹'H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.01(s,3H,CH₃),2.25(s,3H, CH₃, pyrimidine),2.3(s,3H,CH₃),2.5(s,6H,Ar-H),5.00 (s,1H, pyrimidine),6.68-7.55 (m, 8H,Ar-H),9.60, 9.70 (s,2H, 2NH, D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆):178.3(C=S), 164.2(C=N), 99.5(C, C-5),145 (C, C-6),128 (CH₃),149.7,142.3,132,131,130,128.5,126.9,126.8,121.9,112.5 Ar/ olefinic carbon),54(C, C-4),40(NCH₃), 12.18 (S,H₃). Anal. Calcd. for C₂₂H₂₆NS₂ (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-(Dimethylamino-phenyl)-6-methyl-5-(1-(4-acetoxylimino)ethyl)-3,4-dihydro-1H-pyrimidine-2-thione (2c)

Yield: 83%. m.p. 140-142°C. IR(KBr) v (cm⁻¹): 3380 (NH),1590 (C≡N), 1690(C=O), 1215(C=S). MS (EI)m/z:406(M⁺,35%). ¹'H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.20(s,3H,CH₃),2.26(s,3H, CH₃, pyrimidine), 2.40 (s,3H, CH₃,COCH₃), 2.5 (s, 6H, CH₃), 5.16(s,1H, pyrimidine),6.00-7.76(m, 8H,Ar-H), 9.60, 9.78 (s,2H,2NH, D₂O exchangeable). ¹³C NMR (300 MHz,DMSO-d₆):196 (C=O),179 (C=S),165(C=N), 98(C, C-5), 146 (C, C-6),128.5 (CH₃), 112,121,129.1,128.2, 129, 131, 142.2, 135, 153 (Ar/ olefinic carbon),52(C, C-4), 40.3(NCH₃), 22.8 (CH₃), 18.5 (CH₃). Anal. Calcd.C₂₃H₂₆N₂O₅ (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) v (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,CH₃), 2.25 (s, 3H, CH₃, pyrimidine), 2.5 (s,6H,CH₃),5.2 (s, 1H, pyrimidine), 6.90-7.45 (m,8H,Ar-H), 9.92-10.2 (s,2H,2NH, D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 178(C=S), 162(C=N), 97(C, C-5),144(C, C-6),128 (CH₃),112.2,120,122, 127,128,131, 131.5, 135,142.5,150(Ar/olefinic carbon),53(C, C-4), 43.3(NCH₃),18.1(CH₃). Anal.Calcd.for C₂₃H₂₆Cl₂N₂S (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-(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)v (cm⁻¹): 3345 (NH),2986(CH-aliphatic),3167(CH-aromatic), 684 (C=O). MS (EI) m/z: 394(M⁺, 64%). ¹'H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.24 (s,3H, CH₃, pyrimidine), 2.50 (s,6H,2CH₃),3.40 (t,1H,CH₂), 3.73 (t,2H,CH₂), 5.15 (s,1H, pyrimidine),5.90 (s, 1H,NH,D₂O exchangeable), 7.16-8.89(m,9H,Ar-H). 10.84,11.64(s,2H,NH,pyrimidine D₂O exchangeable). ¹³C 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 (NCH₃), 40.0, 45.0 (2CH₃), 21.0 (CH₃). Anal. Calcd for C₂₂H₂₆N₂O₅ (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-(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)v (cm⁻¹): 3344 (NH), 2959 (CH-aliphatic), 3124(CH-aromatic), 1685(C=O). MS (EI) m/z: 408(M⁺, 65%). ¹'H NMR(DMSO-d₆, 300MHz) δ (ppm): 2.49 (s,3H, CH₃, pyrimidine), 2.63 (s,6H,2CH₃),2.83 (s,3H, CH₃,3.03 (t,2H, CH₂), 3.34 (t,2H,CH₂), 5.23(s, 1H,pyrimidine), 5.90(1H, NH, D₂Oexchangeable) ,6.44-8.09(m,8H,Ar-H),9.04,10.09(s,2H,NH, pyrimidine D₂O exchangeable). ¹³C NMR (300
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) ν (cm⁻¹): 3442 (NH); 2934 (CH-aliphatic). 3179 (CH-aromatic). 1687 (C=O). MS (E1) m/z: 377 (M⁺, 67%), 187 (C₆H₄). 139 (C₅H₃), 129 (C₄H₃), 119 (C₃H₂), 109 (C₂H₃), 99 (C₃H₅O), 59 (C₃H₇). Anal. Calcd for C₂₅H₂₃N₃O₢: C, 70.00; H, 6.20; N, 13.09. Found: C, 70.14; H, 6.29; N, 13.07.

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

Yield: 86%. m.p. 265-267°C. IR (KBr) ν (cm⁻¹): 3360 (NH), 2977 (CH-aliphatic), 3179 (CH-aromatic), 1689 (C=O). MS (E1) m/z: 428 (*M⁺, 67%), 430 (M+2, 20.1%). ¹HNMR (DMSO-d₆, 300MHz) δ (ppm): 2.26 (s, 3H, CH₃), 2.5 (s, 6H, 2CH₃), 3.1 (t, 2H, CH₂), 3.2 (t, 2H, CH₂), 5.3 (s, 1H, pyrimidine), 5.8 (1H, NH, D₂O exchangeable), 7.3-7.8 (m, 8H, Ar-H), 9.6, 9.8 (s, 2H, NH, pyrimidine D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 103.2 (C-5), 112.0, 112.4, 125.0, 128.4, 129.0, 131.4, 142.4, 147.0 (Ar olefinic carbon), 43.0 (NCH₃), 40.1, 45.0 (2CH₃), 22.8, 21.0 (2CH₃). Anal. Calcd for C₂₂H₂₀N₃O₢: C, 72.96; H, 5.67; N, 13.95. Found: C, 73.01; H, 5.70; N, 13.93.

General procedure for preparation of compound 4A

A mixture of methyl ketone 1 (0.01 mole) and the appropriate aromatic aldehyde (0.01 mole) in
NMR (DMSO-d₆, 300MHz) δ(ppm): 2.28 (s,3H, CH₃, pyrimidine), 2.5(s,6H, 2CH₃), 4.2 (s,9H, 3OCH₃), 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 D₂O exchangeable). ¹³C 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, 129.9, 131.4, 132,142.2, 148(Ar/olefinic carbon), 56.3,56.6 (OCH₃),53.2 (C, C-4), 43.2(NCH₃), 18(CH₃). Anal. Calcd for C₂₅H₂₃N₃O₄S (467.58): C, 64.22; H, 6.25; N,8.29; S, 7.26%. 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-tetrahydro pyrimidin-5-yl]-prop-2-en-1-one (4d)

Yield: 79%. m.p. 230-232°C. IR(KBr) ν(cm⁻¹): 3388 (NH),2973 (CH-aliphatic), 3167 (CH-aromatic). 1679 (C=O). MS(EI) m/z: 420 (M⁺,77%). ¹H NMR (DMSO-d₆,300MHz) δ (ppm): 2.25 (s,3H, CH₃, pyrimidine), 2.35,2.6 (s,12H, 4CH₂), 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 D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 187.5(C=O), 178.5 (C=S), 159.5(C, C-6) ,129 ,134(CH₂), 116.5(C, C-5),112.5, 113,124,127, 129,131,15,142,1, 143(Ar/ olefinic carbon),53(C, C-4), 43(NCH₃), 18(CH₃). Anal. Calcd for C₂₅H₂₅N₃O₅S (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-tetrahydro pyrimidin-5-yl]-2-oxo-4-(3,4,5-trimethoxy-phenyl)-1,2-dihydro-pyridine-3-carbonitrile (5c)

Yield: 84%. m.p. 260-262°C. IR (KBr) ν(cm⁻¹): 3355 (NH), 2987(CH-aliphatic), 2220(CN), 3177 (CH-aromatic). 1686 (C=O). MS (E1) m/z: 441 (M⁻,77%). ¹H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.26 (s,3H, CH₃, pyrimidine), 2.5 (s,6H, 2CH₃), 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, D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 178.0(C=S) , 162.0 (C=O) , 138.0 (C, C-6) , 112.0(C,C-5),110,126.2, 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(NCH₃), 18.0(CH₃). Anal. Calcd for C₂₅H₂₅N₃O₅S (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%.

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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-tetrahydro pyrimidin-5-yl]-2-oxo-1,2-dihydro pyridine-3-carbonitrile (5d)

Yield: 74%. m.p. 270-272°C. IR(KBr) ν(cm⁻¹): 3376 (NH), 2978(CH-aliphatic), 2221 (CN), 3188(CH-aromatic), 1680(C=O). MS (EI) m/z: 484 (M⁺, 80%). ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.23 (s, 3H, CH₃, pyrimidine), 2.4, 2.53(s,12H, 4CH₂), 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, D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 178.8 (C=S), 162.0 (C=O), 138.0 (C, C), 112.0 (C, C=O), 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 pyridine), 117.0 (CN), 56.3, 56.6 (OCH₃), 54.4(C, C=O), 40.6 (NCH₃), 18.0 (CH₃). Anal. Calcd for C₆H₁₀N₂O₅S (424.41): C, 56.17; H, 7.61; N, 14.25; S, 6.36%. Found C, 56.17; H, 7.61; N, 14.25; S, 6.36%.

General procedure for preparation of compound 6

A mixture of 1 (0.01 mole),malononitrile or ethyl cyanoacetate (0.001 mole) in presence of catalytic amount of triethylamine (4 drops) 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-Dimethylaminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidin-5-yl]ethyl idene]-malononitrile (6a)

Yield: 67%. m.p. 180-2°C. IR(KBr) ν(cm⁻¹): 3357 (NH), 2988(CH-aromatic), 3176(CH-aromatic), 2226(CN). MS(EI) m/z: 377 (M⁺, 67%). ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.24 (s, 3H, CH₃, pyrimidine), 2.4 (s,3H,CH₃), 2.5 (s,6H, 2CH₂), 5.1 (s, 1H pyrimidine), 7.2-7.6 (m,4H, Ar-H), 9.6 9.8 (s,2H, NH, pyrimidine D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 178.0 (C=S), 174.0(CH), 112.2(C, C=O), 138.0(C, C=O), 112.0,128.0,131.0, 142.2(Ar/olefinic carbon), 117.0(CN), 74.0(CH), 55.0(C=O), 40.6(NCH₃), 12.0, 18.0 (CH₃). Anal. Calcd for C₆H₁₀N₂O₅S (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-enolate (6b)

Yield: 70%. m.p. 150-152°C. IR (KBr) ν(cm⁻¹): 3390(NH), 2926(CH-aromatic), 3063(CH-aromatic). 2225(CN), 1704(C=O). MS (EI) m/z: 384 (M⁺, 66%). ¹H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.03(3H, CH₃), 2.25(s,3H,CH₃,pyrimidine), 2.46 (3H,CH₃), 2.50 (s,6H,2CH₂), 3.86(q,2H, CH₂),5.2 (s,1H pyrimid ine), 7.13-7.52(md,4H,aromatic), 9.6,9.9(s, 2H, NH pyrimidine,D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 180.0(C=O), 169.3(CH), 165.6(C=O), 111.9(C, C=O), 111.9, 127.3, 128.1, 145.3(Ar/olefinic carbon), 118.6(CN), 94.3(CH), 56.0(CH₂), 55.0(C, C=O), 40.4(NCH₃), 12.6, 14.9, 18.0 (CH₃). Anal. Calcd for C₂₃H₂₈N₄O₂S (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, diethylenamine (0.1 mol) was added drop wise for 30 min and the reaction mixture was heated (70°C) under stirring in absolute ethanol for 4-6 h. The mixture was left for 24 h at 0°C. The obtained 2-aminothiophene crystalized in the form of yellow powder. The precipitate was filtered, washed with water and recrystallized from ethanol to give 7a, b.

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

Yield: 67%. m.p. 130-132°C. IR(KBr) ν(cm⁻¹): 3257 (NH), 2980(CH-aromatic), 3176(CH-aromatic), 2222 (CN), MS(EI)m/z: 369 (M⁺, 67%). ¹H NMR (DMSO-d₆, 300MHz) δ (ppm): 2.20 (s, 3H, CH₃, pyrimidine), 2.5 (s,6H, 2CH₂), 4.08(q,2H, NH₂, D₂O exchangeable), 5.1 (s,1H pyrimidine), 7.4-7.54 (m, 5H,Ar-H), 9.95 (s, 2H, NH pyrimidine D₂O exchangeable). ¹³C NMR (300 MHz, DMSO-d₆): 178.0(C=O), 110.0(C, C=O), 140.0(C, C=O), 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=O), 40.0(NCH₃), 18.0 (CH₃). Anal. Calcd for C₁₆H₁₅N₃S₅ (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-dimethylaminophenoy)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-yl]-thiophene-3-ethanamine (7b)

Yield: 60%. m.p. 150-152°C. IR(KBr)(cm^-1): 3318 (NH), 2936(CH-aliphatic), 3198 (CH- aromatic), 1736 (C=O). MS (EI) m/z: 416(M^+, 66%). ^1H NMR (DMSO-d_6, 300 MHz) 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, 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). ^13C 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. Calc'd for C_{20}H_{24}N_2O_5S_2: 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:

\[ \text{Swell} = \text{mean difference in rat paw volume between right and left paw} \pm \text{SE} \]

\[ \% \text{ inhibition} = (1 - \frac{rt}{rc}) \times 100 \]

[r = 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
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 ary 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. H₂SO₄ [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 ¹H-NMR showed singlet at 8.8-11.6 ppm corresponding to the D₂O 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 β-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 ¹H-NMR spectra showed triplets of CH₂-CH₂ at the range 3.03-3.34 ppm.

α,β-Unsaturated compounds 4a-d (Chalcones) were prepared via Claisen-Schmidt 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. ¹H-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 ¹H-NMR spectra showed the disappearance of the singlet corresponding to COCH₃ (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 ¹H-NMR spectrum of 6b showed triplet signal at 2 ppm and quartet signals at 3.8 ppm of COOCH₃. Finally, 2-amino thiophene derivatives 7a and 7b were synthesized through Gewald thiophene [51] synthesis via a Knoevenagel 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 IC₅₀ 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-crystalized ligand. Docking results were analyzed using the analyze results tool in Sybyl-X.
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
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 significant 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)

R₃=CN,COOEt

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)
3.4 Molecular Modeling

Molecular docking studies were carried out using SYBYL-X v.2.1 software. Crystal structures from the Protein Databank website (www.rcsb.org) were downloaded. Protocol described in the “Experimental” section.
[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) | 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           |

*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 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 μM) 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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