Ind. J. Pharm. Edu. Res., 2024; 58(2s):s515-s520.
https://www.ijper.org

Original Article

Synthesis and Biological Evaluation of 5,6-Dimethylthieno[2,3-*d*]Pyrimidin-4(3*H*)-One Derivatives as Selective Cyclooxygenase 2 Inhibitors

Lim Tse Mun[1,#], Jamie Kow Kean Hing[1,#], Pran Kishore Deb[2,*], Katharigatta Narayanaswamy Venugopala[3,4], Raghu Prasad Mailavaram[5], Ammena Yahia Binsaleh[6], Sireen Abdul Rahim Shilbayeh[6]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, International Medical University, Kuala Lumpur, MALAYSIA.
[2]Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology (BIT), Mesra, Ranchi, Jharkhand, INDIA.
[3]Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, SAUDI ARABIA.
[4]Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, Durban, SOUTH AFRICA.
[5]Department of Pharmaceutical Chemistry, Shri Vile Parle Kelavani Mandal's Institute of Pharmacy, Samtanagar, Dhule, Maharashtra, INDIA.
[6]Department of Pharmacy Practice, College of Pharmacy, Princess Nourah bint Abdulrahman University, Riyadh, SAUDI ARABIA.
[#]*Both the authors, LTM and JKKH, have equally contributed.*

ABSTRACT

Background: NSAIDs are well-established for treating pain, fever, and inflammation mainly by inhibiting inducible Cyclooxygenase 2 (COX-2) isoenzyme. However, most of the marketed NSAIDs non-selectively inhibit physiological COX-1 and exhibit adverse side effects like GI ulcers, renal toxicity, and platelet disorder. Moreover, cardiac side effects also led to the market withdrawal of some of the potential selective COX-2 inhibitors. Thus, several investigations are underway by researchers from academia and industry in search of safer and more effective COX-2 selective inhibitors devoid of existing side effects. **Materials and Methods:** In this work, four 2-substituted-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (5,6,7,8 and 9) have been synthesized, purified, and characterized based on their physical and spectral data. These compounds were evaluated (*in vitro*) for their affinity and selectivity for human COX-2 enzyme against COX-1 isoenzyme using indomethacin as a positive control. **Results and Discussion:** Compound 5 with *para* fluorophenyl substituent was found to be the most potent, exhibiting better inhibition and selectivity towards COX-2 isoenzyme (IC_{50}=42.19 M, SI=4.81) against COX-1 isoenzyme (IC_{50}=202.96 M, SI=4.81) as compared to the other derivatives (6-8). **Conclusion:** The activity of compound 5 is promising compared to the non-selective drug indomethacin (IC_{50} COX-1=0.68 M, COX-2=18.3 M, SI=0.04). Therefore, compound 8 can be considered a lead molecule for further optimization to develop novel selective COX-2 inhibitors at nanomolar potency.

Keywords: Thieno[2,3-*d*]pyrimidines, Cyclooxygenase 2 (COX-2) inhibitors, Inflammation.

Correspondence:
Dr. Pran Kishore Deb
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology (BIT), Mesra, Ranchi-835215, Jharkhand, INDIA.
Email: prankishore1@gmail.com; prankishoredeb@bitmesra.ac.in
ORCID: 0000-0002-8650-2874

Received: 12-11-2023;
Revised: 14-01-2024;
Accepted: 19-03-2024.

INTRODUCTION

Arachidonic Acid (AA) or eicosatetraenoic acid is an essential 20-carbon polyunsaturated ω6 fatty acid that maintains structural integrity and functional aspects of cell membranes.[1] Apart from this, AA also acts as a substrate for the enzymatic transformations to produce biologically active mediators, including Prostaglandins (PGs), epoxyeicosatetraenoic acids, Leukotrienes (LTs), and endocannabinoids.[2] These lipid mediators are mainly formed via three canonical metabolic pathways, viz. Cyclooxygenase (COX), Lipoxygenase, and cytochrome P450 pathways, mediated by the enzymes found in the nuclear membrane, cytoplasm, mitochondria, and endoplasmic reticulum.[3] The COX pathway utilizes the COX enzyme, also called prostaglandin Endoperoxide synthases or prostaglandin H synthases (EC 1.14.99.1), for the catalysis of the rate-limiting step in the production of prostanoids. All vertebrates, including birds, bony fishes, cartilaginous fishes, and mammals, possess two main isoforms of COX, i.e., COX-1 and COX-2: the former being constitutive and the latter an inducible form.[4] These two isoforms share approximately 60-65% amino acid similarity and are homodimers of 70 kDa subunits that require dimerization for their catalytic activity.[5,6] Apart from these two isoforms, COX-3, a splice variant of COX-1, has also been reported to be highly expressed in micro vessels of the heart and brain.[8] The COX enzymes can exhibit two distinct catalytic reactions: the conversion of AA to PGG2 by bis-dioxygenase (COX) activity and the reduction of PGG2 to PGH2 via

DOI: 10.5530/ijper.58.2s.53

peroxidase action. PGH2 is the precursor for generating other PG isomers by the downstream synthases via oxidation or reduction and isomerization reactions.[8,9] The PG isomers, including prostacyclin (PGI2), prostaglandins like PGD2, PGE2, and PGF2α, and thromboxanes, regulate numerous physiological and pathological processes, such as inflammation, algesia (pain), pyresis (fever), thrombosis, vasoconstriction and vasodilation, ovulation, parturition, etc. by acting on the respective G-protein coupled receptors.[10] Consequently, over the last decades, emphasis has been given to developing cyclooxygenase inhibitors to modulate different pathophysiological conditions.[11] It has been observed that the COX enzymes can remarkably accommodate distinct inhibitory molecules via diversity of binding to the enzyme. Therefore, successful exploitation of the cyclooxygenase modulating compounds may serve as a key approach to obtain dynamic therapeutic agents. In this regard, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have been therapeutically used in the treatment of various inflammatory disorders like rheumatic arthritis, acute fever, and asthma by inhibiting inducible Cyclooxygenase 2 (COX-2) isoenzyme.[12,13] However, most of the marketed NSAIDs non-selectively inhibit physiological COX-1 and exhibit adverse side effects like GI ulcers, renal toxicity, and platelet disorder.[14] Moreover, some of the potential selective COX-2 inhibitors like rofecoxib and valdecoxib were withdrawn from the market in 2004 and 2005 by Merk and Pfizer, respectively due to cardiac side effects. Interestingly, COX-2 selective inhibitors are also playing a vital role in cancer chemotherapy and currently, celecoxib is the only drug available in the market as an adjuvant therapy for the treatment of Familial Adenomatous Polyposis (FAP) which may lead to colon cancer if left untreated.[11,15] Thus, several investigations are underway by researchers from academia and industry in search of safer and more effective COX-2 selective inhibitors for the treatment of inflammatory disorders and cancer devoid of existing side effects.[14,16,17] In continuation to the ongoing research efforts, in the present work we are reporting the synthesis and biological evaluation of four 2-substituted-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (5-9) as potential COX-2 selective inhibitors.

MATERIALS AND METHODS

Experimental

2-Butanone, ethylcyanoacetate (≥98%), dimethylformamide (≥99.5%), ethanol (≥99%), n-hexane, cyanamide and sulphur (≥99.5%) were bought from Acros Chemical (USA). Diethylamine, sodium sulphate anhydrous and analytical grade NaOH pellets were bought from Fisher Scientific (UK). 1,4-dioxane, concentrated HCl, ethylacetate, hexane, chloroform, 4-fluorobenzoyl chloride (98%), 4-methoxyphenyl acetonitrile (≥97%), 4-fluorophenyl acetonitrile (≥99%), and 2-(trifluromethyl)benzoyl chloride were bought from Aldrich Chemicals. TLC Silica gel 60 F254 was obtained from Merck, USA. COX inhibitor screening assay kit (Item no. 560131) was procured from Cayman Chemical, USA.

Identification, progression, purity and R_f values of the synthesized compounds were determined by using silica gel-coated TLC plate. UV-lamp (Perkin Elmer Lambda 25) at 254 nm was used to visualize the TLC spots present on the plate. Melting points of synthesized compounds were carried out in open glass capillary tubes by using Stuart Melting Point Apparatus SMP11. The infrared spectra of synthesized compounds were determined by Shimadzu 8400S spectrophotometer with wavenumbers ranging from 500-4000 cm^{-1}. The proton (^1H) NMR spectra were determined by using VARIAN 500 MHz instrument where the chemical shifts were recorded in (ppm) downfield from Tetramethylsilane (TMS). The splitting patterns were represented as follows: *s*, singlet; *d*, doublet; *m*, multiplet. Mass of the synthesized compounds was confirmed by using Shimadzu GCMS-QP2010 Plus spectrophotometer.

Synthesis of ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate (4)

2-Butanone (0.04 mol, 3.61 mL), ethanol (30 mL), ethylcyanoacetate (0.04 mol, 4.52 mL) and sulphur (0.04 mol, 1.28 g) was added into a conical flask and heated at 60°C. Diethylamine (0.04 mol, 4 mL) was then added dropwise, stirred for 5 hr and cooled to room temperature. The solution was kept into the fridge at 4°C for 24 hr. The crystals formed were filtered, dried and re-crystalized with ethanol to obtain compound (4).[18,19]

Yield: 60%. Orange yellow crystals, mp. 89-91, R_f ($CHCl_3$: Acetone, 9:1)=0.89, FTIR (KBR, cm^{-1}): 3398.57, 3294.42 (NH_2); 2980.02, 2926.01 (aliphatic C-H); 1643.35 (C=O); 1593.20 (C=C); 1573.91 (N-H bend); 1479.40 (CH_2 bend); 1375.25 (CH_3 bend); 1262.32 (C-N). ^1H NMR ($CDCl_3$) δ ppm: 4.288-4.246 (m, 2H, 3-CH_2); 2.159 (s, 3H, 5-CH_3); 2.135 (s, 3H, 4-CH_3); 1.353-1.325 (t, 3H, 3-CH_3). ESI-MS (*m/z*; %)=199.07 (M^+ + 1; 100).

Synthesis of 2-(4-Fluorobenzyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (5)

Compound 4 (0.02 mol; 2.00 g) and 4-fluorophenyl acetonitrile (0.03 mol; 1.80 mL) in 1,4-dioxane (0.02 mol; 8.00 mL) were taken in conical flask and a stream of dry hydrochloric acid gas was passed through the mixture for 24 hr. Then, neutralization was carried out with sufficient dilute sodium hydroxide solution (10% NaOH). The resultant precipitate was filtered, dried, and recrystallized from ethanol to yield the compound 5.

Yield: 88.68 %. White powder, mp. 291, R_f (Hexane: EtAc, 6:4)=0.53, FTIR (KBR, cm^{-1}): 3304 (2^0 Amides N-H), 3068 (Aromatic=C-H), 3005 (Alkanes C-H), 2920 (Alkanes C-H), 1645 (Amides C=O), 1600 (Aromatic C=C), 1504 (2^0 Amines N-H), 1471 (Aromatic C=C), 1207 (Amines C-N), 1381 (Alkanes CH_3), 1029 (Fluorides C-F), 858 (para-disubstituted rings). ^1H NMR ($CDCl_3$) δ ppm: 4.288-4.246 (*q*, 2H, 3-COOC$\underline{H}_2$),

2.159 (*s*, 3H, 5-CH_3), 2.135 (*s*, 3H, 4-CH_3), 1.353-1.325 (t, 3H, 3-$COOCH_2CH_3$). ESI-MS (*m/z*; %)=288.0 (M^+ + 1; 100).

Synthesis of 2-(4-Methoxybenzyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (6)

Compound 4 (0.02 mol; 2.00 g) and 4-methoxyphenyl acetonitrile (0.03 mol; 2.00 mL) in 1,4-dioxane (0.02 mol; 8.00 mL) were taken in conical flask and a stream of dry hydrochloric acid gas was passed through the mixture for 24 hr. Then, neutralization was carried out with sufficient dilute sodium hydroxide solution (10% NaOH). The resultant precipitate was filtered, dried and recrystallized from ethanol to yield the compound 6.

Yield: 85.37 %. White powder, mp. >300, R_f (Hexane: EtAc, 6:4)=0.55, FTIR (KBR, cm^{-1}): 3304 (2^0 Amides N-H), 3005 (Alkanes C-H), 3095 (Aromatic =C-H), 1581 (2^0 Amines N-H Bending), 1651 (Amides C=O), 1608 (Aromatic C=C), 1508 (Alkanes CH_2), 1475 (Aromatic C=C), 1381 (Alkanes CH_3), 1298 (Amines C-N), 1242 (Ethers C-O), 817 (para-disubstituted rings). ^{1}H NMR ($CDCl_3$) δ ppm: 4.288-4.246 (*q*, 2H, 3-$COOCH_2$), 2.159 (*s*, 3H, 5-CH_3), 2.135 (*s*, 3H, 4-CH_3), 1.353-1.325 (t, 3H, 3-$COOCH_2CH_3$). 12.363 (*s*, 1H, 3-NH), 7.240-6.844 (*m*, 4H, 2-$CH_2C_6H_4$), 3.804 (*s*, 2H, 2-CH_2), 3.689-3.554 (*s*, 3H, 2-$CH_2C_6H_4OCH_3$), 2.480 (*s*, 3H, 6-CH_3), 2.329-2.292 (*s*, 3H, 5-CH_3). ESI-MS (*m/z*; %)=300.0 (M^+ + 1; 100).

Synthesis of 2-amino-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (7)

The compound (4) (0.01 mol, 2 g), cyanamide (2 g) and concentrated HCl solution (2 mL) was added into a round bottom flask and refluxed for 12 hr at 45°C. Then, the reaction mixture was neutralized with diluted NaOH (10%) solution. The precipitate formed was filtered, dried and re-crystalized with ethanol to obtain pure product (7).[20]

Yield: 90 %. White powder, mp. >300, R_f ($CHCl_3$: Acetone, 9:1)=0.05, FTIR (KBR, cm^{-1}): 3408.22 (NH_2); 3309.85 (N-H stretch); 2902.87 (aliphatic C-H); 1654.92 (C=O); 1614.42 (N-H bend); 1591.27 (C=C); 1263.52 (C-N). ^{1}H NMR ($CDCl_3$) δ ppm: 10.736 (s, 1H, 3-NH); 6.380 (s, 2H, 2-NH_2); 2.462 (s, 3H, 6-CH_3); 2.188 (s, 3H, 5-CH_3). ESI-MS (*m/z*; %)=195.05 (M^+ + 1; 100).

Synthesis of 2-(4-fluorobenzamido)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (8)

The compound **(7)** (0.002 mol, 0.390 g) was completely dissolved into DMF (5 mL) in a round bottom flask with constant stirring. The mixture was cooled to room temperature and placed into an ice bath (0-5°C) followed by the addition of NaH (0.008 mol, 0.192 g). After an hour, 4-fluorobenzoyl chloride (0.003 mol, 0.35 mL) was added dropwise with a syringe and stirred for 24 hr. The reaction mixture was neutralized with diluted NaOH (10%) solution and extracted with ice. The precipitate formed was filtered and dried. The aqueous layer was extracted further with ethyl acetate and evaporated. The crude reaction compound was purified using column chromatography (Hexane: Ethylacetate: 9.2:0.8) to obtain a pure product (8).

Yield: 30 %. White powder, mp. >300, R_f ($CHCl_3$: Acetone, 9:1)=0.85, FTIR (KBR, cm^{-1}): 3317.56, 3230.77 (N-H stretch), 3120.82 (aromatic C-H); 2912.51, 2848.86 (aliphatic C-H); 1674.21 (C=O); 1651.07 (aromatic C=C); 1589.34 (N-H bend); 1381.03 (C-H); 1309.67 (C-N); 1249.38 (C-F); 849.78 (*para*-substituted ring). ^{1}H NMR ($CDCl_3$) δ ppm: 12.101 (s, 1H, 2NH); 11.887 (s, 1H, 3NH); 8.118-7.353 (m, 4H, $2C_6H_4$); 2.518 (s, 3H, $6CH_3$); 2.354 (s, 3H, $5CH_3$). ESI-MS (*m/z*; %)=317.06 (M^+ + 1; 100).

Synthesis of 2-(trifluromethylbenzamido)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (9)

The compound (7) (0.002 mol, 0.390 g) was completely dissolved into DMF (5 mL) in a round bottom flask with constant stirring. The mixture was cooled to room temperature and placed into an ice bath (0-5°C), followed by the addition of NaH (0.008 mol, 0.192 g). After an hour, 2-(trifluoromethyl)benzoyl chloride (0.003 mol, 0.44 mL) was added dropwise with a syringe and stirred for 24 hr. The reaction mixture was neutralized with diluted NaOH (10%) solution and extracted with ice. The precipitate formed was filtered and dried. The aqueous layer was extracted further with ethyl acetate and evaporated. The crude reaction compound was purified using column chromatography (Hexane: Ethylacetate:9.2:0.8) to obtain a pure product (9).

Yield: 25 %. White powder, mp. >300, R_f ($CHCl_3$: Acetone, 9:1)=0.85, FTIR (KBR, cm^{-1}): 3224.98 (N-H stretch); 3186.40 (C-H sp^2 stretch); 2954.95, 2916.37, 2848.86 (aliphatic C-H); 1654.92 (C=O); 1597.06 (aromatic C=C); 1556.55 (N-H bend); 1369.46 (C-N); 1311.59 (C-F); 1261.45 (in plane C-H bend); 756.10 (*ortho*-disubstituted ring). ^{1}H NMR ($CDCl_3$) δ ppm: 12.142 (s, 1H, 2-NH); 11.877 (s, 1H, 3-NH); 7.864-7.688 (m, 4H, 2-C_6H_4); 2.365 (s, 3H, 6-CH_3); 2.321 (s, 3H, 5-CH_3). ESI-MS (*m/z*; %)=367.06 (M^+ + 1; 100).

In vitro cyclooxygenase inhibition activity

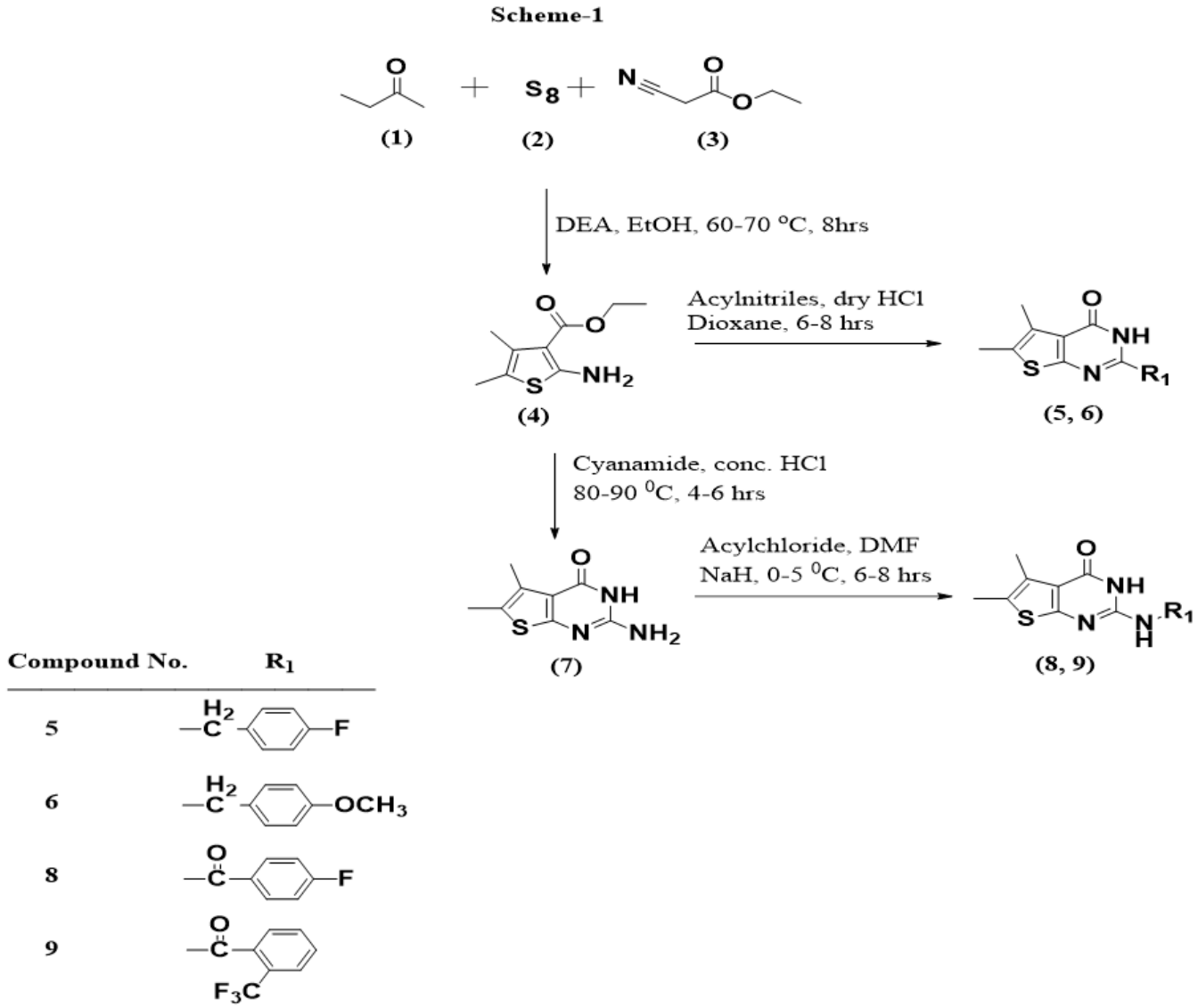
The COX (ovine) Inhibitor Screening Assay was performed using the COX Inhibitor Screening Assay Kit (Item No. 560131, Cayman Chemical, USA), mainly to determine the selective inhibition of tested compounds (5, 6, 8, and 9) towards the ovine COX-1 and human recombinant COX-2 isoenzymes according to manufacturer instructions. All reagents and buffers were prepared in ultrapure water. All the stock solutions of the compounds and the positive control (Indomethacin) were dissolved in DMSO. The addition of Arachidonic Acid (AA) helped to convert the COX component into PGG_2, followed by the reduction of PGG_2 into PGH_2. Then the addition of $SnCl_2$ converted PGH_2 into PGF_2. The assay directly measures the PGF_2 via Enzyme Immunoassay (EIA) using a broadly specific antiserum that is bound to all PG

tracers. Depending on the tested compounds' inhibitory effect, the concentration of PGF_2 in each well varied and was inversely proportional to the amount of PG tracers that were able to bind to the anti-serum. PG tracers-antiserum complex was left to bind to the mouse monoclonal anti-rabbit antibody that had been coated to the well during the 18 hr of incubation. Ellman's reagent was added to give distinct yellow coloration to the products from this enzymatic reaction, which absorbed strongly at 412 nm. The intensity of the color was proportional to the amount of PG tracers-antiserum complex bound to the well, which was inversely proportional to the concentration of PGF_2 present. The inhibitory activity of tested compounds was determined based on IC_{50} values by screening the compounds at six different concentrations of 0.01, 0.1, 1.0, 10, 100, and 500 µM, whereas the selectivity was based on their Selectivity Indices (SI), which are defined as COX-1 IC_{50}/COX-2 IC_{50}.

RESULTS AND DISCUSSION

Chemistry

The intermediate compound ethyl-2-amino-4,5-dimeth ylthiophene-3-carboxylate (4) was synthesized by following well-established Gewald's reaction,[21] via condensation of 2-butanone with elemental sulfur and ethylcyanoacetate in the presence of excess diethylamine as the base as shown in Scheme 1. It was interesting to note that the reaction product decreased drastically at temperatures below 60°C and above 70°C. Further reaction of compound 4 with 4-fluorophenyl acetonitrile and 4-methoxyphenyl acetonitrile, respectively, in the presence of dry HCl gas led to the formation of compounds 5 and 6 in quantitative yields. The cyclization reaction of compound 4 with cyanamide in the presence of concentration hydrochloric acid resulted in the formation of compound 7.[22] Compound 7 on treatment with 4-fluoro benzoyl chloride and 2-(trifluoromethyl)benzoyl



Scheme 1: Synthesis of 5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives.

Table 1: *In vitro* catalytic inhibition data for the compounds 5-9 against COX-1 and COX-2 isoenzymes using indomethacin as positive control.

Compound code	Compound Structure	COX-1 [a]IC_{50} (μM)	COX-2 [a]IC_{50} (μM)	SI[b]
5	2	202.96	42.19	4.81
6	2	586.07	142.71	4.11
8	2	111.79	103.47	1.08
9	2	21.84	189.05	0.12
Indomethacin	--	0.68	18.3	0.04

[a]IC_{50} is the concentration needed to cause 50% inhibition of the OX-2 enzymatic activity measured at six different concentrations (n=6). [b]SI: Selectivity index.

chloride, respectively, in the presence of NaH, maintaining the temperature between 0-5°C resulted in the formation of target compounds 8 and 9, respectively. All the synthesized compounds were characterized based on their physical and spectral data (IR, ^{1}H, ^{13}C NMR, and Mass) analysis, as mentioned in the experimental section.

Cyclooxygenase inhibition activity (*in vitro*)

The target compounds (5, 6, 8, and 9), were screened against the COX-1 and COX-2 isoenzymes at six different concentrations of 0.01, 0.1, 1.0, 10, 100, and 500 μM, to determine their IC_{50} values using indomethacin as a reference standard as shown in Table 1. In the assay system, indomethacin was used as a non-selective COX inhibitor standard. Compound 5 with *para* fluorophenyl substituent was found to be the most potent, exhibiting better inhibition and selectivity towards COX-2 Isoenzymes (IC_{50}=42.19 M, SI=4.81) as compared to the other derivatives (6,8 and 9). Interestingly, compound 5 showed better selectivity for COX-2 than indomethacin (SI=0.04). Introduction of electron donating methoxy substituent at the *para* position of the phenyl group decreased the affinity towards COX-2 for compound 6 (IC_{50}=142.71 M, SI=4.11). The difference in electronegativity affecting the dipole moment for fluorine atom in 5 and carbon atom in 6 might contribute to the molecule's ability to be engaged in intermolecular interactions at the hydrophobic pocket of COX-2 active site. Presence of amide group at the second position of the thienopyrimidine scaffold exhibited moderate inhibitory activity and decreased selectivity towards COX-2 isoenzyme for compound 8 (IC_{50}=103.47 M, SI=1.08). It is also observed that substituent at *para* position of phenyl ring attached to second position of the thienopyrimidine scaffold is required for inhibitory activity and selectivity towards COX-2 isoenzyme, because introduction of trifuoromethyl substituent at the *ortho* position of phenyl ring of the compound 9 drastically decreased the affinity and selectivity for COX-2 (IC_{50}=189.05 M, SI=0.12).

CONCLUSION

In this work, four 2-substituted-5,6-dimethylthieno [2,3-*d*]pyrimidin-4(3*H*)-one derivatives (5,6,8 and 9)have been synthesized following the Scheme 1 and evaluated (*in vitro*) for their affinity and selectivity for human COX-2 enzyme against COX-1 isoenzyme using indomethacin as a positive control. Compound 5 with *para* fluorophenyl substituent was found to be the most potent, exhibiting better inhibition and selectivity towards COX-2 isoenzyme (IC_{50}=42.19 M, SI=4.81) against COX-1 isoenzyme (IC_{50}=202.96 M, SI=4.81) as compared to the other derivatives (6,8 and 9). This compound can be considered as a lead molecule for further optimization to develop novel selective COX-2 inhibitors at nanomolar potency.

ACKNOWLEDGEMENT

The authors extend their appreciation to the School of Pharmacy, IMU, for providing the necessary laboratory facilities and funding (Project ID: IMU 293/2013) to carry out this research project. The current work was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R419), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AA: Arachidonic Acid; **COX-1:** Cyclooxygenase 1; **COX-2:** Cyclooxygenase 2; **FAP:** Familial Adenomatous Polyposis; **LTs:** Leukotrienes; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **PGs:** Prostaglandins.

REFERENCES

1. Saini RK, Keum Y-S. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance-A review. Life sciences. 2018;203:255-67.
2. adimon L, Vilahur G, Rocca B, Patrono C. The key contribution of platelet and vascular arachidonic acid metabolism to the pathophysiology of atherothrombosis. Cardiovascular Research. 2021;117(9):2001-15.
3. Zhang Y, Liu Y, Sun J, Zhang W, Guo Z, Ma Q. Arachidonic acid metabolism in health and disease. MedComm. 2023;4(5):e363.
4. Chandrasekharan N, Simmons DL. The cyclooxygenases. Genome biology. 2004;5:1-7.
5. Xiao G, Chen W, Kulmacz RJ. Comparison of structural stabilities of prostaglandin H synthase-1 and-2. Journal of Biological Chemistry. 1998;273(12):6801-11.
6. Blobaum AL, Marnett LJ. Structural and functional basis of cyclooxygenase inhibition. Journal of medicinal chemistry. 2007;50(7):1425-41.
7. Chandrasekharan N, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, *et al.* COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proceedings of the National Academy of Sciences. 2002;99(21):13926-31.
8. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. Journal of lipid research. 2009;50:S29-34.

9. Smith WL, Urade Y, Jakobsson P-J. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chemical reviews. 2011;111(10):5821-65.
10. Tsuboi K, Sugimoto Y, Ichikawa A. Prostanoid receptor subtypes. Prostaglandins & other lipid mediators. 2002;68:535-56.
11. El-Malah AA, Gineinah MM, Deb PK, Khayyat AN, Bansal M, Venugopala KN, *et al.* Selective COX-2 inhibitors: road from success to controversy and the quest for repurposing. Pharmaceuticals. 2022;15(7):827.
12. Deb PK, Mailabaram RP, Al-Jaidi B. Molecular basis of binding interactions of NSAIDs and computer-aided drug design approaches in the pursuit of the development of cyclooxygenase-2 (COX-2) selective inhibitors. Nonsteroidal Anti-Inflammatory Drugs. 2017;2:64.
13. Balakumar C, Lamba P, Kishore DP, Narayana BL, Rao KV, Rajwinder K, *et al.* Synthesis, anti-inflammatory evaluation and docking studies of some new fluorinated fused quinazolines. European journal of medicinal chemistry. 2010;45(11):4904-13.
14. Deb PK, Kaur R, Chandrasekaran B, Bala M, Gill D, Kaki VR, *et al.* Synthesis, anti-inflammatory evaluation, and docking studies of some new thiazole derivatives. Medicinal Chemistry Research. 2014;23:2780-92.
15. Smith M-L, Hawcroft G, Hull M. The effect of non-steroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. European journal of cancer. 2000;36(5):664-74.
16. Venugopala KN, Chandrashekharappa S, Tratrat C, Deb PK, Nagdeve RD, Nayak SK, *et al.* Crystallography, molecular modeling, and COX-2 inhibition studies on indolizine derivatives. Molecules. 2021;26(12):3550.
17. Dhingra MS, Deb PK, Chadha R, Singh T, Karan M. Synthesis, evaluation, and molecular docking studies of cycloalkyl/aryl-3, 4, 5-trimethylgallates as potent non-ulcerogenic and gastroprotective anti-inflammatory agents. Medicinal Chemistry Research. 2014;23:87-106.
18. Gewald A. Schinke KE; Bottcher, H. Chem Ber. 1966;99:94-100.
19. Prasad MR, Kishore DP. Multistep, microwave assisted, solvent free synthesis and antibacterial activity of 6-substituted-2, 3, 4-trihydropyrimido [1, 2-c] 9, 10, 11, 12-tetrahydrobenzo [b] thieno [3, 2-e] pyrimidines. Chemical and pharmaceutical bulletin. 2007;55(5):776-9.
20. Deb PK, Mailavaram R, Chandrasekaran B, Kaki VR, Kaur R, Kachler S, *et al.* Synthesis, adenosine receptor binding and molecular modelling studies of novel thieno [2, 3-d] pyrimidine derivatives. Chemical biology & drug design. 2018;91(4):962-9.
21. Rao KV, Balakumar C, Narayana BL, Kishore DP, Rajwinder K, Rao AR. Transesterification of trimethyl orthoacetate: an efficient protocol for the synthesis of 4-alkoxy-2-aminothiophene-3-carbonitriles. Tetrahedron Letters. 2013;54(10):1274-8.
22. Venugopala KN, Deb PK, Mailavaram R, Al-shar'i NA, Ganguly S, Gorain B, *et al*. "Thieno[2,3-d]pyrimidines as COX-2 inhibitors." U.S. Patent 11,932,656, issued March 19, 2024.

Cite this article: Mun LT, Hing JKK, Deb PK, Venugopala KN, Mailavaram RP, Binsaleh AY, *et al*. Synthesis and Biological Evaluation of 5,6-Dimethylthieno[2,3-*d*] Pyrimidin-4(3*H*)-One Derivatives as Selective Cyclooxygenase 2 Inhibitors. Indian J of Pharmaceutical Education and Research. 2024;58(2s):s515-s520.