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Synthesis of thio-Pyrimidine, Benzoxazole, Benzothiazole and Triazole Analogues from Baylis-Hillman Bromides as Potent Cyclooxygenase-2 Inhibitors

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ABSTRACT

Non-steroidal anti-inflammatory drugs play a major role in pain management. This article discusses the synthesis of thio-Pyrimidine, Benzoxazole, Benzothiazole and Triazole Analogues from Baylis-Hillman Bromides and their in-vitro testing for the activity of cyclooxygenase (COX) inhibition. The results are very encouraging and we can proceed for further investigations regarding their safety, in-vivo issues etc.

Key Words: Non-steroidal anti-inflammatory drugs, cyclooxygenase, thio-Pyrimidine, Benzoxazole, Benzothiazole and Triazole Analogues, Baylis-Hillman Bromides

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) were used in the treatment of pain, inflammation and different types of Joint pains. The main target of these drugs is to inhibit cyclooxygenase (COX) enzyme which catalyses oxygenation of arachidonic acid (AA) to prostaglandins (PGs) which are responsible for inflammation. Cyclooxygenase exists in mammalian tissues in two isoforms, namely, COX-1 and COX-2. COX-1 is housekeeping enzyme and expressed constitutively in many tissues, and plays a physiological role in the production of prostaglandins which are involved in cytoprotection of gastrointestinal tract (lowering the acid secretions), renal blood flow regulation and platelet aggregation. COX-2 is present only in kidney; brain; endothelial cells and reproductive tissues, and its expressions are induced by various kinds of inflammatory mediators such as pro-inflammatory cytokines, fibroblast growth factors, luteinizing hormones and haemostasis disorders, which results in increasing the production of pain-mediating prostaglandins in neoplastic and inflamed tissues. The excess production of PGs influences a variety of physiological processes like inflammation, Alzheimer's disease, heart failure, hypertension and carcinogenesis. Therefore, the main focus is on the inhibition of COX-2 isoform selectively.

NSAIDS such as Indomethacin, Ibuprofen, Diclofenac and Aspirin are nonselective inhibitors of both COX-1 and COX-2. But, these drugs show adverse effects like damaging the gastrointestinal tissues and leads to ulcers. Rofecoxib, Valdecoxib and Celecoxib drugs are well known selective COX-2 inhibitors in market, but are associated with risk of cardiovascular symptoms. So there is a need to develop new molecules which are completely devoid of side effects and are capable of being selective COX-2 inhibitors without affecting COX-1 isoform. Very few molecules have been reported to be free of side effects. DUP69, selective COX-2 inhibitors virtually devoid of gastrointestinal toxicity and renal damage. SC58152 is also a selective COX-2 inhibitor and has no effect on PG production in stomach and do not cause gastric toxicity.

Heterocyclic compounds comprising of pyrimidine, benzoxazole, benzothiazole and triazole rings have received special attention due to their broad range of biological activities. Pyrimidine core with two nitrogen atoms help to control the lipophilicity of molecule and act as template to bind COX-2 pocket. Aurelio *et al* have designed and synthesized 2-(4-methyl sulponyl phenyl) pyrimidine derivatives and evaluated as highly potent and specific COX-2 inhibitors. New quinazolinone-pyrimide compounds have been as potent COX-2 inhibitor. Many pyrimidine derivatives as anti-inflammatory agents have been patented. A number of highly substituted benzoxazole, benzothiazole and triazole compounds have been reported as selective COX-2 inhibitors.

In order to gain more insight into selective COX-2 inhibitors and to minimize the risk of unwanted side effects, we have conducted exploratory research to find new molecules. In the present study, we have used Baylis-Hillman (BH) chemistry to synthesize allyl phenyl substituted thio derivatives of pyrimidine, benzoxazole, benzothiazole and triazole. These four series of newly synthesized compounds were then evaluated for COX-1 and COX-2 inhibition property. Majority of compounds found to be selective COX-2 inhibitors.

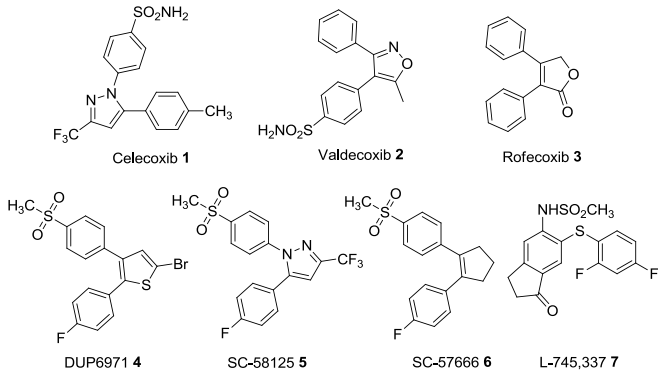


Figure.1.Examples of COX-2 selective anti-inflammatory compounds

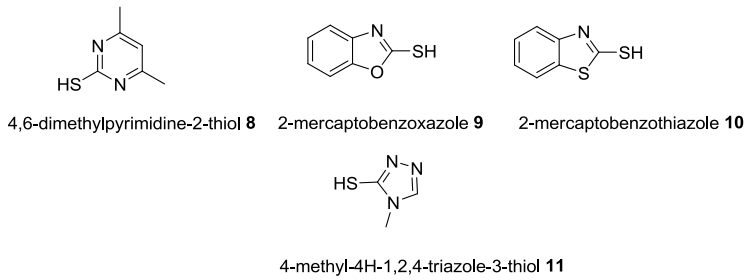
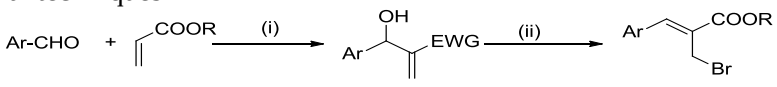


Figure.2.Various thiol compounds

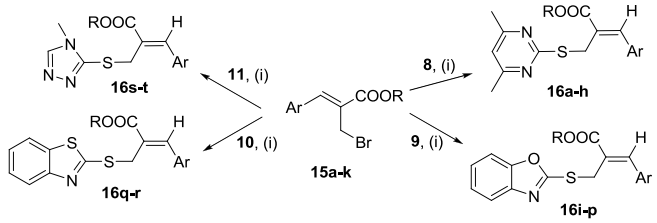
RESULTS AND DISCUSSION

Chemistry: Baylis-Hillman adducts 14a-k were prepared by treating aromatic aldehydes with acrylates and DABCO as catalyst (20-50 mol %) under solvent-free condition at room temperature in very good yields (Scheme 1). Thus prepared BH adducts were then treated with conc. H_2SO_4 and HBr (48% in acetic acid) at 0 °C in dichloromethane solution to get the corresponding BH bromides 15a-k in good yields having (*Z*)-configuration. These bromides served as key intermediates in the synthesis of target compounds. Treatment of these allyl bromides with various thiol compounds like 4, 6-dimethylpyrimidine-2-thiol 8, 2-mercaptobenzoxazole 9, 2-mercaptobenzothiazole 10, 4-methyl-4*H*-1, 2, 4-triazole-3-thiol 11 in DMF using K_2CO_3 as a base at room temperature furnished the final products16a-t in good yields (Scheme 2). All the products synthesized were characterized by spectral techniques



15a R = Me, Ar = *p*-fluorophenyl; **15b** R = Me, Ar = *p*-Chlorophenyl; **15c** R = Me, Ar = *p*-methoxyphenyl; **15d** R = Me, Ar = 2-napthyl; **15e** R = Et, Ar = *m*- Chlorophenyl; **15f** R = Et, Ar = *p*-bromophenyl; **15g** R = Et, Ar = *p*-methylphenyl; **15h** R = Et, Ar = *p*-isopropylphenyl; **15i** R = Me, Ar = *p*-trifluoromethylphenyl; **15j** R = Et, Ar = *m*-fluorophenyl; **15k** R = Me, Ar = *p*-ethylphenyl.

Scheme.1.Reagents and conditions: (i) DABCO (20-50 mol %), neat, rt, 60-90%; (ii) Conc. H_2SO_4, HBr, 0 °C-rt, overnight, 80-90%



Scheme.2.Reagents and conditions: (i) DMF, K_2CO_3, rt, 78-93%.

Table.1.Synthesis of thio derivatives

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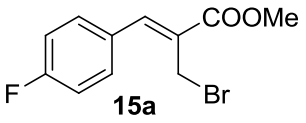
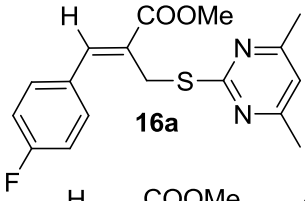
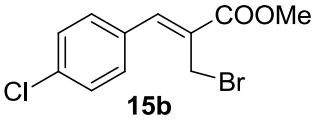
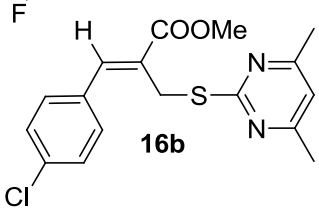
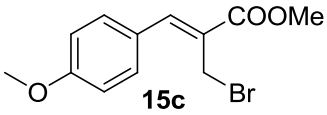
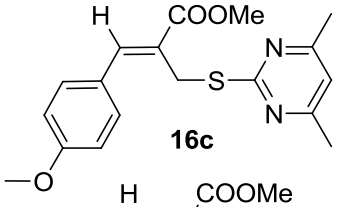
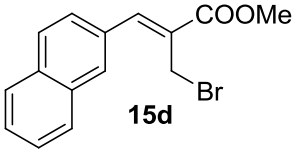
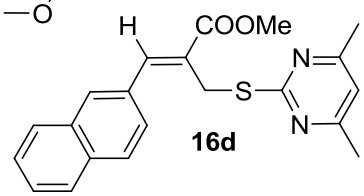
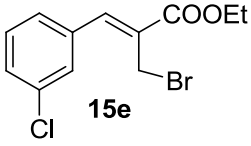
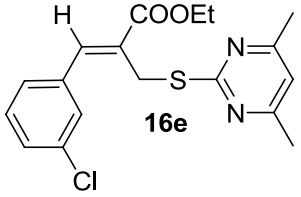
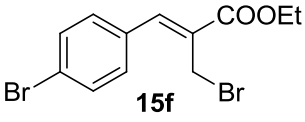
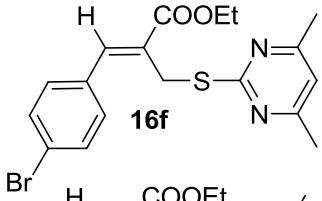
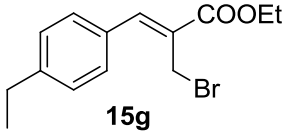
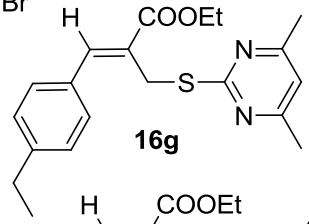
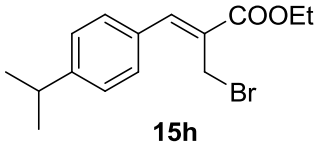
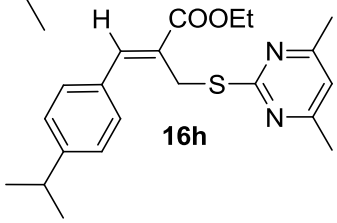
1	 **15a**	**8**	 **16a**	88
2	 **15b**	**8**	 **16b**	91
3	 **15c**	**8**	 **16c**	85
4	 **15d**	**8**	 **16d**	93
5	 **15e**	**8**	 **16e**	80
6	 **15f**	**8**	 **16f**	81
7	 **15g**	**8**	 **16g**	85
8	 **15h**	**8**	 **16h**	89

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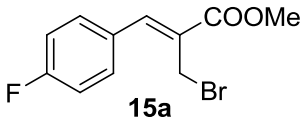
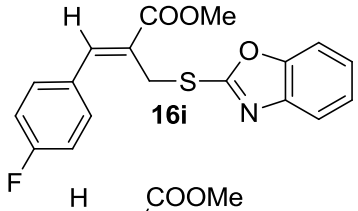
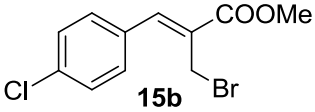
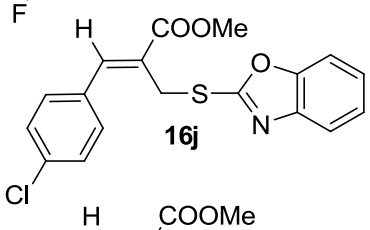
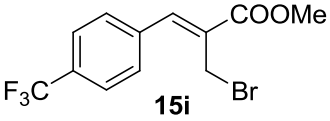
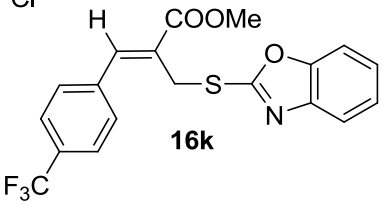
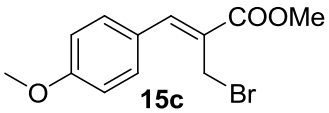
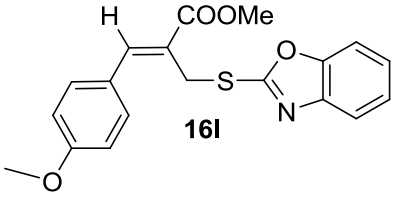
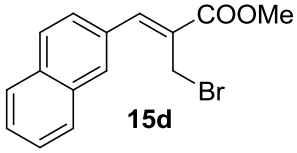
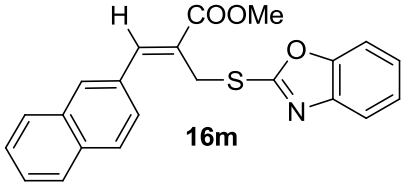
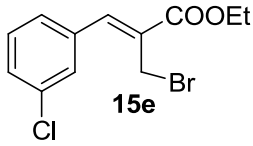
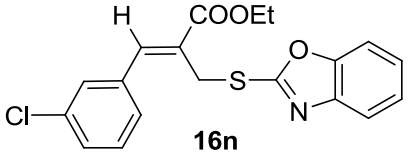
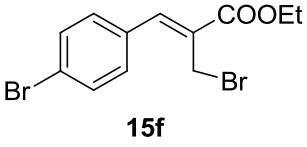
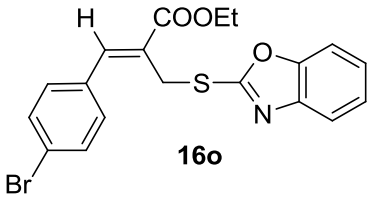
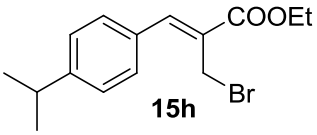
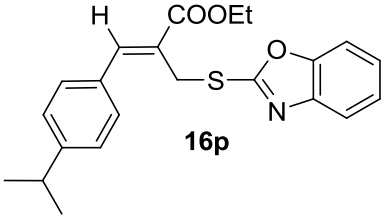
Sl no.	BH allyl bromides	Thiol compound	Products	Yield (%)
9	 **15a**	**9**	 **16i**	82
10	 **15b**	**9**	 **16j**	91
11	 **15i**	**9**	 **16k**	80
12	 **15c**	**9**	 **16l**	78
13	 **15d**	**9**	 **16m**	85
14	 **15e**	**9**	 **16n**	78
15	 **15f**	**9**	 **16o**	89
16	 **15h**	**9**	 **16p**	84

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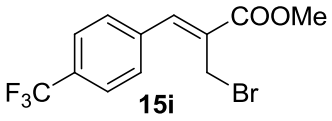
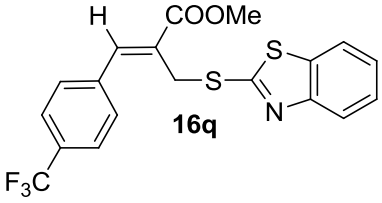
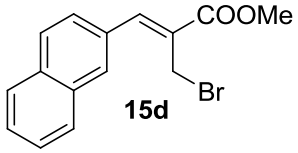
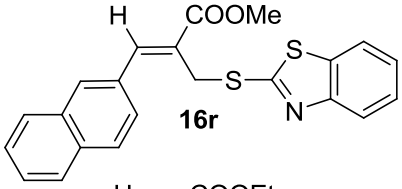
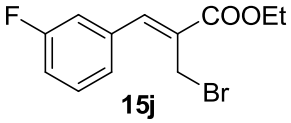
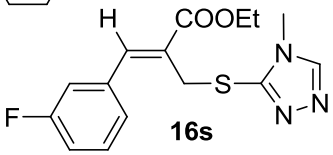
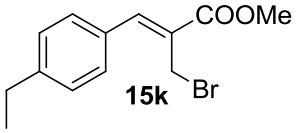
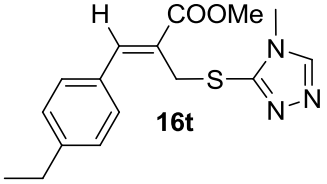
Sl no.	BH allyl bromides	Thiol compound	Products	Yield (%)
17	 **15i**	**10**	 **16q**	85
18	 **15d**	**10**	 **16r**	87
19	 **15j**	**11**	 **16s**	83
20	 **15k**	**11**	 **16t**	93

Pharmacology: All the synthesized compounds were then evaluated for their inhibition ability and selectivity on both COX isoforms using the calorimetric COX inhibitor screening assay. The *in vitro* activity results are reported as a percentage of inhibition of the purified enzymes at different concentrations 100 µM, 10 µM and their IC_{50} values were calculated from the concentration-inhibition curves.

Among the all newly synthesized compounds except 16b, 16o and 16t showed very good degree of inhibition of COX2 (more than 70% as against 82% inhibition showed by the standard). More than 90% of pyrimidine and benzoxazole derivatives displayed very good biological profile. Both the benzothiazole derivatives and one of the triazole compounds are also active (Fig. 3). When IC_{50} values were determined, seven compounds (16e, 16f, 16k, 16l, 16m, 16r and 16s) exhibited potent activity at micromolar concentration (Table 2). Benzoxazole derivative 16k (IC_{50} of 2.95 µM) and benzothiazole derivative 16r (IC_{50} of 2.93 µM) are as active as the standard Celecoxib (IC_{50} of 2.66 µM). Four compounds i.e. 16e, 16f, 16k and 16s have halogens (Cl or Br or CF_3) on the Ph ring of BH part. Compound 16l has a *p*-methoxy substituent on Ph ring. So there may be some interaction like electronic interaction of these groups with the enzyme. Compounds 16g, 16h, 16p and 16t have alky substituents present on Ph ring and are comparably less active. Presence of napthyl ring lead to excellent biological profile for compounds 16m and 16r. As the napthyl ring is highly rigid it might have a better interaction with the enzyme.

As we want to have compounds with selective COX-2 inhibition property, all the compounds were then evaluated for COX-1 inhibitory activity at 100 µM and 10 µM concentrations. Here it is desirable that the compounds should not inhibit COX-1 enzyme to a greater extent and much better if do not inhibit COX-1. In the present study, the most active compounds against COX-2 showed no inhibition or very less inhibition against COX-1(Fig. 4). Compounds 16e, 16f, 16k, 16r and 16s showed very little inhibition towards COX-1 and compounds 16l and 16m are virtually inert to COX-1. Remaining compounds also showed less inhibition for COX-1. So, most of the compounds have selective inhibition affinity against COX-2.

Figure.3.COX-2 inhibitory activity of thiopyrimidine, thiobenzoxazole, and thiobenzthiazole derivatives (100 µM in DMSO). Standard Drug: Celecoxib (100 µM in DMSO).

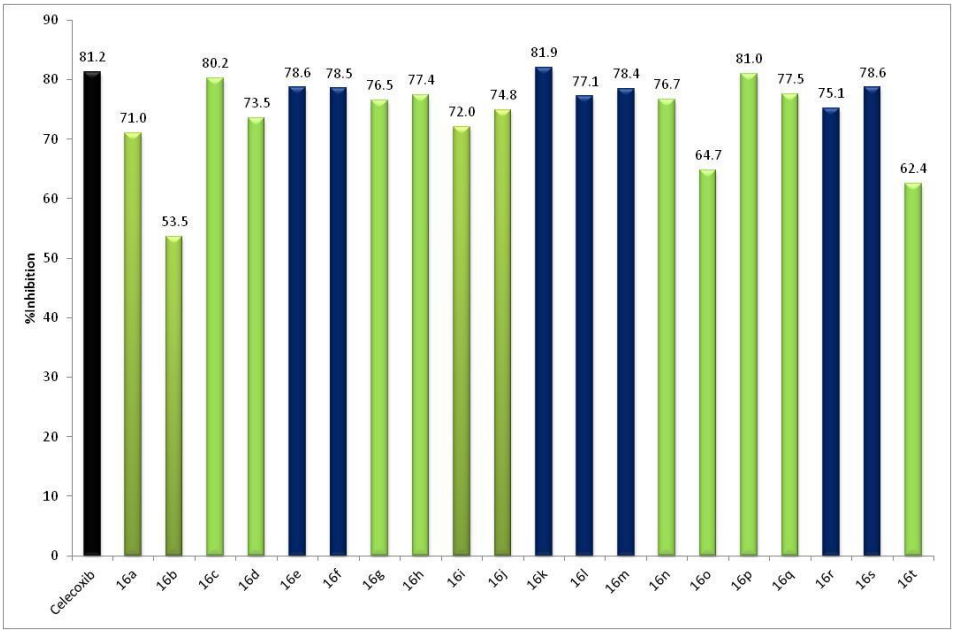


Table.2.Cox-2 inhibitory activity (IC50) of thiobenzoxazole, thiobenzthiazole derivatives

Compound	Ic50
16e	5.88µM
16f	3.17µM
16k	2.95µM
16l	4.76µM
16m	4.34µM
16r	2.93µM
16s	3.88µM
Celecoxib	2.66µM

General procedure for preparation of Baylis-Hillman bromides: 15a-k: To a stirred solution of BH adduct (41.1 mmol) in dichloromethane, HBr (48%, 13.5 mL) was added drop wise followed by concentrated H_2SO_4 (11.9 mL) at 0 °C. After stirring for overnight at room temperature, the reaction mixture was carefully diluted with dichloromethane (40 mL) and H_2O (20 mL). The aqueous phase was then extracted with dichloromethane (30 mL × 2). The combined organic phase was washed with H_2O (15 mL × 2), dried over Na_2SO_4, concentrated under vacuum and the residual oil was purified by column chromatography (eluent hexane/ethyl acetate (10:1).

General procedure for synthesis of Baylis-Hillman substituted thiobenzoxazole, thiobenzothiazole, thiopyrimidine, thiotriazole derivatives: To a stirred solution of thiobenzoxazole/thiobenzothiazole/thiopyrimidine/thiotriazole (1 mmol) and potassium carbonate (2 mmol) in DMF, a solution of BH bromide (1 mmol) in dimethylformamide (3 mL) was added and stirred at room temperature for 6 h (monitored by TLC). The reaction was quenched by addition of ice cold water. The organic layer was extracted with ethyl acetate and dried over anhydrous sodium sulphate. The solvent was evaporated to obtain the crude product which was purified by column chromatography over silica gel (60-120 mesh) using hexane and ethyl acetate as an eluent.

Experimental Section: Characterization Data:

(*Z*)-methyl 2-((4,6-dimethylpyrimidin-2-ylthio)methyl)-3-(4-fluorophenyl)acrylate 16a: Yield: 88%; Cream solid; mp 90-95 °C; IR (KBr): 3467, 1715, 1633, 1428, 1279, 994 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.78 (s, 1H), 7.57-7.52 (m, 2H), 7.10 (t, 2H, *J* = 9.07 Hz), 6.69 (s, 1H), 4.37 (s, 2H), 3.84 (s, 3H), 2.34 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.45, 167.56, 166.92, 164.53, 161.21, 140.74, 140.29, 131.82, 131.71, 130.89, 127.33, 115.74, 115.47, 52.24, 28.39, 23.68; Mass (ESI-MS): *m/z* 333 $[M+H]^+$; HRMS (ESI): *m/z* 333 $[M+H]^+$ calculated for $C_{17}H_{18}FN_2O_2S$: 333.1069, found 333.1068.

(*Z*)-methyl 3-(4-chlorophenyl)-2-((4,6-dimethylpyrimidin-2-ylthio)methyl)acrylate 16b: Yield: 91%; cream solid; mp 100-105 °C; IR (KBr): 3436, 1698, 1585, 1428, 1265, 1167, 1075, 870, 790 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.49 (d, 2H, *J* = 8.39 Hz), 7.36 (d, 2H, *J* = 8.55 Hz), 6.68 (s, 1H), 4.36 (s, 2H), 3.84 (s, 3H), 2.33 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.37, 167.45, 166.95, 140.48, 134.92, 133.27, 131.02, 128.75, 128.29, 115.76, 52.30, 28.35, 23.68; Mass (ESI-MS): *m/z* 349 $[M+H]^+$; HRMS (ESI): *m/z* 349 $[M+H]^+$ calculated for $C_{17}H_{18}ClN_2O_2S$: 349.0776, found 349.0772.

(*Z*)-methyl 2-((4,6-dimethylpyrimidin-2-ylthio)methyl)-3-(4-methoxyphenyl)acrylate 16c: Yield: 85%; White solid; mp 90-95 °C; IR (KBr): 3447, 2946, 1706, 1604, 1579, 1513, 1433, 1338, 1269, 1179, 1151 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.79 (s, 1H), 7.56 (d, 2H, *J* = 8.69 Hz), 6.92 (d, 2H, *J* = 8.88 Hz), 6.68 (s, 1H), 4.42 (s, 2H), 3.83 (s, 6H), 2.35 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.83, 167.98, 166.89, 160.29, 141.99, 131.79, 127.33, 124.82, 115.65, 114.03, 55.26, 52.15, 28.84, 23.73; Mass (ESI-MS): *m/z* 345 $[M+H]^+$; HRMS (ESI): *m/z* 345 $[M+H]^+$ calculated for $C_{18}H_{21}N_2O_3S$: 345.1270, found 345.1267.

CONCLUSION

It is concluded that the synthesized thio-Pyrimidine, Benzoxazole, Benzothiazole and Triazole Analogues from Baylis-Hillman Bromides have shown considerable in-vitro inhibition of cyclooxygenase (COX). The activity of the COX inhibition of the newly synthesized compounds were studied in comparison with NSAIDS and the results were encouraging.

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