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Design, synthesis and biological evaluation of benzoxazole derivatives as cyclooxygenase inhibitors

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Abstract

We have synthesized a series of methyl-2-(2-(arylideneamino)thiazole-4-ylamino)benzoxazole-5-carboxylate derivatives and investigated their ability to inhibit human cyclooxygenase-2 enzyme (COX-2). The active compounds were screened for cyclooxygenase-1 (COX-1) inhibition. Compound VI 6 is 379-fold and VI 12 is more than 465 fold selective towards COX-2 compared to COX-1. Thus, this class of compounds may serve as excellent candidates for selective COX-2 inhibition.

Key words: Benzaxozoles, synthesis, cyclooxygenase, evaluation, COX-2, COX-1

INTRODUCTION

Cyclooxygenase (COX; prostaglandin endoperoxide synthase) metabolizes arachidonic acid to prostaglandin (PG) H_2, which serves as the precursor for the biosynthesis of various PGs, thromboxanes, and prostacyclin [1]. COX activity originates from two distinct and independently regulated isozymes, COX-1 and COX-2 [2]. COX-1 is a constitutive enzyme, whereas COX-2 is inducible and short-lived. COX-2 is the product of an immediate-early gene, and its expression is stimulated by a host of growth factors, cytokines, and mitogens [3]. COX-1 appears responsible for the biosynthesis of PGs in the gastric mucosa and in the kidney, whereas COX-2 appears responsible for biosynthesis in inflammatory cells and the central nervous system [4]. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the two isoforms to different extents, and this feature accounts for their shared therapeutic properties and side effects [5]. The differential tissue distribution of the COX isozymes has provided a rationale for the development of COX-2-selective inhibitors as nonulcerogenic, anti-inflammatory, and analgesic agents [6]. Most selective COX-2 inhibitors, including the recently approved drugs celecoxib [7] and rofecoxib [8] belong to the diarylheterocycle class of compounds [9–11]. Diarylheterocycles

have been investigated extensively as COX-2 inhibitors since the description of the 2,3-diarylthiophene, DuP 697, as a nonulcerogenic anti-inflammatory agent [12]. In addition 2-Oxo-3H-benzoxazole derivatives exhibit a broad range of biological properties [13-16] including analgesic and anti-inflammatory activity [17-22]. Among them, especially 3-substituted-2-oxo-3H-benzoxazoles are known to exhibit analgesic and anti-inflammatory properties [23]. It has also been reported that mannich bases of 6-acyl-2-oxo-3H-benzoxazoles resulted in compounds with potent analgesic activity [20]. Additional studies with some 3-aminoalkyl-2-oxo-3H-benzoxazole derivatives also demonstrated potent analgesic and anti-inflammatory activity, and showed that these compounds exerted their *in vivo* activity by inhibiting the synthesis of prostaglandin E72. (2-oxo-3H-benzoxazol-3-yl)propanamides also showed potent analgesic and anti inflammatory activity [24-27]. (6-acyl-2-oxo-3H-benzoxazol-3-yl)alkanoic acids possessed potent analgesic and anti-inflammatory activity with reduced gastric toxicity [28]. In general, most of the research on this class of compounds included substitutions on positions 3 and 6 of the 2-oxo-3H-benzoxazole nucleus. As a result, 2-oxo-3H-benzoxazoles bearing N-alkyl, N-acyl, N-diaminoalkyl and 6-acyl substituents were reported to have higher anti-inflammatory activity [29 – 30]. Berna *et al* described the synthesis of two novel 4-phenyl-and 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-3(2H)-pyridazinone derivatives and showed potent anti-inflammatory activities without causing gastric lesions in the tested animals [31]. In 2003 we synthesized series of Schiff's bases (N'-benzylidene-2-alkylbenzoxazole-5-carbohydrazide) moiety which possess significant anti inflammatory activity screened by carragenen induced rat paw edema method [32].

Hence these observations prompted us to synthesize a series of methyl-2-(2-(arylideneamino)thiazole-4-ylamino) benzoxazole-5-carboxylate derivatives (VI 1-VI15) and to evaluate their COX – 2 activity. The required starting material, methyl-3-amino-4-hydroxybenzoate (II) was synthesized in good yield (85%) according to reported procedure [33].The starting material (II) on cyclization with cyanogen bromide on rapid stirring at room temperature gave the product, methyl-2-aminobenzoxazole-5-carboxylate (III). The compound (III) on reaction with chloroacetyl chloride in dry benzene yields the compound, methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (IV). The compound (IV) on reaction with thiourea gave the compound methyl-2-(2-aminothiazol-4-ylamino) benzoxazole-5-carboxylate (V), finally which on reaction with various aromatic aldehydes conveniently converted into the targeted compounds methyl-2-(2-(arylideneamino) thiazole-4-ylamino) benzoxazole-5-carboxylate derivatives (VI).

The synthesized compounds were tested for their ability to inhibit human cyclooxgenase-2 (COX-2) enzyme and the more active compounds were tested for cyclooxgenase-1 (COX-1) inhibition in human whole blood assay.[34] Rofecoxib was used as active control in cyclooxygenase inhibition assay.

The compound which shown IC_{50}less than 10 mM concentration were tested for COX-1 inhibition. Interestingly two compounds, namely VI 6 and VI 12 have exhibited good activity with high selectivity towards COX-2 inhibition when compared to rest of the compounds. Compound VI 6 is 379 times more selective towards COX-2 when compared to COX-1 (COX-1 IC_{50}=384mM; COX-2 IC_{50}=1mM). Surprisingly compound VI 12 is 465 times more selective towards COX-2 inhibition than COX-1 (COX-1 IC_{50}=>500; COX-2 IC_{50}=1.06 mM),

interestingly 100 fold more selective than compound VI 6. However they are more selective and less potent than rofecoxib in human whole blood assay. Although compounds VI 4, VI 5, VI 7, VI 8 and VI 14 possess good selectivity, they have shown moderate activity towards COX-2. In conclusion, these classes of compounds may serve as excellent candidates for selective COX-2 inhibition.

MATERIALS AND METHODS

Chemistry

All melting points were taken in open capillaries on a veego VMP-1 apparatus and are uncorrected IR spectra were recorded as KBr pellets on a Perkin-Elmer FT IR 240-c spectrometer. The ^{1} H NMR spectra were recorded on Varian-Gemini 200 MHz spectrometer in DMSO-d_6 using TMS as an internal standard and mass spectras were recorded on Schimadzu QP 5050A spectrometer.

In the present work, 15 benzoxazole derivatives (VI1–15) were synthesized (Scheme I). The structures of the obtained compounds were elucidated by spectral data. IR spectroscopic data and ^{1} H-NMR of the compounds proved its structure.

1. Synthesis of Methyl-3-nitro-4-hydroxybenzoate (I)

To a solution of aluminium nitrate (40g) in acetic acid- acetic anhydride (1:1) mixture (160mL), was added an appropriate phenol (40g) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 1.5 h while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted to complete the nitration. The resulting brown solution was diluted with ice-cold water and acidified with concentrated Nitric acid to get a bulky, yellow precipitate. It was filtered washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline solid (44g, 85%), m.p 73°C.

2. Synthesis of Methyl-3-amino-4-hydroxybenzoate (II)

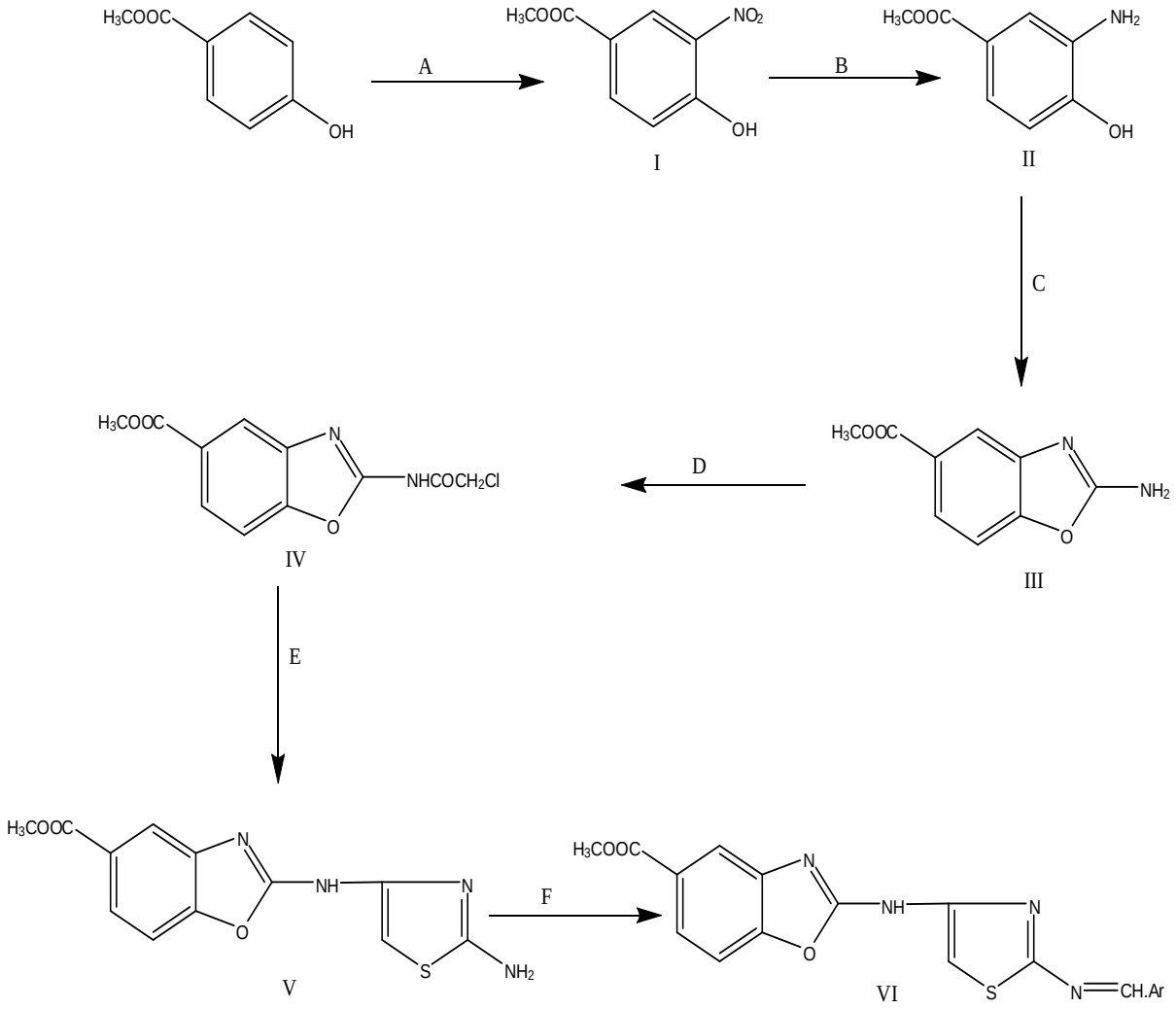
4-carbomethoxy-2-nitrophenol (I, 10 g) was dissolved in boiling alcohol (50%, 100mL) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colourless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with crushed ice. The resulting colourless, shiny product was filtered, and dried in the air. Its purification was effected by recrystallization from benzene to get colourless, shiny scales (5.1 g; 60%) m.p 143°C [33].

3. Synthesis of methyl-2-aminobenzoxazole-5-carboxylate (III)

4-carbomethoxy-2-aminophenol (II, 1.3mol) was dissolved in 1 lit. methyl alcohol and cooled the solution to 5°C by adding chopped ice. A cold suspension of cyanogenbromide (1.5mol) in 1 lit. of water was added over a period of 5min with rapid stirring. The reaction mixture was stirred for 0.75h at room temperature, solid sodium bicarbonate (1.3 mol) in small portions over a period of 1.5 h was added to bring the p^{H} 6.5 -7.0. Stirring was continued for another 1h. The solid was separated by filtration, washed with cold water and on recrystallization from ethyl alcohol has resulted white solid, yield 70% and m.p is 238°C.

4. Synthesis of methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (IV)

A mixture of methyl-2-aminobenzoxazole-5-carboxylate (III, 0.01mol) and chloroacetyl chloride (0.01mol) was taken in 20 mL of dry benzene and the reaction mixture was refluxed for 5 h on a water bath. The solvent was evaporated and the residue was washed first with benzene and then with Petroleum ether. The compound was recrystallized from suitable solvent(s). The compound was found to be containing yield 72% and m.p is 177°C.



A= Ac_2O + AcOH/ Al_2NO_3
B= $Na_2S_2O_4$/ 50% MeOH
C= CMBr/ MeOH
D= $ClCH_2COCl$/ dry benzene
E= Thiourea/ EtOH
F= Ar.CHO/ EtOH + AcOH

Scheme-1

5. Microwave synthesis of methyl 2-(2-aminothiazol-4-ylamino) benzoxazole-5-carboxylate (V)

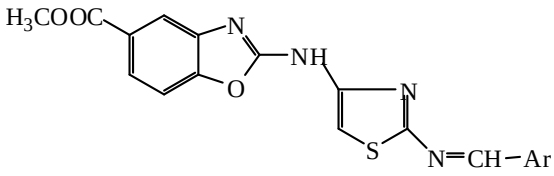
Methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (IV, 0.01mol) and thiourea (0.01mol) were dissolved in 10mL of absolute alcohol in conical flask. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 6min in LG-Microwave oven. The reaction was monitored by TLC. After the completion of the reaction the contents were cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture found to be containing yield 97% and m.p 199°C.

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3450 (NH_2), 3146 (NH), 1672 (C=O), 1626(C=C), 1528 (C=N), 1342 (C-O-C), 1142 (C=S). ^{1}NMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 8.3(s, 1H, Ar-H), 7.8 (d, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 7.0 (s, 1H, CH, thiazole ring), 6.3 (s, 2H, NH_2), 5.5 (s, 1H, NH), 3.9 (s, 3H, CH_3).

6. Microwave synthesis of methyl 2-(2-(arylideneamino) thiazole-4-ylamino) benzoxazole-5-carboxylates (VI1-15)

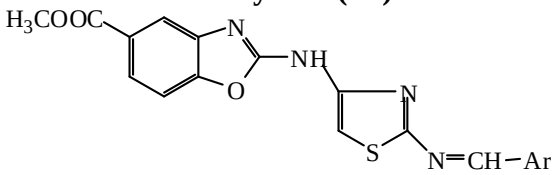
Methyl-2-(2-aminothiazol-4-ylamino)benzoxazole-5-carboxylate (V,0.01mol) and appropriate aromatic aldehydes viz. 4-dimethylaminophenyl, *4-t*-butylphenyl, Anisyl, phenyl, 4-hydroxyphenyl,4-nitrophenyl, Veratryl , Cinnamyl , 3,4,5-trimethylphenyl, 4-tolyl, 2-hydroxyphenyl, 4-bromophenyl, 4-chlorophenyl, 2-naphthyl, 1-naphthyl (0.015mol) were taken into a conical flask and were dissolved in 10mL of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 5min in LG-Microwave oven. The reaction was monitored by TLC. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture. The compounds were characterized by spectral data (Physical Data presented in Table-1 and Spectral Data presented in Table-2).

Table1: Physical data of methyl 2-(2-(arylideneamino) thiazol-4-ylamino) benzoxazole-5-carboxylates (VI)



Compound (VI)	Ar	Molecular Formula	Melting Point (°C)	Yield (%)
1	4-dimethylamino phenyl	$C_{21}H_{19}N_5O_3S$	208	90
2	*4-t*-butylphenyl	$C_{23}H_{22}N_4O_3S$	301	93
3	4-methoxyphenyl	$C_{20}H_{16}N_4O_4S$	280	98
4	Phenyl	$C_{19}H_{14}N_4O_3S$	201	92
5	4-hydroxy phenyl	$C_{19}H_{14}N_4O_4S$	228	95
6	4-nitrophenyl	$C_{19}H_{13}N_5O_5S$	299	97
7	3,4 dimethoxyphenyl	$C_{19}H_{18}N_4O_5S$	226	96
8	Cinnamyl	$C_{21}H_{15}N_4O_3S$	240	94
9	3,4,5-trimethylphenyl	$C_{22}H_{20}N_4O_3S$	238	95
10	4-methylphenyl	$C_{20}H_{16}N_4O_3S$	303	91
11	2-hydroxyphenyl	$C_{19}H_{14}N_4O_4S$	234	91
12	4-bromophenyl	$C_{19}H_{13}BrN_4O_3S$	305	90
13	4-chlorophenyl	$C_{19}H_{13}ClN_4O_3S$	222	99
14	2-naphthyl	$C_{23}H_{16}N_4O_3S$	341	90
15	1-naphthyl	$C_{23}H_{16}N_4O_3S$	322	91
Compound (VI)	Ar	Molecular Formula	Melting Point (°C)	Yield (%)
1	4-dimethylamino phenyl	$C_{21}H_{19}N_5O_3S$	208	90
2	*4-t*-butylphenyl	$C_{23}H_{22}N_4O_3S$	301	93
3	4-methoxyphenyl	$C_{20}H_{16}N_4O_4S$	280	98
4	Phenyl	$C_{19}H_{14}N_4O_3S$	201	92
5	4-hydroxy phenyl	$C_{19}H_{14}N_4O_4S$	228	95
6	4-nitrophenyl	$C_{19}H_{13}N_5O_5S$	299	97
7	3,4 dimethoxyphenyl	$C_{19}H_{18}N_4O_5S$	226	96
8	Cinnamyl	$C_{21}H_{15}N_4O_3S$	240	94
9	3,4,5-trimethylphenyl	$C_{22}H_{20}N_4O_3S$	238	95
10	4-methylphenyl	$C_{20}H_{16}N_4O_3S$	303	91
11	2-hydroxyphenyl	$C_{19}H_{14}N_4O_4S$	234	91
12	4-bromophenyl	$C_{19}H_{13}BrN_4O_3S$	305	90
13	4-chlorophenyl	$C_{19}H_{13}ClN_4O_3S$	222	99
14	2-naphthyl	$C_{23}H_{16}N_4O_3S$	341	90
15	1-naphthyl	$C_{23}H_{16}N_4O_3S$	322	91

Table2: Spectral data of methyl 2-(2-(arylideneamino) thiazol-4-ylamino) benzoxazole-5-carboxylates (VI)



Compounds	IR Spectral data (cm $^{-1}$)	NMR Spectral data(δ)
VI1	3096 (NH), 1683 (C=O), 1640(C=C), 1576 (C=N), 1442(C-O-C), 1383(C=S).	8.8 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.0 (s, 6H, $(CH_2)_3$).
VI2	3091 (NH), 1681 (C=O), 1642 (C=C), 1577 (C=N), 1443 (C-O-C), 1381 (C=S).	δ 8.7 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.1(d, 2H, ArH), 6.0 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.9 (s, 3H, CH_3), 1.3(s, 9H, $(CH_3)_3$).
VI3	3068 (NH), 1687 (C=O), 1645 (C=C), 1512 (C=N), 1432 (C-O-C), 1371 (C=S).	8.9 (s, 1H, CH), 8.7 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.8 (d, 2H, ArH), 7.1 (d, 2H, ArH), 6.2 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3).
VI4	3076 (NH), 1679 (C=O), 1646(C=C), 1580 (C=N), 1448(C-O-C), 1373 (C=S).	8.6 (s, 1H, ArH), 8.1(s, 1H, CH), 8.0 (d, 1H,ArH), 7.9 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.0(t, 3H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.8 (s, 3H, CH_3).
VI5	3091 (NH), 1699 (C=O), 1676 (C=C), 1580 (C=N), 1455 (C-O-C), 1371 (C=S).	9.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 4.6(s, 1H, NH), 3.7 (s, 3H, CH_3).
VI6	3091 (NH), 1692 (C=O), 1684 (C=C), 1582 (C=N), 1449 (C-O-C), 1373 (C=S).	8.6 (s, 1H, CH), 8.4 (s, 1H,ArH), 8.3 (d, 2H, ArH),8.1 (d, 1H, ArH), 7.9 (d, 1H, ArH), 7.8 (d, 2H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 5.5 (s, 1H, NH), 3.8 (s, 3H, OCH_3).
VI7	3071 (NH), 1687 (C=O), 1663 (C=C), 1575 (C=N), 1451 (C-O-C), 1373 (C=S).	8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (s, 1H, ArH), 7.4 (d, 1H, ArH), 6.9 (d, 1H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 9H, $3OCH_3$).
VI8	3088 (NH), 1681 (C=O), 1667 (C=C), 1573 (C=N), 1455 (C-O-C), 1371 (C=S).	8.8 (s, 1H, ArH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (t, 2H, ArH), 7.5 (s, 1H, CH), 7.4 (t, 2H, ArH), 7.3(t, 1H, ArH),7.0 (s, 1H, CH), 6.4 (s, 1H, ArH, thiazole ring), 5.6 (s, 1H, CH), 4.2 (s, 1H, NH), 3.7 (s, 3H, OCH_3).
VI9	3090 (NH), 1674 (C=O), 1660 (C=C), 1569 (C=N), 1449 (C-O-C), 1367 (C=S).	8.7 (s, 1H, ArH), 8.6 (s, 1H,CH), 8.1 (d, 1H, ArH),8.0 (d, 1H, ArH), 7.3 (d, 2H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 3H, OCH_3), 2.3 (s, 6H, $2CH_3$), 2.1 (s, 1H, CH_3).
VI10	3092 (NH), 1695 (C=O), 1689	8.8 (s, 1H, CH), 8.6 (s, 1H,ArH), 8.1 (d, 1H, ArH),8.0

	(C=C), 1589 (C=N), 1458 (C-O-C), 1371 (C=S).	(d, 1H, ArH), 7.7 (d, 2H, ArH), 7.2 (d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.1 (s, 1H, NH), 4.0(s, 3H, OCH_3), 2.3 (s, 3H, CH_3).
VI11	3088 (NH), 1697 (C=O), 1666 (C=C), 1578 (C=N), 1454 (C-O-C), 1375 (C=S).	11.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (t, 1H, ArH), 7.2 (t, 1H, ArH), 7.0 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3).
VI12	3093 (NH), 1693 (C=O), 1679 (C=C), 1583 (C=N), 1446 (C-O-C), 1376 (C=S).	.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.0 (d, 1H,ArH), 7.9(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (d, 2H, ArH), 7.3 (d, 2H, ArH), 5.9 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3).
VI13	3094 (NH), 1691 (C=O), 1680 (C=C), 1581 (C=N), 1447 (C-O-C), 1374 (C=S).	8.8 (s, 1H, CH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 1H, ArH), 7.5 (d, 1H, ArH), 7.4 (t, 1H, ArH), 7.3 (t, 1H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.6 (s, 3H, CH_3).
VI14	3090 (NH), 1692 (C=O), 1683 (C=C), 1585 (C=N), 1453 (C-O-C), 1370 (C=S).	8.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.4 (d, 1H,ArH), 8.3 (t, 1H, ArH),8.2 (d, 1H, ArH), 8.1(d, 1H, ArH), 8.0(d, 1H, ArH), 7.9(d, 1H, ArH), 7.7(t, 1H, ArH), 7.4 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 4.1 (s, 1H, NH), 3.9 (s, 3H, OCH_3).
VI15	3093 (NH), 1689 (C=O), 1687 (C=C), 1581 (C=N), 1458 (C-O-C), 1365 (C=S).	8.8 (s, 1H, CH), 8.5 (s, 1H, CH), 8.6 (d, 1H,ArH), 8.2 (t, 1H, ArH),8.0 (d, 1H, ArH), 7.9(d, 1H, ArH), 7.8(d, 1H, ArH), 7.7 (d, 1H, ArH), 7.6 (t, 1H, ArH), 7.5 (t, 1H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.4 (s, 3H, OCH_3).

Biological Evaluation

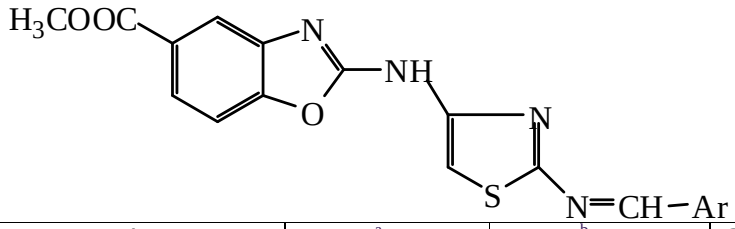
Cyclooxygenase Inhibitory Screening

The compounds synthesized were tested for cyclooxygenase–1 and cyclooxygenase–2 inhibitory activity. The method of Copeland *et al*. [34] was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of N,N,N′,N′-tetramethyl-*p* phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX–1 and COX–2 enzymes. COX–1 enzyme is from Ram seminal vesicles (microsomal fraction) and COX–2 is Recombinant human enzyme purified from SF_9 cells (microsomal fraction) were used in the assay. The compounds were dissolved in DMSO and stock solution is diluted to required assay concentration. The assay mixture consists of Tris-HCl buffer (pH 8.0, 100 mM), hematin (15 µM), EDTA (3 µM), enzyme (COX-1 or COX-2, 100µg) and test compound. The mixture was pre-incubated at 25°C for 15 min and then the reaction was initiated by the addition of arachidonic acid (100µM) and TMPD (120µM) in total volume of 1.0 mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 seconds of the reaction following the increase in absorbance at 603 nm. IC_{50} values are calculated from four parameter least squares non-linear regression analysis of the log dose vs. percentage inhibition plot.

RESULTS AND DISCUSSION

The target compounds were synthesized as outlined in Scheme 1. The required starting material, Methyl-3-amino-4-hydroxybenzoate (II) was prepared in good yield (85%) according to reported procedure [33].The Methyl-3-amino-4-hydroxybenzoate (II) on cyclization with cyanogen bromide on rapid stirring at room temperature gave the product, Methyl 2-aminobenzoxazole-5-carboxylate (III). The compound Methyl 2-aminobenzoxazole-5-carboxylate (III) on reaction with chloroacetyl chloride in dry benzene yields the compound, Methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (IV**)**. The compound Methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (IV) on reaction with thiourea gave the compound methyl-2-(2-aminothiazol-4-ylamino) benzoxazole-5-carboxylate (V), finally which on reaction with various aromatic aldehydes conveniently converted into the targeted compounds methyl-2-(2-(arylideneamino) thiazole-4-ylamino) benzoxazole-5-carboxylate derivatives (VI).

Table 3. Inhibitory effect on COX-2 and COX-1 activity



Compound (VI)	Ar	COX-2[a] IC_{50}mM	COX-1[b] IC_{50}mM	COX-1/COX-2
1	4-dimethylamino phenyl	>10	nt*	--
2	*4-t*-butylphenyl	>10	nt*	--
3	4-methoxyphenyl	>10	nt*	--
4	Phenyl	2.63	>500	>190
5	4-hydroxy phenyl	2.27	>500	>220
6	4-nitrophenyl	1.0	379	379
7	3,4 dimethoxyphenyl	1.47	>500	>340
8	Cinnamyl	6.25	>500	>80
9	3,4,5-trimethylphenyl	>10	nt*	--
10	4-methylphenyl	>10	nt*	--
11	2-hydroxyphenyl	3.84	>500	>130
12	4-bromophenyl	1.06	>500	>465
13	4-chlorophenyl	>10	nt*	--
14	2-naphthyl	2.77	>500	>220
15	1-naphthyl	>10	nt*	

[a]COX-2 activity was evaluated in human whole blood as LPS induced PGE_2generation.[b]COX-1 activity was measured in Human whole blood as TXB_2generation. IC_{50}values were estimated from dose–response curve analysed by nonlinear regression using GraphPad software and values are average of three determinations, nt* samples those have IC_{50}> 10 mM for COX-2 inhibition are not tested for COX-1 inhibition.

In the case of compound VI 4 having a simple phenyl group and compound VI 5 having hydroxyl group on 4-position of phenyl ring showed moderate activity towards COX-2 inhibition. Compound VI 12 bromo group at 4-position of phenyl ring exhibited more inhibition (IC_{50}=1.06 mM) when compare to compound VI 4. In the case of compound VI 14, which bears naphthalene ring exhibited 2.5-fold less inhibition compared to compound VI 4. In the case of

compound VI 7 two methoxy groups at 3- and 4-positions on the phenyl ring showed 1.8-fold more inhibition with reference to compound VI 4.Compound VI 8 bearing unsaturated group on phenyl ring exhibited very weak inhibition (IC_{50}=6.25mM).Compound VI 11 having hydroxyl group on the phenyl ring same as compound VI 5 exhibited similar inhibition. Compound VI 6 possessing nitro group on the phenyl ring exhibited highest activity (IC_{50}=1mM) among tested compounds. Remaining compounds are less active with an IC_{50}more than 10 mM.The compound which shown IC_{50}less than 10 mM concentration were tested for COX-1 inhibition. Interestingly two compounds, namely VI 6 and VI 12 shown good activity with high selectivity towards COX-2 inhibition when compared to rest of the compounds. Compound6 is 379 times more selective towards COX-2 when compared to COX-1 (COX-1 IC_{50}=384mM; COX-2 IC_{50}=1mM). Surprisingly compound VI 12 is 465 times more selective towards COX-2 inhibition than COX-1 (COX-1 IC_{50}=>500; COX-2 IC_{50}=1.06 mM), interestingly 100 fold more selective than compound 6. However they are more selective and less potent than rofecoxib in human whole blood assay. Although compounds VI 4, VI 5, VI 7, VI 8 and VI 14 possess good selectivity, they have shown moderate activity towards COX-2 (results presented in Table-3). In conclusion, these classes of compounds may serve as excellent candidates for selective COX-2 inhibition.

CONCLUSION

This study reports the successful synthesis of the title compounds in good yields and moderate to potent COX-2 of these derivatives containing benzoxazole moiety which is comparable with standard drug. It has been observed that the increased COX-2 inhibitory activity is attributed to the presence of pharmacologically active substituents like 2-(dialkylamino) acetamido group.

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