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Synthesis and *invitro* evaluation of novel benzoxazole derivatives as specific cyclooxygenase – 2 inhibitors

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Abstract

We have synthesised a series of methyl-2-{(2-(dialkylamino) acetamido)}-benzoxazole-5-carboxylates from Methyl 3-amino-4-hydroxybenzoate and investigated their ability to inhibit human cyclooxygenase-2 enzyme (COX-2). The IC50 values of Compounds Compound VIIf and Compound VIIb were found to be comparable to that of standard - refecoxib. Thus, this class of compounds apart from the ease of synthesis and higher yield may serve as excellent candidates for selective COX-2 inhibition

Key words: Novel benzaxozoles, synthesis, COX -2 inhibition.

Introduction

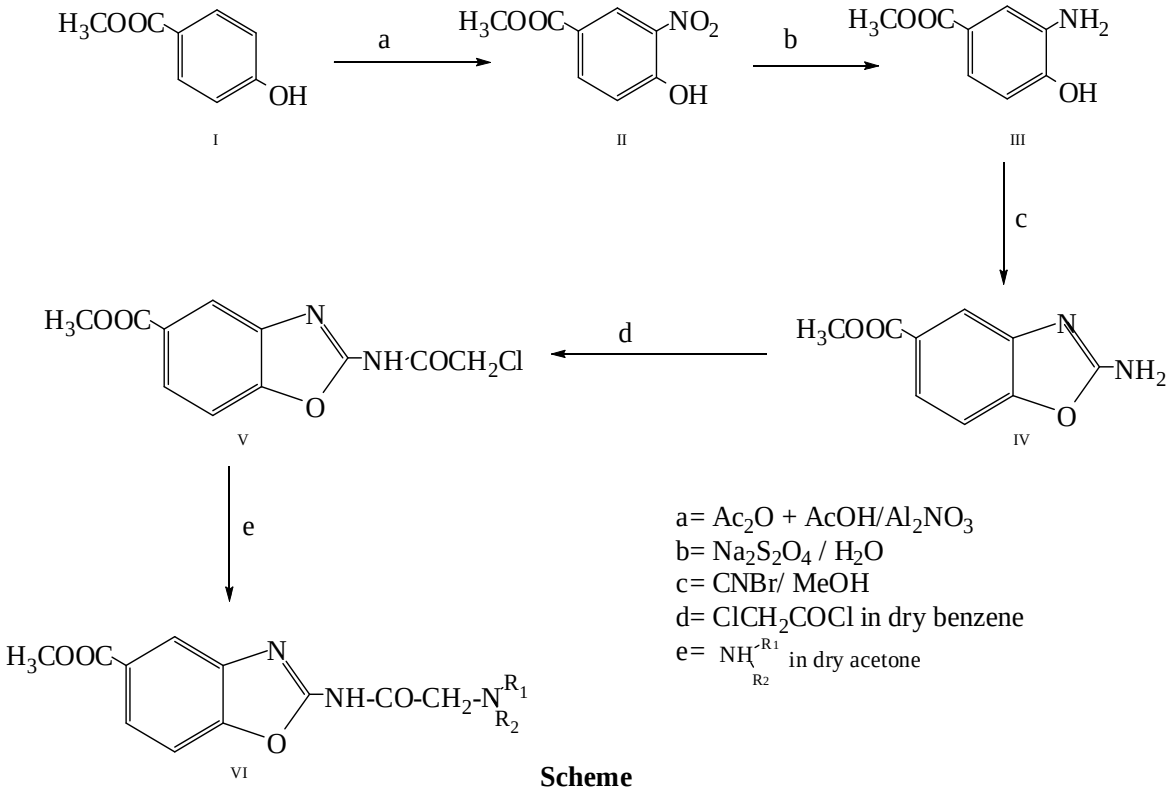
Cyclooxygenase (COX) is the key enzyme which catalyses the conversion of arachidonic acid to prostaglandins and thromboxanes[1,2]. There are two types of cyclooxygenase enzymes, COX-1 and COX-2. COX-1 is a constitutive enzyme, produced in many tissues such as the kidney and the gastrointestinal tract, while COX-2 is inducible and is expressed during inflammation at a site of injury [3-5].

Treatment of inflammation with steroids (i.e., glucocorticoids) is associated with severe side effects leading, at times, to heart, liver, and kidney damages [6]. Presently, non steroidal anti-inflammatory drugs (NSAID) are the preferred agents for the treatment of pain and inflammation, particularly arthritis. Currently available NSAIDS have been characterized as dual

COX-1 and COX-2 inhibitors [7]. Even these NSAIDS are found to have some side effects, due to their inhibitory activity against COX-1[8]. Celecoxib [9] Rofecoxib [10], Valdecoxib [11], and Etoricoxib [12].

Materials and Methods

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.



Scheme

a. Synthesis of 4-carbomethoxy-2-nitrophenol (II)

To a solution of aluminium nitrate (40grms) in acetic acid- acetic anhydride (1:1) mixture (160ml), was added an appropriate phenol (I, 40grms) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 1.5 hours 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^{O}C [13].

b. Synthesis of 4-carbomethoxy-2-aminophenol (III)

4-carbomethoxy-2-nitrophenol (II, 10 grams) 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, washed with cold water 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^{O}C [14].

c. Synthesis of methyl-2-aminobenzoxazole-5-carboxylate (IV)

4-carbomethoxy-2-aminophenol (III, 1.3 moles) was dissolved in 1lit. methyl alcohol and cooled the solution to 5^{O}C by adding chopped ice.A cold suspension of cyanogenbromide (1.5 moles) in 1lit.of water was added over a period of 5min with rapid stirring. The reaction mixture was stirred for 0.75hrs at room temperature, solid sodium bicarbonate (1.3 moles) in small portions over a period of 1.5 hrs was added to bring the p^{H} 6.5 -7.0. Stirring was continued for another 1hour. 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^{O}C.

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3364(NH_2), 1783 (C=O), 1576 (C=N), 1085 (C-O-C); PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 7.4(d, 1H, Ar-H), 7.3(d, 1H, Ar-H), 7.2 (s, 1H, Ar-H) 4.7(s, 2H, NH_2), 3.4(s, 3H, CH_3).

d. Synthesis of methyl-2-(2-chloroacetamido) benzoxazole-5-carboxylate (VI)

A mixture of methyl-2-aminobenzoxazole-5-carboxylate (IV, 0.01mol) and chloroacetyl chloride (0.01mol) was taken in 20 ml of dry benzene and the reaction mixture was refluxed for 5hrs 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^{O}C.

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 1775(C=O), 1682 (C=O), 1575 (C=N), 1560(C=C), 1085 (C-O-C); ^{1}H NMR Spectrum (DMSO-d_6) has been found to exhibit characteristic proton signals (δ, ppm) at: 8.3 (s, 1H, Ar-H), 7.9-8.0 (dd, 2H, Ar-H), 7.8 (s,1H, NH), 4.4 (s,2H, CH_2), 3.8 (s, 3H, CH_3).

e. Synthesis of methyl-2-{(2-(dialkylamino) acetamido)}-benzoxazole-5-carboxylates (VII)

To a solution of Methyl 2-(2-chloroacetamido) benzoxazole-5-carboxylate (V, 0.01mol) in 20 ml of dry Acetone, N, N-dialkylamine (0.01mol) was added and the reaction mixture was refluxed for 5hrs on a water bath. The colorless products formed were recrystallized by suitable solvents. The compounds were characterized as the methyl 2-{(2-dialkylamino)acetamido)}-benzoxazole-5-carboxylates (VII) by their spectral data.

Compound VIIa

IR (KBr, cm-1): 1760 (C=O), 1610 (C=C), 1582 (C=N), 1340 (C-N), 1249 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.8 (s, 1H, Ar-H), 8.2 (d, 1H, Ar-H), 8.1 (d, 1H, Ar-H), 8.0 (s, 1H, NH), 3.8 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 2.6 (q, 4H, 2 CH_2), 1.0 (t, 6H, 2CH_3); MS (*m/z):* M+ calculated 305.1, found 304.1.

Compound VIIb
IR (KBr, cm-1): 1759 (C=O), 1612 (C=C), 1592 (C=N), 1344 (C-N), 1254 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.6 (s, 1H, Ar-H), 8.2 (d, 1H, Ar-H), 8.1 (d, 1H, Ar-H), 7.9 (s, 1H, NH), 4.0 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 2.7 (s, 6H, $2CH_3$); MS (*m/z):* M+ calculated 278.1, found 277.1.

Compound VIIc
IR (KBr, cm-1): 1751 (C=O), 1611 (C=C), 1579 (C=N), 1314 (C-N), 1233 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.6 (s, 1H, Ar-H), 8.1 (d, 1H, Ar-H), 8.0 (d, 1H, Ar-H), 8.0 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.3 (s, 2H, CH_2), 2.4 (q, 4H, 2 CH_2), 1.5 (m, 6H, $3CH_2$); MS (*m/z):* M+ calculated 318.1, found 317.1.

Compound VIId
IR (KBr, cm-1): 1743 (C=O), 1621 (C=C), 1597 (C=N), 1333 (C-N), 1201 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.8 (s, 1H, Ar-H), 8.4 (d, 1H, Ar-H), 8.3 (d, 1H, Ar-H), 8.2 (s, 1H, NH), 3.6 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 2.6 (t, 4H, 2 CH_2), 2.3 (t, 4H, 2 CH_2), 2.0 (s, 1H, NH (piperizine ring)); MS (*m/z):* M+ calculated 319.1, found 318.1.

Compound VIIe
IR (KBr, cm-1): 1719 (C=O), 1601 (C=C), 1549 (C=N), 1324 (C-N), 1241 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.9 (s, 1H, Ar-H), 8.4 (d, 1H, Ar-H), 8.2 (d, 1H, Ar-H), 7.4 (s, 1H, NH), 3.4 (s, 3H, CH_3), 3.1 (s, 2H, CH_2), 1.1-2.2 (m, 20H, 10 CH_2 (di cyclohexyl groups)); MS (*m/z):* M+ calculated 414.1, found 413.1.

Compound VIIf
IR (KBr, cm-1): 1717 (C=O), 1610 (C=C), 1557 (C=N), 1320 (C-N), 1246 (C-O-C); ^{1}H-NMR (DMSO-d6) δ: 8.6 (s, 1H, Ar-H), 8.1 (d, 1H, Ar-H), 8.0 (d, 1H, Ar-H), 7.2 (s, 1H, NH), 3.7 (s, 3H, CH_3), 3.6 (t, 4H, 2 CH_2), 3.2 (s, 2H, CH_2), 2.5 (t, 4H, 2 CH_2); MS (*m/z):* M+ calculated 320.1, found 319.1.

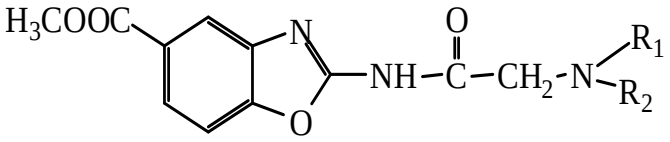
Cyclooxygenase -2 Inhibitory Screening

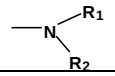
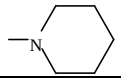
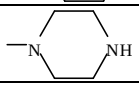
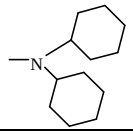
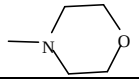
The compounds prepared were tested for cyclooxygenase– 2 inhibitory activity. The method of Graph Pad Prism was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of of N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX–2 enzymes. 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 (*p*H 8.0, 100 mM), hematin (15 μ*M*), EDTA (3 μ*M*), 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 (100μ*M*) and TMPD (120μ*M*) 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 v/s percentage inhibition plot. The results were given in table:2

Table 1: Physical data of methyl-2-(2-(dialkylamino) acetamido)-benzoxazole-5-carboxylates (VII)



S.No	Compound		Chemical formula	MeltingPoint (^{O}C)	Yield (%)
1	**VIIa**	-N(CH_2CH_3)$_2$	$C_{15}H_{19}N_3O_4$	170	70
2	**VIIb**	-N(CH_3)$_2$	$C_{15}H_{17}N_3O_5$	206	75
3	**VIIc**		$C_{16}H_{19}N_3O_4$	198	66
4	**VIId**		$C_{15}H_{18}N_3O_4$	188	65
5	**VIIe**		$C_{23}H_{31}N_3O_4$	183	50
6	**VIIf**		$C_{13}H_{15}N_3O_4$	164	65

Results and Discussion

The targeted compounds were synthesized by reaction of Methyl 2-(2-chloroacetamido) benzoxazole-5-carboxylate with different secondary amines to give the corresponding methyl 2-{(2-dialkylamino) acetamido)}-benzoxazole-5-carboxylates (VIIa - VIIf). The yield was found to be 50-75 %. The structures of the synthesized compounds were characterized by IR, 1HNMR, mass spectral analysis. All the benzoxazole derivatives synthesized were shown good to moderate activity when comparing with the IC_{50} value of Refecoxib (standard) i.e. 7.79. The compounds VIIa, VIId, and VIIe shown moderate activity with the IC_{50} values of 12.69, 20.13, 23.85 and 21.09 respectively. Interestingly Compound VIIe and VIIf showed a very potent COX-2 inhibitory activity when comparing with the standard with the IC50 values of 10.72 and 10.85 respectively.

Interestingly two compounds, namely VIIc and VIIb (IC_{50} = 6.40 and 9.39 respectively) shown good activity with high selectivity towards COX-2 inhibition when compared to rest of the compounds.

Although compounds VIIa, VIId, VIIe, and VIIf possess good activity, they have shown moderate activity towards COX-2. In conclusion, these classes of compounds may serve as excellent candidates for selective COX-2 inhibition (Table2).

Table2: COX-2 inhbition activity of methyl-2-(2-(dialkylamino) acetamido)-benzoxazole-5-carboxylates (VII)

S.No	Compound	IC_{50} Value
01	VIIa	12.69
02	VIIb	09.39
03	VIIc	06.40
04	VIId	20.13
05	VIIe	10.72
06	VIIf	10.85
	Refecoxib	07.79

Conclusion

This study reports the successful synthesis of the title compounds in good yields and moderate to potent anti inflammatory activity of these derivatives containing benzoxazole moiety which is comparable with standard drug.

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