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Note

Synthesis of 4*H*-imidazo[2,1-*c*][1,4]benzoxazin-4-yl acetic acids and esters as possible COX-2 inhibitors

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A series of 4*H*-imidazo[2,1-*c*][1,4]benzoxazin-4-yl acetic acids and esters (**7** and **8**) have been synthesized from methyl α-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl)acetates **5** and their COX-2 inhibition activities are evaluated.

Keywords: Imidazo acetic acids, benzoxazin acetates, COX-2 inhibition activity

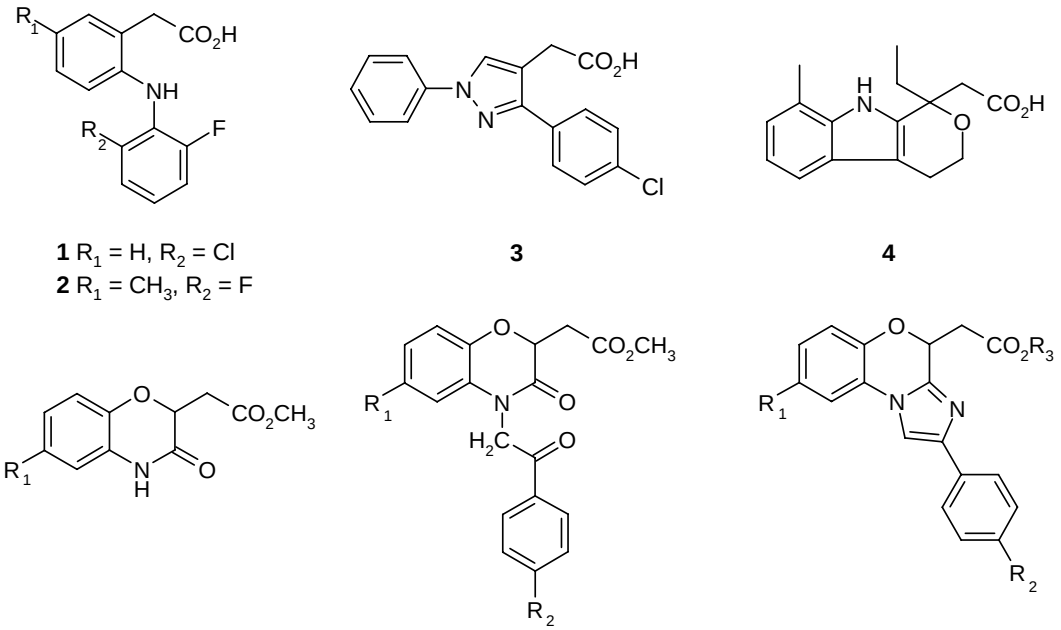
IPC: **Int.Cl.**[7] **C 07 D**

Although, non-steroidal anti-inflammatory drugs (NSAIDS), have been used in the treatment of various inflammatory diseases, their usage is limited by the side effects produced by them, thereby necessitating the need for searching new molecular entities[1]. A number of aryl and heteroaryl substituted acetic acids such as Diclofenac[1] **1**, Lumiracoxib[2] **2**, Lonazolac[3] **3**, Etodolac[1] **4** have been commercialized as NSAIDS. Several imidazoazines have been reported to possess significant pharmacological activities[4]. Otsuka *et al.*[5] isolated a number of 1,4-benzoxazinone derivatives from the roots of *Coix lachryma – jobi* which have been used in treatment of neuralgia, rheumatism and inflammatory diseases. Furthermore, recently several 2-substituted benzoxazinones have been reported as smooth muscle relaxants[6], anticoagulant[7] and antibacterial agents[8]. In addition, a new series of benzoxazinones were reported useful in the treatment of type **2** diabetes which does not contain thiazolidenedione[9]. In view of these observations and in continuation of our work on new benzoxazines[10], it was considered of interest to synthesize some new imidazobenzoxazinyl acetic acids and evaluate their COX-2 inhibitor activity.

Methyl α-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl)acetates **5** required as starting materials in the present work, were prepared by the reaction of substituted 2-aminophenols with maleic anhydride in refluxing methanol in the presence of triethylamine according to the procedure described earlier[11]. Reaction of **5** with various ω-haloacetophenones in refluxing acetone in the presence of anhydrous potassium carbonate and tetrabutylammonium bromide as phase transfer catalyst gave the N-alkylated benzoxazinones **6** in excellent yields. ^{1}H NMR spectra of **6** exhibited characteristic signals around δ 3.76-3.78 (CO_2CH_3), 2.98-3.06 (2 × dd, $CH_2CO_2CH_3$), 5.0-5.03 (m, OCH) and 5.31-5.34(ABq, -NCH_2-CO) apart from other aromatic protons. IR spectra of **6** exhibited characteristic ester, amide and ketone carbonyl in the region 1735, 1690 cm^{-1}. Cyclocondensation of **6** in acetic acid in presence of ammonium acetate gave the targeted imidazobenzoxazinyl acetates **7** in good yields (IR 1737 cm^{-1}). Hydrolysis of representative **7** in aqueous methanolic NaOH furnished the corresponding acetic acids **8** as colourless crystalline solids (**Scheme I**). Structures of compounds **7** were established based on their ^{1}H NMR, IR, mass spectra and correct elemental analyses. In the ^{1}H NMR spectra of **7**, the ester group appeared as a singlet at δ 3.76, the methylene protons of ester group appeared as two sets of double doublets at δ 3.06 and 3.42 whereas OCH proton appeared as a double doublet at δ 5.74 apart from C_1-imidazoproton (δ 7.4-7.5, singlet) and other aromatic protons.

COX-2 Inhibitory activity

All the compounds (**7a-j**, **8b**, **8e**, **8g** and **8h**) were tested for cycloxygenase-2 inhibitory activity. The method of Copeland *et al.*[10] was followed to determine the IC_{50} values. The enzyme activity was measured using chromogenic assay based on oxidation of N,N,N′,N′-tetramethylparaphenylene-



Scheme I

diamine (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 was diluted to required assay concentration. The assay mixture consists of tris buffer (*p*H 8.0), EDTA solution and hematin as cofactor, the enzyme and the drug of assay concentration in DMSO. The assay mixture was pre-incubated at 25°C and then TMPD in ethanol was added. The enzyme activity was measured by estimating the initial velocity during the first 25 seconds by measuring the absorbance at 603 nm. IC_{50} values were calculated from four parameter least squares nonlinear regression analysis of the log dose vs percentage inhibition plot. However, none of the compounds reported herein exhibited significant inhibition up to 100 μg/mL when compared to standard Celecoxib.

Experimental Section

Melting points were determined in open capillaries and are uncorrected. The purity of all the compounds was routinely checked by TLC on silica gel coated plates. IR spectra were recorded in KBr pellets on a Perkin-Elmer system 2000 FTIR spectrometer; ^{1}H NMR spectra on a Varian 200 MHz instrument with TMS as internal standard (chemical shifts in δ, ppm); and mass spectra on a Hewlett Packard mass spectrometer operating at 70eV.

Preparation of 4-chloro-ω-(6-methyl-2-methoxycarbonyl-3-oxo-3,4-dihydro-1,4-benzoxazin-4-yl)-acetophenone 6f. To a mixture of methyl α-(3,4-dihydro-3-oxo-2*H*-6-methyl-1,4-benzoxazin-yl)acetate (5, R_1 = CH_3, 2.35 g, 0.01 mole), anhyd. potassium carbonate (10 g) and 4-chloro-ω-bromoacetophenone (2.33 g, 0.01 mole) in acetone (100 mL) was added a catalytic amount of tetrabutylammoniumbromide (PTC) and the reaction mixture was refluxed for 4-6 hr. At the end of the reaction as monitored by TLC, it was cooled, filtered and solvent removed *in vacuo* to give a solid. It was recrystallized from methanol to give pure **6f** as crystalline solid, IR: 3076, 2942, 1735, 1689, 1601 cm $^{-1}$.

Compounds **6a-j** were prepared similarly and their characterization data are listed in **Table I**.

Preparation of methyl [8-methyl-2-(4-chlorophenyl)-4*H*-imidazo[2,1-*c*][1,4]-benzoxazin-4-yl]acetate 7f. A mixture of **6f** (3.87 g, 0.01 mole) and ammonium acetate (0.1 mole) in gl. acetic acid (8 mL) was refluxed for 4 hr. The solution was cooled, poured onto crushed ice, neutralized with ammonia solution and the solid obtained was filtered and

Table I — Characterization data of compounds **6** and **7**

Compd*	R_1	R_2	m.p. °C	Yield (%)	Mol. formula	Found (Calcd) % C	H	N	^{1}H NMR (δ, ppm)
6a	H	H	116	72	$C_{19}H_{17}NO_5$	67.57 (67.25	5.32 5.01	4.39 4.12)	3.01(dd, 1H), 3.17(dd, 1H), 3.78(s, 3H), 5.08(m, 1H), 5.19(d, 1H), 5.49(d, 1H), 6.61(d, 1H), 6.96(m, 3H), 7.61(m, 3H), 8.03(m, 2H)
6b	H	F	131	67	$C_{19}H_{16}FNO_5$	63.62 (63.86	4.82 4.48	4.23 3.92)	2.96(dd, 1H), 3.12(dd, 1H), 3.76(s, 3H), 5.07(m, 2H), 5.41(d, 1H), 6.56(m, 1H), 6.91(m, 3H), 7.21(m, 2H), 8.03(m, 2H)
6c	H	Cl	120	78	$C_{19}H_{16}ClNO_5$	61.23 (61.04	4.63 4.28	3.82 3.74)	2.99(dd, 1H), 3.15(dd, 1H), 3.78(s, 3H), 5.08(m, 2H), 5.41(d, 1H), 6.58(d, 1H), 6.94(m, 3H), 7.51(d, 2H), 7.97(d, 2H)
6d	CH_3	H	131	82	$C_{20}H_{19}NO_5$	68.21 (67.98	5.26 5.09	4.27 3.96)	2.21(s, 3H), 2.92(dd, 1H), 3.13(dd, 1H), 3.78(s, 3H), 4.98(m, 1H), 5.07(d, 1H), 5.41(d, 1H), 6.36(s, 1H), 6.81(2d, 2H), 7.58(m, 3H), 8.02(d, 2H)
6e	CH_3	F	128	75	$C_{20}H_{18}FNO_5$	65.61 (65.22	5.01 4.85	4.01 3.77)	2.21(s, 3H), 2.92(dd, 1H), 3.10(dd, 1H), 3.76(s, 3H), 4.99(m, 1H), 5.07(d, 1H), 5.38(d, 1H), 6.37(s, 1H), 6.81(2d, 2H), 7.22(m, 2H), 8.03(m, 2H)
6f	CH_3	Cl	124	76	$C_{20}H_{18}ClNO_5$	62.67 (62.45	5.23 5.09	4.23 3.96)	2.22(s, 3H), 2.94(dd, 1H), 3.11(dd, 1H), 3.67(s, 3H), 4.98(m, 1H), 5.07(d, 1H), 5.35(d, 1H), 6.32(s, 1H), 6.81(2d, 2H), 7.44(d, 2H), 7.83(d, 2H)
6g	CH_3	CH_3	125	81	$C_{21}H_{21}NO_5$	68.82 (68.66	6.02 5.72	4.12 3.81)	2.22(s, 3H), 2.43(s, 3H), 2.95(dd, 1H), 3.12(dd, 1H), 3.76(s, 3H), 4.99(m, 1H), 5.01(d, 1H), 5.42(d, 1H), 6.39(s, 1H), 6.81(Abq, 2H), 7.24(d, 2H), 7.83(d, 2H)
6h	Cl	H	122	69	$C_{19}H_{16}ClNO_5$	61.36 (61.04	4.51 4.28	3.96 3.74)	2.96(dd, 1H), 3.11(dd, 1H), 3.73(s, 3H), 5.04(m, 2H), 5.42(d, 1H), 6.51(s, 1H), 6.92(s, 2H), 7.58(m, 3H), 8.01(m, 2H)
6i	Cl	F	127	72	$C_{19}H_{15}ClFNO_5$	58.51 (58.23	4.21 3.83	3.71 3.57)	2.95(dd, 1H), 3.17(dd, 1H), 3.78(s, 3H), 5.12(m, 2H), 5.43(d, 1H), 6.59(s, 1H), 7.12(m, 4H), 8.07(m, 2H)
6j	Cl	Cl	132	75	$C_{19}H_{15}Cl_2NO_5$	56.12 (55.88	4.03 3.67	3.67 3.43)	2.94(dd, 1H), 3.12(dd, 1H), 3.77(s, 3H), 5.06(m, 2H), 5.41(d, 1H), 6.52(s, 1H), 6.83(s, 2H), 7.44(d, 2H), 7.93(d, 2H)
7a	H	H	155	67	$C_{19}H_{16}N_2O_3$	71.46 (71.25	5.26 5.00	8.31 8.75)	3.08(dd, 1H), 3.43(dd, 1H), 3.76(s, 3H), 5.72(dd, 1H), 6.92-7.20(m, 6H), 7.53(s, 1H), 7.67(m, 3H)
7b	H	F	141	65	$C_{19}H_{15}FN_2O_3$	67.51 (67.46	4.59 4.44	8.66 8.28)	3.07(dd, 1H), 3.42(dd, 1H), 3.78(s, 3H), 5.73(dd, 1H), 6.93(m, 2H), 7.21(m, 3H), 7.54(s, 1H), 7.66(m, 3H)
7c	H	Cl	161	71	$C_{19}H_{15}ClN_2O_3$	64.65 (64.32	4.56 4.28	8.21 7.90)	3.06(dd, 1H), 3.43(dd, 1H), 3.76(s, 3H), 5.74(dd, 1H), 7.01(m, 3H), 7.21(m, 2H), 7.45(s, 1H), 7.66(m, 3H)
7d	CH_3	H	117	73	$C_{20}H_{18}N_2O_3$	72.03 (71.86	5.53 5.39	8.52 8.38)	2.31(s, 3H), 3.06(dd, 1H), 3.42(dd, 1H), 3.78(s, 3H), 5.73(dd, 1H), 6.94(m, 2H), 7.19(m, 3H), 7.55(s, 1H), 7.68(m, 3H)
7e	CH_3	F	137	68	$C_{20}H_{17}FN_2O_3$	67.86 (68.18	4.71 4.83	8.27 7.95)	2.32(s, 3H), 3.06(dd, 1H), 3.42(dd, 1H), 3.76(s, 3H) 5.74(dd, 1H), 6.95(m, 2H), 7.18(m, 3H), 7.53(s, 1H) 7.66(m, 2H)
7f	CH_3	Cl	129	77	$C_{20}H_{17}ClN_2O_3$	64.92 (65.13	4.86 4.61	7.43 7.60)	2.32(s, 3H), 3.03(dd, 1H), 3.39(dd, 1H), 3.77(s, 3H), 5.76(dd, 1H), 6.93(m, 2H), 7.13(m, 3H), 7.56(s, 1H), 7.69(d, 2H)
7g	CH_3	CH_3	125	76	$C_{21}H_{20}N_2O_3$	72.63 (72.41	6.01 5.75	8.29 8.05)	2.32(s, 6H), 3.06(dd, 1H), 3.41(dd, 1H), 3.76(s, 3H), 5.74(dd, 1H), 6.95(m, 2H), 7.19(m, 3H), 7.55(s, 1H), 7.68(d, 2H)
7h	Cl	H	152	72	$C_{19}H_{15}ClN_2O_3$	64.67 (64.32	4.56 4.28	8.23 7.90)	3.06(dd, 1H), 3.43(dd, 1H), 3.76(s, 3H), 5.76(dd, 1H), 7.06(m, 3H), 7.24(m, 2H), 7.46(s, 1H), 7.67(m, 3H)
7i	Cl	F	167	69	$C_{19}H_{14}ClFN_2O_3$	61.38 (61.20	4.12 3.76	7.39 7.52)	3.08(dd, 1H), 3.41(dd, 1H), 3.76(s, 3H), 5.76(dd, 1H), 7.02(m, 3H), 7.24(m, 2H), 7.43(s, 1H), 7.66(m, 2H)
7j	Cl	Cl	172	68	$C_{19}H_{14}Cl_2N_2O_3$	58.82 (58.61	4.02 3.60	7.53 7.20)	3.06(dd, 1H), 3.42(dd, 1H), 3.76(s, 3H), 5.76(dd, 1H), 7.03(m, 3H), 7.24(m, 2H), 7.43(s, 1H), 7.68(m, 2H)

recrystallized from methanol to get pure **7f** as crystalline solid, IR: 2948, 1737 cm $^{-1}$.

The characterization data of **7a-j** prepared by similar procedure, are listed in **Table I**.

Preparation of 2-aryl-4*H*-imidazo[2,1-*c*][1,4]-benzoxazin-4-yl acetic acids 8. General procedure. A mixture of **7** (0.01 mole), NaOH (1N, 10 mL) and MeOH (5 mL) was heated to reflux for 1 hr and then acidified to *p*H 5 with 1*N* HCl. The separated solid was filtered, washed with water and recrystallized from MeOH to give pure **8** as crystalline solid.

The following derivatives of **8** were prepared as per the procedure described above. **8b** (R_1 = H, R_2 = F): yield 81%, m.p. 224^o; **8e** (R_1 = CH_3, R_2 = F): yield 82%, m.p. 177^o; **8g** (R_1 = R_2 = CH_3): yield 84%, m.p. 264^o; **8h** (R_1 = Cl, R_2 = H): yield 81%, m.p. 164^o.

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