SYNTHESIS OF METHYL-(3-OXO-2*H*-[1,4]-BENZOXA /THIAZIN-6-YL)-PYRAZOLE-5-CARBOXYLATES & ISOXAZOLE-3-CARBOXYLATES AS POSSIBLE COX-2 / 5-LOX INHIBITORS

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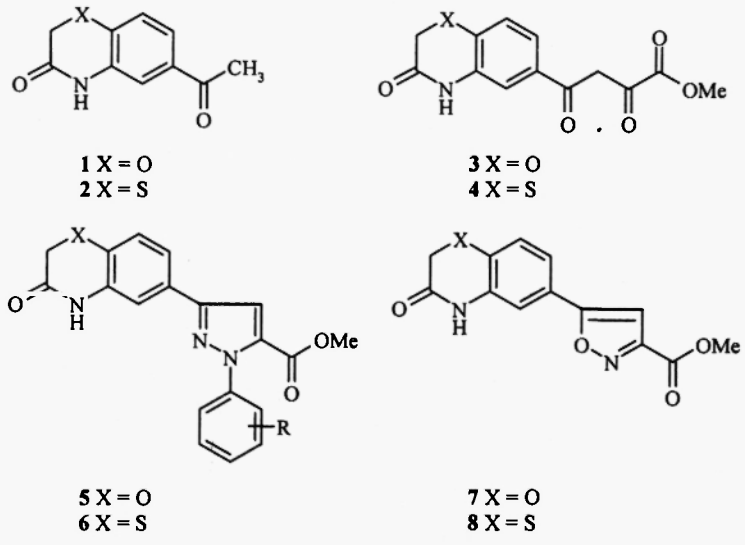
Abstract: A series of Methyl-(3-oxo-2*H*-[1,4]benzoxa/thiazin-6-yl)pyrazole-5-carboxylates (**5** & **6**) and isoxazole-3-carboxylates (**7** & **8**) have been synthesized and tested for their COX-2 / 5-LOX inhibitory activities.

Introduction

1,4-Benzoxazines are new emerging class of heterocyclic compounds with significant medicinal properties like anticancer[1], anticoagulant[2], antidiabetic[3] and antibacterial activities[4]. These are also reported useful in the treatment of respiratory tract, CNS, neurogenerative diseases and G1 tract disorders[5]. Benzoxazine forms core structural unit in *Ofloxacin*[6], a third generation quinolone antibacterial and in a number of natural products with antitumor[7] and anti T.B activities[8]. Several heterocyclic substituted benzoxazinones have been reported to possess interesting biological activities. For example *Bemoradan*[9] is a pyridazinylbenzoxazinone useful in the treatment of congestive heart failure. Also 1,4-benzothiazines are useful as Ca^{2+} antagonists, blood platelet aggregation inhibitors and anticoagulant agents[10]. *Semotiadil*[11] is a 2-aryl-1,4-benzothiazinone derivative with antihypertensive activity.

Pyrazoles and isoxazoles are known for their wide application in pharmaceutical and agricultural industry. Arylpyrazole derivatives such as *Celecoxib*[12] is a well known COX-2-inhibitor. Recently some of the arylpyrazoles have been reported useful in prostate cancer chemotherapy[13] and possess non-nucleoside HIV-I reverse transcriptase inhibitory activity[14]. In view of the above observations, it was considered of interest to synthesize some new Benzoxa(thia)zinyl pyrazole and isoxazole carboxylates and evaluate their COX-2/5-LOX inhibitory activity.



1 X = O
2 X = S

3 X = O
4 X = S

5 X = O
6 X = S

7 X = O
8 X = S

R = H, Br, Cl, F, 2,4-diF, 3-Cl,4-F

Scheme-1

Thus claisen reaction of 6-acetylbenzoxa/thiazin-3-ones (**1** & **2**) with diethyloxalate in presence of sodium methoxide gave the respective 3-oxo-benzoxa/thiazin-6-yl β-diketones (**3** & **4**) in good yields. Cyclization of **3** & **4** with substituted arylhydrazines in refluxing isopropanol in presence of acetic acid gave respective Benzoxa/thiazinyl pyrazole carboxylates in fair yields. ^{1}H-NMR spectra of **5** & **6** are characterized by the presence of three singlets around δ 3.9 (ester), 3.4/4.5 (-SCH_2/-OCH_2 of benzoxa/thiazine ring), 7.0 (pyrazole H) apart from other aromatic and ring NH protons. Similarly cyclization of **3** & **4** with hydroxylamine hydrochloride gave corresponding isoxazole carboxylates **7** & **8** in fair yields. All the compounds have been characterized by IR, NMR, Mass and elemental analyses.

COX-2 / 5-LOX inhibitory activity

All the compounds **5, 6, 7** & **8** were tested for their COX-2/5-LOX inhibitory activity. The method of Copeland et.al was adopted for determination of IC_{50} values as reported earlier[15]. *Potato lipoxygenase* was used as enzyme source for testing 5-LOX inhibitory activity. The compounds were dissolved in DMSO and stock solution was diluted to required assay concentration. The assay mixture consists of 50 mm phosphate buffer (pH 6.8). the enzyme and the drug at assay concentration in DMSO. The assay mixture was preincubated at 25° and then substrate was added. The enzyme activity was measured by estimating the initial velocity during the first 25 seconds by measuring the absorbance at 235 nm. IC_{50} values were calculated from four parameter least square nonlinear regression analysis of the log dose vs percentage inhibition plot. Compounds **5a, 5g, 6a, 6e** & **7a**, exhibited significant inhibition at 10, 12, 70 & 5 μM when compared to standard Nordihydroguairetic acid which inhibited at 1.5 μM. None of the compounds reported herein exhibited any COX-2 inhibitory activity.

Experimental

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 FT IR spectrometer, ^{1}H NMR spectra in $CDCl_3$ / DMSO-d_6 on a Varian 200 MHz instrument with TMS as internal standard (chemical shifts in δ, ppm) and mass spectra on a Hewelett Packard mass spectrometer operating at 70 eV.

Methyl-2-hydroxy-4-oxo-4-(3-oxo-[1,4]-benzoxazin-6-yl)-2-butenoate 3

To a mixture of sodium methoxide prepared from sodium (0.2 mol) in methanol (100 ml) was added drop wise, a solution of 6-acetyl-[1,4]-benzoxazin-3-one (**1**, 0.1 mol) and diethyl oxalate dissolved in methanol (100 ml). The mixture was heated at reflux for 2 hr. It was cooled, solvent removed *in vacuo* and the residue dissolved in water and acidified with conc. HCl. The separated solid was filtered and recrystallized from methanol to give pure **3** as crystalline solid. Mp 242°C, Yield 67%. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H), 4.71(s, 2H), 6.98(s, 1H), 7.06(d, 1H), 7.57(d, 1H), 7.72(dd, 1H), 10.88(bs, 1H).

Methyl 2-hydroxy-4-oxo-4-(3-oxo-1,4-benzothiazin-6-yl)-2-butenoate 4

To a mixture of sodium methoxide prepared from sodium (0.2 mol) in methanol (100 ml) was added drop wise, a solution of 6-acetyl-[1,4]-benzothiazin-3-one (**2**, 0.1 mol) and diethyl oxalate dissolved in methanol (100 ml). The mixture was heated at reflux for 2 hr. It was cooled, solvent removed *in vacuo* and the residue dissolved in water, and acidified with conc. HCl. The separated solid was filtered and recrystallized from methanol to give pure 4 as crystalline solid. mp. 243°C, yield: 69%. ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 3.44(s, 2H), 3.94(s, 3H), 7.02(s, 1H), 7.37(d, 1H), 7.57(dd, 1H), 7.67(d, 1H), 10.31(bs, 1H).

Methyl [1-phenyl-3-(3-oxo-[1,4]benzoxazin-6-yl)-1H-pyrazole]carboxylate 5a

A mixture of **3** (0.1 mole), phenylhydrazine (0.11 mole), isopropanol (100 ml) acetic acid (2 ml) was refluxed for 4-5 hrs, At the end of the reaction as monitored by TLC (Hexane:ethylacetate, 8:2), solvent was removed *in vacuo*. The solvent was taken up in water, filtered and recrystallized from methanol to give pure **5a**.

Methyl [5-(3-oxo-[1,4]benzoxa/thiazin-6-yl]isoxazole-3-carboxylates 7 & 8

A mixture of **3** (0.1 mole), hydroxylamine hydrochloride (0.11 mole) isopropanol (100 ml) was refluxed for 4-5 hrs. At the end of the reaction as monitored by TLC solvent was removed *in vacuo*. The residue was diluted with water. The separated solid was filtered and recrystallized from MeOH to give pure 7 & **8**.

The physical and spectral data of **5, 6, 7 & 8** are listed in **Table-I**.

Table-1: Physical and spectral data of **5**, **6**, **7** & **8**

Compd*	R	m.p °C	Yield %	Mol. formula	^{1}H NMR, δ ppm ($CDCl_3$ + DMSO-d_6)	MS m/e [M^+]
5a	H	187	76	$C_{19}H_{15}N_3O_4$	3.97(s, 3H), 4.69(s, 2H), 6.74(m, 2H), 6.79(d, 1H), 7.00(s, 1H), 7.35(m, 5H), 8.93(bs, 1H)	349
5b	F	223	75	$C_{19}H_{14}FN_3O_4$	3.96(s, 3H), 4.68(s, 2H), 6.72(m, 2H), 7.01(s, 1H), 7.35(m, 5H), 8.94(bs, 1H)	367
5c	Cl	219	74	$C_{19}H_{14}ClN_3O_4$	3.98(s, 3H), 4.69(s, 2H), 6.73(m, 2H), 7.02(s, 1H), 7.32(m, 5H), 8.96(bs, 1H)	383
5d	Br	235	77	$C_{19}H_{14}BrN_3O_4$	3.97(s, 3H), 4.67(s, 2H), 6.73(m, 2H), 7.00(s, 1H), 7.31(m, 5H), 8.93(bs, 1H)	429
5e	2,4-diF	229	72	$C_{19}H_{13}F_2N_3O_4$	3.97(s, 3H), 4.69(s, 2H), 6.72(m, 2H), 7.00(s, 1H), 7.32(m, 4H), 9.11(bs, 1H)	385
5f	3-Cl, 4-F	207	71	$C_{19}H_{13}ClFN_3O_4$	3.97(s, 3H), 4.68(s, 2H), 6.72(m, 2H), 7.00(s, 1H), 7.32(m, 4H), 9.09(bs, 1H)	402
5g	2-F	237	73	$C_{19}H_{14}FN_3O_4$	3.95(s, 3H), 4.54(s, 2H), 6.66(d, 1H), 6.79(d, 1H), 6.91(d, 1H), 7.00(s, 1H), 7.21(m, 2H), 7.46(m, 2H), 10.48(bs, 1H)	367
6a	H	264	69	$C_{19}H_{15}N_3O_3S$	3.42(s, 2H), 3.93(s, 3H), 6.73(m, 2H), 6.78(d, 1H), 7.01(s, 1H), 7.34(m, 5H), 10.31(bs, 1H)	365
6b	F	219	70	$C_{19}H_{14}FN_3O_3S$	3.43(s, 2H), 3.94(s, 3H), 6.72(m, 2H), 7.01(s, 1H), 7.34(m, 5H), 10.32(bs, 1H)	383
6c	Cl	220	68	$C_{19}H_{14}ClN_3O_3S$	3.42(s, 2H), 3.92(s, 3H), 6.72(m, 2H), 7.00(s, 1H), 7.32(m, 5H), 10.31(bs, 1H)	400
6d	Br	186	73	$C_{19}H_{14}BrN_3O_3S$	3.44(s, 2H), 3.94(s, 3H), 6.73(m, 2H), 7.00(s, 1H), 7.31(m, 5H), 10.31(bs, 1H)	446
6e	CH_3	228	67	$C_{20}H_{17}N_3O_3S$	2.38(s, 3H), 3.42(s, 2H), 3.93(s, 3H), 6.72(m, 2H), 7.00(s, 1H), 7.31(m, 5H), 10.32(bs, 1H)	379
7	-	254	71	$C_{13}H_{11}N_2O_5$	4.00(s, 3H), 4.63(s, 2H), 6.84(s, 1H), 7.02(d, 1H), 7.37(m, 2H), 10.54(s, 1H)	
8	-	223	74	$C_{13}H_{11}N_2O_4S$	3.42(s, 2H), 4.00(s, 3H), 6.93(s, 1H) 7.40(m, 3H), 10.42(s, 1H)	

*All the compounds gave satisfactory C, H and N analyses.

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