SYNTHESIS OF METHYL [6-(2-AMINO-1,3-THIAZOL-4-YL)-3-OXO-1,4-BENZOXAZIN-2-YL]ACETATES AS POSSIBLE COX-2 / 5-LOX INHIBITORS

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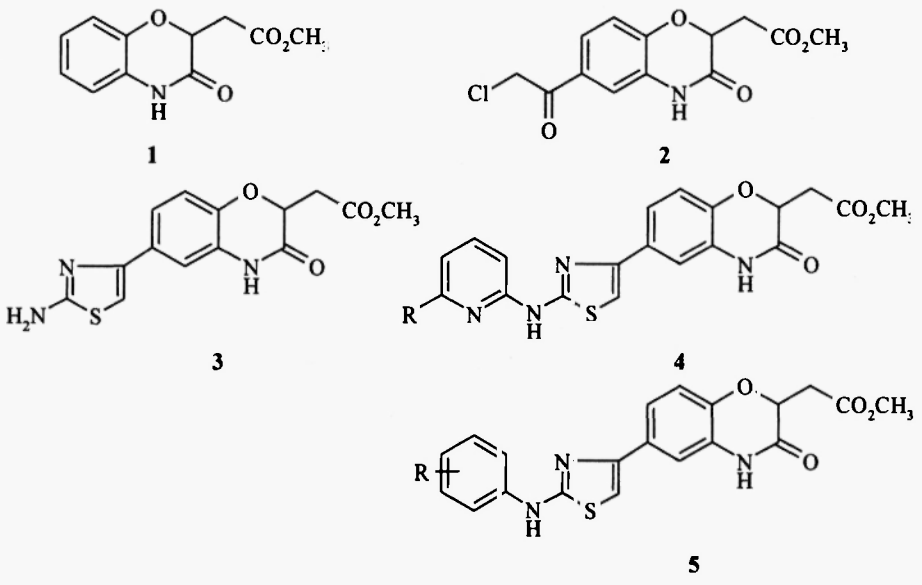
Abstract : A series of methyl [6-(2-aminothiazol-4-yl)-3-oxo-1,4-benzoxazin-2-yl]acetates (3-5) have been synthesized and tested for COX-2 (Cyclooxygenase) / 5-LOX (Lipoxygenase) inhibitory activity. Some of the compounds exhibited notable 5-LOX inhibitory activity.

Introduction

The introduction of *Celecoxib* and *Rofecoxib* as antiinflammatory agents with better gastric tolerance has created interest in the development of selective COX-2 inhibitors[1]. Aminothiazole ring system has broad application in the treatment of allergies[2], hypertension[3], inflammation[4], schizophrenia[5], bacterial[6] and HIV infections[7]. *Fanetizole*[4] (4-phenyl-2-phenethylaminothiazole) is an antiinflammatory agent under phase II clinical trials for the treatment of rheumatoid arthritis. Recently, compounds with pyridylaminothiazole as core unit have been reported as anticancer agents[8], KDR Kinase inhibitors[9], CNS, mood disorder, brain tumour, epilepsy, anxiety, depression and Alzheimeirs disease[10]. A number of 1,4-benzoxazine derivatives have been reported as smooth muscle relaxants[11], anticoagulants[12] and antibacterial agents[13]. Furthermore, several aryl and heteroaryl acetic acids represented by indomethacin, diclofenac, sulindac, fentiazac and Lonazolac enjoy clinical status as antiinflammatory agents[1]. Prompted by these observations and in continuation of our work on new benzoxazines[14] we report herein the synthesis and COX-2 / 5-LOX inhibitory activity of some new aminothaizolylbenzoxazinyl acetates.

Methyl α-(3,4-dihydro-3-oxo-2H-1,4-benzoxazin-2-yl)acetate (**1**) required as starting material in the present work, was prepared by the reaction of 2-aminophenol with maleic anhydride in refluxing methanol in the presence of triethylamine according to procedure described earlier[15]. The reaction of **1** with chloroacetyl chloride in the presence of aluminium chloride under Friedel-Crafts reaction conditions gave the hitherto unreported 6-chloroacetylbenzoxazin-2-ylacetate **2** in good yields. ^{1}H NMR spectra of **2** exhibited signals at δ 2.9 (m, 2H, $CH_2CO_2CH_3$), 3.61(s, 3H, $CH_2CO_2CH_3$), 5.17 (m, 3H, OCH & CH_2CO) and 10.02 (bs, NH) apart from three aromatic protons. IR spectra of **2** exhibited characteristic absorption bands around 1738 cm^{-1} (CO_2CH_3), 1714 & 1716 cm^{-1} (C=O).



Scheme-1

Various pyridyl and arylthioureas required in the present work were prepared by reaction of the corresponding amines with benzoylisothiocyanate followed by hydrolysis. The targeted compounds **3**, **4** & **5** were obtained by Hantzsch cyclization of **2** with suitable thioureas in good yields (**Scheme-1**). Structures of the compounds **3**, **4** & **5** were elucidated based on their ^{1}H NMR, IR, mass spectra and correct elemental analyses. For example ^{1}H NMR spectra of **4a,** the aminothiazole and lactam NH protons appeared as two broad singlets at δ 10.25 & 10.42. The methylester appeared as a singlet at δ 3.61, –OCH & CH_2CO_2 protons appeared as three sets of double doublets at δ 4.81, 2.99 & 2.84 respectively apart from thiazole, pyridine and aromatic protons in the region δ 6.70 - 8.21 (8H). IR spectra of **4a** exhibited absorption bands around 1741 (CO_2CH_3) and 1705 cm^{-1} (NHCO).

COX-2 / 5-LOX inhibitory activity

All the compounds **3**, **4** & **5** (**Table-1**) were tested for their COX-2 / 5-LOX inhibitory activity. The method of Copeland[16] *et. al* was adopted for determination of IC_{50} values as reported earlier. *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 mm. IC_{50} values were calculated from four parameter least square nonlinear regression analysis of the log dose vs percentage inhibition plot. Four compounds, amino-**3**, methylpyridylamino-**4b** and tolylaminothiazole derivatives **5b** & **5c** exhibited significant inhibition at 8, 10 & 5 μM when compared to standard nor dihydroguairetic acid which inhibited at 1.5 μM. Compounds **5a** & **5e-h** exhibited a low order of activity ranging from 15μM-60μM (**Table–1**). None of the compounds reported herein exhibited any COX-2 inhibitory activity.

Table-1 : Characterization data of compounds **3**, **4** & **5**

Compd*	R	m.p. °C	Yield (%)	IC_{50} μM 5-LOX	Mol. formula	Found (Calc.) % C	H	N	^{1}H NMR (δ, ppm) $CDCl_3$	MS m/z [M^+]
3	-	193	72	8	$C_{14}H_{13}N_3O_4S$	52.78 (52.66	4.21 4.07	13.42 13.16)	2.96(dd, 1H), 3.12(dd, 1H), 3.74(s, 3H), 4.94(dd, 1H), 6.55(s, 1H), 6.67(bs, 2H), 6.87(d, 1H), 7.24(d, 1H), 7.31(d, 1H), 10.36(bs, 1H)	319
4a	H	199	69	>100	$C_{19}H_{16}N_4O_4S$	57.73 (57.57	4.26 4.04	14.51 14.14)	2.84(dd, 1H), 2.99(dd, 1H), 3.61(s, 3H), 4.81(dd, 1H) 6.70(m, 2H), 6.81(d, 1H), 6.91(d, 1H), 7.26(m, 2H), 7.45(m, 1H), 8.21(d, 1H), 10.25(bs, HH), 10.42(bs, 1H)	396
4b	CH_3	146	68	10	$C_{20}H_{18}N_4O_4S$	58.76 (58.53	4.53 4.39	13.78 13.66)	2.81(s, 3H), 2.85(dd, 1H), 2.98(dd, 1H), 3.61(s, 3H), 4.82(dd, 1H), 6.71(m, 2H), 6.82(d, 1H), 6.91(d, 1H), 7.26(m, 1H), 7.45(m, 1H), 8.21(d, 1H), 10.25(bs, 1H), 10.42(bs, 1H)	410
5a	H	171	74	60	$C_{20}H_{17}N_3O_4S$	61.02 (60.76	4.46 4.30	10.82 10.63)	2.94(dd, 1H), 3.12(m, 1H), 5.73(s, 3H), 4.94(dd, 1H), 6.56(s, 1H), 6.64-7.76(m, 8H), 10.25(bs, 1H), 10.41(bs, 1H)	395
5b	4-CH_3	180	72	5	$C_{21}H_{19}N_3O_4S$	61.89 (61.61	4.91 4.64	10.09 10.26)	2.32(s, 3H), 2.96(m, 1H), 3.12(m, 1H), 3.73(s, 3H), 4.95(m, 1H), 6.55(s, 1H), 6.67-7.81(m, 7H), 10.23(bs, 1H), 10.42(bs, 1H)	409
5c	3-CH_3	218	67	5	$C_{21}H_{19}N_3O_4S$	61.92 (61.61	4.83 4.64	10.54 10.26)	2.31(s, 3H), 2.94(dd, 1H), 3.12(m, 1H), 3.73(s, 3H), 4.96(m, 1H), 6.56(s, 1H), 6.64-7.83(m, 7H), 10.22(bs, 1H), 10.42(bs, 1H)	
5d	2-CH_3	200	65	40	$C_{21}H_{19}N_3O_4S$	61.86 (61.61	4.72 4.64	10.41 10.26)	2.32(s, 3H), 2.95(dd, 1H), 3.12(m, 1H), 3.74(s, 3H), 4.95(m, 1H), 6.56(s, 1H), 6.67-7.84(m, 7H), 10.23(bs, 1H), 10.43(bs, 1H)	
5e	4-F	182	77	15	$C_{20}H_{16}FN_3O_4S$	58.34 (58.11	4.02 3.87	10.43 10.16)	2.89(dd, 1H), 3.12(dd, 1H), 3.67(s, 3H), 5.17(m, 1H), 6.44(d, 2H), 6.61(s, 1H), 6.72(d, 2H), 6.81(d, 1H), 7.11(dd, 1H), 7.75(d, 1H), 10.25(bs, 1H), 10.42(bs, 1H)	413

Table-1 (Continued) : Characterization data of compounds **3, 4 & 5**

Compd*	R	m.p. °C	Yield (%)	IC_{50} uM 5-LOX	Mol. formula	Found (Calc.) % C	H	N	^{1}H NMR (δ, ppm) $CDCl_3$	MS m/z [M^+]
5f	2-F	178	74	50	$C_{20}H_{16}FN_3O_4S$	58.42 (58.11	4.21 3.87	10.37 10.16)	2.93(dd, 1H), 3.09(m, 1H), 3.74(s, 3H), 4.93(m, 1H), 6.55(s, 1H), 6.64-7.75(m, 7H), 10.25(bs, 1H), 10.43(bs, 1H)	
5g	2,4-diF	184	78	40	$C_{20}H_{15}F_2N_3O_4S$	55.82 (55.68	3.71 3.48	9.68 9.44)	2.86(dd, 1H), 2.99(dd, 1H), 3.73(s, 3H), 4.94(m, 1H), 6.55(s, 1H), 6.67-7.92(m, 6H), 10.24(bs, 1H), 10.43(bs, 1H)	
5h	3-Cl,4-F	238	79	55	$C_{20}H_{15}ClFN_3O_4S$	53.78 (53.63	3.62 3.35	9.72 9.38)	2.88(dd, 1H), 2.98(dd, 1H), 3.74(s, 3H), 4.94(m, 1H), 6.55(s, 1H), 6.71-7.86(m, 6H), 10.21(bs, 1H), 10.43(bs, 1H)	
5i	4-Cl	190	82	-	$C_{20}H_{16}ClN_3O_4S$	56.12 (55.87	3.56 3.75	9.89 9.77)	2.89(dd, 1H), 3.12(dd, 1H), 3.71(s, 3H), 5.01(m, 1H), 6.44(d, 2H), 6.58(s, 1H), 6.72-7.81(m, 5H), 10.21(bs, 1H), 10.46(bs, 1H)	431
5j	4-OCH_3	168	79	-	$C_{21}H_{19}N_3O_5S$	59.47 (59.29	4.68 4.47	9.67 9.88)	2.87(dd, 1H), 3.11(dd, 1H), 3.71(s, 3H), 3.81(s, 3H), 5.02(m, 1H), 6.56(s, 1H), 6.42-7.75(m, 7H), 10.11(bs, 1H) 10.39(bs, 1H)	

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 on KBr pellets on a Perkin-Elmer 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 Hewelett Packard mass spectrometer operating at 70eV.

Preparation of Methyl [6-chloroacetyl-3-oxo-1,4-benzoxazin-2-yl]acetate 2:

Chloroacetylchloride (12.43 gm, 0.11 mol) in anhydrous dichloromethane (20 mL) was added to a stirred mixture of methyl [3-oxo-1,4-benzoxazin-2-yl]acetate[15] (**1**, 22.1 gm, 0.1 mol) and anhydrous aluminium chloride (26.7 gm, 0.02 mol) in DCM at 0-10°C and the stirring was continued at room temperature for 4 hrs. Solvent was removed *in vacuo* and the residue poured onto crushed ice. The separated solid was filtered washed with water, dried and recrystallized from ethylacetate to give **2** as crystalline solid (22.61 gm, 76%); m.p. 155°C; IR: 1676, 1714 & 1733 cm^{-1} (C=O & CO_2CH_3); ^{1}H NMR ($CDCl_3$): δ 2.90(m, 2H, CH_2CO_2), 3.61 (s, 3H, CO_2CH_3), 5.17(m, 3H, OCH & CH_2CO), 7.03(d, 1H, ArH), 7.47(m, 1H, ArH), 7.61(dd, 1H, ArH) and 10.21(bs, 1H, NH). (Found: C, 52.68; H, 4.17; N, 4.96; $C_{13}H_{12}ClNO_5$ requires C, 52.43; H, 4.03; N, 4.70%).

Preparation of Methyl [6-(2-aryl/pyridylamino-1,3-thiazol-4-yl)-3-oxo-1,4-benzoxazin-2-yl]acetates 3, 4 & 5: General procedure:

A mixture of **2** (0.01 mol) and thiourea/pyridylamino thiourea/arylamino thiourea (0.01 mol) in methanol (50 mL) was refluxed for 4-5 hrs. At the end of the reaction as monitored by TLC, solvent was removed *in vauo*, the residue was treated with $NaHCO_3$ solution to neutral pH. The separated solid was filtered washed with water, dried and recrystallized from ethylacetate to give pure **3**, **4** & **5** as crystalline solids.

The physical and spectral data of **3**, **4** & **5** are listed in **Table–1**.

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