

Note

Synthesis of methyl-5-[2-arylamino-4-(3-oxo-1,4-benzoxazin-6-yl)thiazole acetates and 7*H*-[3-aryl-6-(3-oxo-1,4-benzoxazin-6-yl)-*s*-triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl]acetates as possible COX-2-inhibitors

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A series of methyl 5-[2-arylamino-4-(3-oxo-[1,4]benzoxazin-6-yl)thiazoleacetates **5a-h** and 7*H*-[3-aryl-6-(3-oxo-[1,4]benzoxazin-6-yl)-*s*-triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl]acetates **6a-e** have been synthesized and tested for their COX-2 inhibitor activity.

Keywords: Benzoxazin, *v*-bromobenzoxazinyl butanoate, thiazolyl-benzoxazinyl acetates, triazolothiadiazinyl benzoxazinyl acetate, COX-2 inhibitors.

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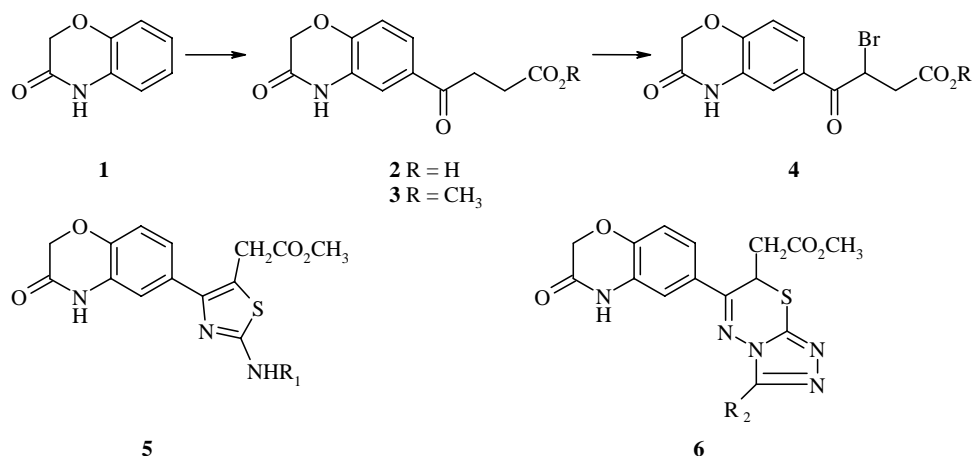
Thiazole ring system plays an important role in a number of heterocycles with wide spectrum of biological activity such as antibacterial, antifungal and antiviral¹. Thiazole containing natural products are known for their antibiotic and antifungal activities². A number of marine products with thiazole ring exhibit antineoplastic and cytotoxic activities³. Thiazole acetic acids like Fentiazac and Fenclozic acid are known for their anti-inflammatory activities⁴. Several *s*-triazolothiadiazines have found potential application as antibacterial, antifungal and anti-inflammatory agents⁵. Furthermore, 3-oxo-1,4-benzoxazines substituted with a heterocycle in the aromatic ring have shown excellent pharmacological profiles and are found useful in the treatment of congestive heartfailure⁶, breast cancer⁷, CNS diseases⁸ and as inhibitors of transforming growth factors⁹. In

view of this and in continuation of our work on benzoxazines¹⁰, we report herein the synthesis of benzoxazinylthiazolyl acetates and triazolothiadiazinylacetates as possible COX-2 inhibitors.

Fridel-Crafts acylation of 3-oxo-3,4-dihydro-2*H*-[1,4]-benzoxazine **1** with succinic anhydride and aluminium chloride according to the reported procedure⁶ gave the oxobutyric acid **2**. Esterification of **2** followed by bromination in carbon tetrachloride gave the α -bromoketoester **4** in excellent yields. ¹H NMR spectra of **4** exhibited three sets of double doublets at δ 3.06, 3.42 and 5.46 for CH₂-CH-Br protons and two singlets at δ 3.67 and 4.64 for methyl ester and -OCH₂ protons of benzoxazine ring. The lactam NH of benzoxazine ring appeared as a broad singlet at δ 10.73. Reactions of **4** with various substituted thioureas and 3-mercapto-4-amino-*s*-triazoles in refluxing methanol gave the corresponding benzoxazinylthiazolylacetates **5** and benzoxazinyltriazolo-thiadiazinyl acetates **6** respectively in good yields (**Scheme I**). The formation of thiazolyl **5** and triazolothiadiazinyl benzoxazines **6** is evident from the disappearance of three sets of double doublets for -CO-CHBr-CH₂ system present in **4** and appearance of three singlets around δ 3.67, 3.78 and 4.60 for CH₂CO₂CH₃, CO₂CH₃ and -OCH₂ of benzoxazine ring protons in **5** whereas triazolothiadiazinylbenzoxazines **6** were characterized by the presence of signals around δ 2.8 (m, CH₂CO₂CH₃), 3.6 (s, CO₂CH₃), 4.8 (dd, -SCH) apart from benzoxazine ring protons around 4.6 (s, -OCH₂) and 10.2 (bs, NH). All the compounds reported in **Table I** were based on IR, ¹H NMR, mass spectra and correct elemental analysis.

COX-2 Inhibitor activity

All the compounds reported in **Table I** were tested for cyclooxygenase-2 inhibitory activity. The method of Copeland *et al.*¹¹ was followed to determine the IC₅₀ values. The enzyme activity was measured using chromogenic assay based on oxidation of N,N,N',N'-tetramethylparaphenylenediamine (TMPD) during the reduction of prostaglandin G₂ to prostaglandin H₂ 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



Scheme I

Table I — Characterization data of compounds **5a-h** and **6a-f**

Compd*	R ₁	m.p. °C	Yield (%)	Mol. formula	Found (Calcd) %			¹ H NMR (δ, ppm) CDCl ₃ +DMSO- <i>d</i> ₆	MS m/z [M ⁺]
					C	H	N		
5a	H	193	69	C ₁₄ H ₁₃ N ₃ O ₄ S	52.78 (52.66)	4.26 4.07	13.41 13.16)	3.69(s, 2H), 3.78(s, 3H), 4.56(s, 2H), 7.06(m, 3H), 8.65(bs, 2H), 10.67(bs, 1H)	319
5b	C ₆ H ₅	179	74	C ₂₀ H ₁₇ N ₃ O ₄ S	61.03 (60.75)	4.32 4.03	10.86 10.63)	3.64(s, 2H), 3.76(s, 3H), 4.62(s, 2H), 7.14-7.63(m, 8H), 10.18(bs, 1H), 10.76(bs, 1H)	395
5c	4-CH ₃ C ₆ H ₄	157	72	C ₂₁ H ₁₉ N ₃ O ₄ S	61.83 (61.61)	4.79 4.64	10.43 10.26)	2.32(s, 3H), 3.67(s, 2H), 3.72(s, 3H), 4.57(s, 2H), 6.78-7.17(m, 7H), 9.37(bs, 1H), 9.78(bs, 1H)	409
5d	4-CH ₃ OC ₆ H ₄	173	71	C ₂₁ H ₁₉ N ₃ O ₅ S	59.26 (59.01)	4.62 4.44	10.21 9.83)	3.65(s, 2H), 3.71(s, 3H), 3.80(s, 3H), 4.59(s, 2H), 6.81-7.19(m, 7H), 9.41(bs, 1H), 9.81(bs, 1H)	425
5e	4-ClC ₆ H ₄	183	76	C ₂₀ H ₁₆ ClN ₃ O ₄ S	56.13 (55.87)	4.06 3.72	9.53 9.77)	3.65(s, 2H), 3.79(s, 3H), 4.61(s, 2H), 7.16(m, 5H), 7.63(m, 2H), 10.14(bs, 1H), 10.78(bs, 1H)	429
5f	4-FC ₆ H ₄	181	77	C ₂₀ H ₁₆ FN ₃ O ₄ S	58.42 (58.11)	4.21 3.87	10.43 10.16)	3.66(s, 3H), 3.82(s, 2H), 4.60(s, 2H), 7.12(m, 5H), 7.65(m, 2H), 10.16(bs, 1H), 10.79(bs, 1H)	413
5g	2,4-diFC ₆ H ₃	237	78	C ₂₀ H ₁₅ F ₂ N ₃ O ₄ S	55.82 (55.68)	3.64 3.48	10.12 9.74)	3.67(s, 2H), 3.76(s, 3H), 4.62(s, 2H), 7.15-7.62(m, 6H), 10.17(bs, 1H), 10.78(bs, 1H)	431
5h	3-Cl,4-FC ₆ H ₃	197	76	C ₂₀ H ₁₅ ClFN ₃ O ₄ S	53.89 (53.63)	3.62 3.35	9.74 9.38)	3.66(s, 2H), 3.78(s, 3H), 4.61(s, 2H), 7.14-7.63(m, 6H), 10.16(bs, 1H), 10.76(bs, 1H)	447
6a	C ₆ H ₅	175	72	C ₂₁ H ₁₇ N ₅ O ₄ S	58.21 (57.93)	4.19 3.90	16.26 16.09)	2.67(m, 2H), 3.62(s, 3H), 4.63(s, 2H), 4.84(dd, 1H), 7.43-7.68(m, 8H), 10.54(bs, 1H)	434
6b	4-CH ₃ C ₆ H ₄	183	74	C ₂₂ H ₁₉ N ₅ O ₄ S	58.92 (58.79)	4.56 4.23	15.72 15.59)	2.34(s, 3H), 2.66(m, 2H), 3.64(s, 3H), 4.62(s, 2H), 4.82(dd, 1H), 7.44-7.71(m, 7H), 10.67(bs, 1H)	448
6c	4-ClC ₆ H ₄	199	76	C ₂₁ H ₁₆ ClN ₅ O ₄ S	53.78 (53.67)	3.46 3.40	15.21 14.90)	2.68(m, 2H), 3.68(s, 3H), 4.60(s, 2H), 4.81(dd, 1H), 7.38-7.67(m, 7H), 10.68(bs, 1H)	468

Contd

Table I — Characterization data of compounds **5a-h** & **6a-f** — *Contd*

Compd*	R ₁	m.p. °C	Yield (%)	Mol. formula	Found (Calcd) %			¹ H NMR (δ, ppm) CDCl ₃ +DMSO- <i>d</i> ₆	MS m/z [M ⁺]
					C	H	N		
6d	4-FC ₆ H ₄	195	77	C ₂₁ H ₁₆ FN ₅ O ₄ S	55.84 (55.62)	3.76 3.53	15.78 15.45	2.66(m, 2H), 3.67(s, 3H), 4.54(s, 2H), 4.84(dd, 1H), 7.41-7.69(m, 7H), 10.63(bs, 1H)	452
6e	2,4-diClC ₆ H ₃	233	75	C ₂₁ H ₁₅ Cl ₂ N ₅ O ₄ S	50.26 (50.00)	3.26 2.97	13.61 13.88	2.67(m, 2H), 3.66(s, 3H), 4.61(s, 2H), 4.84(dd, 1H), 7.45-7.67(m, 6H), 10.69(bs, 1H)	503
6f	4-C ₅ H ₅ N	247	68	C ₂₀ H ₁₆ N ₆ O ₄ S	55.37 (55.09)	3.93 3.66	19.41 19.26	2.61(m, 2H), 3.65(s, 3H), 4.64(s, 2H), 4.83(dd, 1H), 7.42-7.91(m, 7H), 10.71(bs, 1H)	435

SF₉ 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 (pH 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 250°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. IC₅₀ values are calculated from four parameter least squares nonlinear regression analysis of the log dose versus percentage inhibition plot. However, none of the compounds reported herein exhibited significant inhibitions up to 100 μM except **5a** which exhibited a low order of inhibitions (31%) when compared to Celecoxib with 98% inhibition at 5 μM.

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 system 2000 FT IR spectrometer. ¹H NMR spectra on a Varian 200 MHz instrument with TMS as internal standard and chemical shifts expressed in δ ppm and mass spectra on Hewlett Packard mass spectrometer operating at 70 eV.

Methyl-[v-bromo-β-oxo-(2H - 3-oxo-1,4-benzoxazin-6-yl)]butanoate 4. To a mixture of methyl-β-oxo-(2H-3-oxo-1,4-benzoxazin-6-yl)butanoate (**3**, 2.61 g, 0.01 mole) in dichloromethane (200 mL) was added bromine (1.8 moles) in dichloromethane (15 mL) at room temperature. The mixture was refluxed for 3-4 hr. At the end of the reaction as monitored by TLC it was poured onto ice water. The organic layer was separated, washed with water (2 × 50 mL), dried

(Na₂SO₄) and solvent removed to get a solid. It was recrystallized from dichloromethane to give pure **4** as light yellow crystalline solid. M.p. 189°C, yield 85%. IR (KBr): 1720, 1690 cm⁻¹; ¹H NMR (CDCl₃+DMSO-*d*₆): δ 3.06 (dd, 1H), 3.42 (dd, 1H), 3.67 s, 3H), 4.64 (s, 2H), 5.46 (dd, 1H), 7.01 (d, 1H), 7.32 (m, 2H), 10.73 (bs, 1H); MS m/z [M⁺]: 341 (Found: C, 45.83; H, 3.76; N, 4.21, C₁₃H₁₂BrNO₅ requires C, 45.62; H, 3.51; N, 4.09%).

General procedure for the preparation of methyl- 5-[2 - arylamino-4-(3 - oxo-1,4-benzoxazin-6-yl)thiazole acetates 5. A mixture of **4** (0.01 mole) and arylamino thiourea in methanol (50 mL) was refluxed for 4-5 hr. At the end of the reaction as monitored by TLC, solvent was removed *in vacuo*, the residue was treated with NaHCO₃ solution to neutral pH. The separated solid was filtered and recrystallized with methanol to give pure **5** as crystalline solids.

Compounds **5a-h** were prepared similarly and their characterization data are listed in **Table I**.

General procedure for the preparation of methyl-[7H-3-aryl-6-(3-oxo-1,4-benzoxazin-6-yl)-s-triazolo[3,4-b][1,3,4]thiadiazin-7-yl]acetates 6. A mixture of **4** (0.01 mole) and 3-mercapto-4-amino-5-aryl-s-triazole (0.01 mole) in methanol (50 mL) was refluxed for 4-5 hr. At the end of the reaction as monitored by TLC, solvent was removed *in vacuo*, the residue was treated with NaHCO₃ solution to neutral pH. The separated solid was filtered and recrystallized from methanol to give pure **6** as crystalline solids.

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

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