Indian Journal of Chemistry
Vol. 46B, June 2007, pp. 1033-1037

Note

Synthesis of 2-aminophenyl-5-phenyl-4-[3-oxo-1,4-benzoxazin-6-yl] thiazoles as potential COX-2 inhibitors

T Venkateshwar Kumar, K Srinivasa Rao & P K Dubey*

Department of Chemistry, College of Engineering
J.N.T.University, Hyderabad 500 072, India

and

K Anil Kumar & P Reddanna*

School of Life Sciences, University of Hyderabad
Hyderabad 500 046, India

Email: venkatkumar76@rediffmail.com

Received 6 October 2005; accepted (revised) 16 January 2007

A series of 2-aminophenyl-5-phenyl-4-[3-oxo-1,4-benzoxazin-6-yl]thiazoles **5a-n** have been prepared and evaluated for their COX-2 inhibition activity.

Keywords: COX-2 inihibitors, inflammation, 2H-1,4-benzoxazin-3(4H)-one, aminothiazoles, phenylthioureas.

A number of benzoxazinones with a variety of substituted heterocycles like imidazolylamino[1], imidazolylmethyl[2], tetrahydrophthalimido[3] and pyridazines[4] have been shown to exhibit a wide range of biological activities such as anti-fungal, α-adrenoceptor, anti-inflammatory and action on congestive heart failure activities. Furthermore, 2-aminothiazole[5] forms an important ring system that is present in a number of synthetic medicinal agents and natural products. In view of the recent trend of evaluating vicinal diaryl heterocycles[6] as COX-2 inhibitors, it was considered of interest to synthesize some new 2-aminothiazole derivatives with a phenyl and active pharmacophore benzoxazinone in the vicinal position.

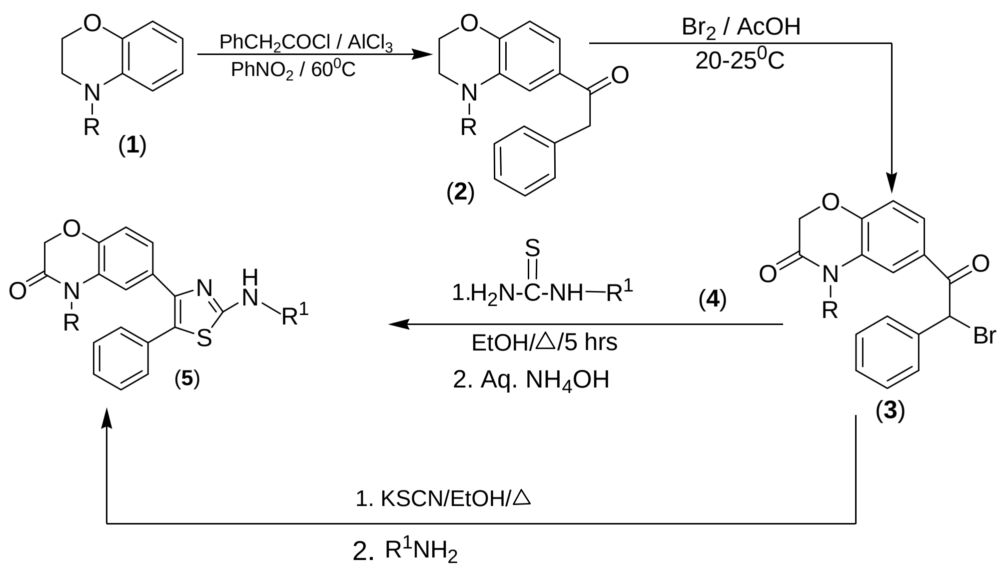
Results and Discussion

Thus, 3-oxo-1,4-benzoxazine (**1**, R=H, CH_3) (ref.7) was reacted with phenylacetyl chloride under Friedel-Crafts conditions to give the ketone **2** (R=H, CH_3) in good yields. The latter on treatment with Br_2/AcOH at RT gave the corresponding α-bromoderivatives **3**. These α-bromoketones were subjected to Hantzsch reaction with various phenyl thioureas **4** (ref.8) in refluxing ethanol to give the desired diaryl heterocycles **5** in good yields (**Scheme I**). Compounds **5** were characterized by IR, ^{1}H NMR and mass spectra (**Table I**). Thus, **5** exhibited in their ^{1}H NMR (DMSO-d_6/TMS), characteristic absorption for benzoxazinone protons as singlet around at δ 4.40(2H,CH_2) and absence of the signal due to –COCHBr proton of **3** apart from aromatic protons. All the compounds reported in **Table I** showed satisfactory elemental analysis. Compounds **5** could also be prepared from **3** by treatment with potassium thiocyanate in refluxing ethanol followed by condensation of the intermediary formed thiocyanate derivatives *in situ* with aromatic amines. All the above reactions are shown in the **Scheme I**.

Biological activity

The compounds prepared were tested for cyclooxygenase–1 and cyclooxygenase–2 inhibitory activity. The method of Copeland[11] *et al*. was followed to determine the IC_{50} values. The enzyme activity was measured using chromogenic assay based on oxidation of N,N,N′,N′-tetramethyl-*p*-phenylene-diamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX–1 and COX–2 enzymes. COX–1 enzyme from Ram seminal vesicles (microsomal fraction) and COX–2 from 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-HCl buffer (*p*H 8.0, 100 m*M*), hematin (15 μ*M*), EDTA (3 μ*M*), enzyme (COX-1 or COX-2, 100 μg) 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 (100 μ*M*) and TMPD (120 μ*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 s 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 *vs* percentage inhibition plot. The compounds studied here showed little inhibition at 100 μ*M* concentration, but this inhibition was not dose dependent when compared to standard inhibitors Indomethacin (for COX-1) and Celecoxib (for COX-2).



Scheme I

Experimental Section

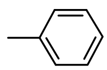
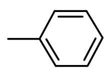
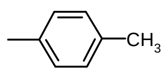
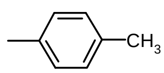
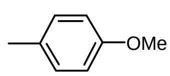
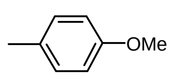
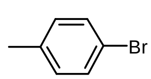
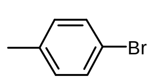
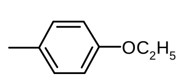
Melting points were determined using open capillary tubes on a Polmon Melting Point apparatus and are uncorrected. The IR spectra of all compounds were recorded on a Perkin-Elmer-FT-IR 240-c spectrometer with KBr optics. The ^{1}H NMR spectral data of all the compounds were recorded on Varian-Gemini-200 MHz spectrometer in DMSO-d_6 using TMS as an internal standard. The mass spectra of these compounds were recorded on a Shimadzu QP 5050A spectrometer operating at 70eV.

Preparation of 2a(R=H): To a suspension of 2H-1,4-benzoxazin-3(4H)-one **1a** (R=H, 10.0 g, 67 mmoles) in nitrobenzene (60 mL) was added phenyl-acetyl chloride (11.40 g, 73 mmoles) dropwise at 25-30°C. Anhydrous aluminium chloride (22.3 g, 167 mmoles) was added in small lots during 45 min, with 15 min intervals for each lot at 0-5°C. The reaction mixture was stirred at 5-10°C for 1 hr and then at room temperature for 2 hr. Then, it was stirred at 60-65°C for 5 hr. After completion of the reaction, as monitored by TLC, it was poured into ice water (300 g) containing 15 mL of hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with ethylenedichloride (2 × 100 mL). The combined organic layers were distilled under reduced pressure. To the crude residue, methanol (100 mL) was added and the mixture filtered to obtain the separated solid. The crude product was recrystallized from methanol to give pure product **2a** (R=H). Yield: 8.0g (44.4%). m.p. 208-210°C. IR (KBr): 3057, 2888, 1687, 1600 cm^{-1}. ^{1}H NMR: (DMSO-d_6): δ 4.20 (s, 2H, CH_2); 4.60 (s, 2H, CH_2); 6.80-7.00 (m, 1H, aryl); 7.20–7.40 (m, 5H, aryl); 7.50–7.70 (m, 2H, aryl); 10.90 (br s, 1H, NH).

Preparation of 2b(R=CH_3): To a solution of **1b** (R=CH_3, 10.0 g, 61 mmoles) in dichloromethane (100 mL) was added phenylacetyl chloride (10.4 g, 67 mmoles) dropwise at 10-12°C. Anhydrous aluminium chloride (16.3 g, 122 mmoles) was added in small lots during a 40 min period at 0-5°C. The reaction mixture was stirred at 0-5°C for further 1.5 hr and then at room temperature for 3 hr. After completion of reaction, as monitored by TLC, the reaction mixture was poured into ice water (300 mL) containing hydrochloric acid (15 mL). The organic layer was separated and washed first with saturated sodium bicarbonate solution (50 mL), and then with saturated brine solution (100 mL). The organic layer was dried over anh. sodium sulphate and the solvent removed *in vacuo* to obtain a residue. Methanol (50 mL) was added to this residue, the suspended solid was filtered and washed with methanol (20 mL). The crude product was purified by column chromatography (silica gel 100-200 mesh: hexane:ethyl acetate 80:20) to get pure **2b** (R=CH_3). Yield: 8.2 g (47.5%). m.p. 123-125°C. IR (KBr): 2905, 1681, 1606 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 3H, CH_3); 4.20 (s, 2H, CH_2); 4.60 (s, 2H, CH_2); 6.80–7.00 (m, 1H, aryl); 7.20 (m, 5H, aryl); 7.60 (m, 2H, aryl).

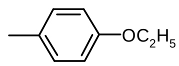
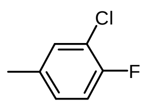
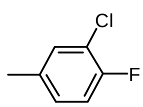
Preparation of 3a: To a suspension of **2a** (R=H, 5.0 g, 18.7 mmoles) in acetic acid (30 mL), bromine (3.0 g, 18.7 mmoles) was added slowly at 20-25°C during 30 min. The reaction mixture was stirred

Table I — Spectral data for compound **5**

Compd	IR (KBr) cm^{-1}	Yield (%)	R	R[1]	m.p. (°C)	^1H NMR (δ ppm)	Mass (% abundance)	% Inhibition COX-1	% Inhibition COX-2
5a	3310 1668 1558	65.2	H		268-70	4.50(s, 2H, OCH_2CO); 6.60-6.70(m, 1H, aryl); 6.80-6.90(m, 2H, aryl); 7.10-7.20(m, 8H, aryl); 7.45-7.55(m, 2H, aryl); 9.70(bs, 1H, NH); 10.40 (bs, 1H, NH).	399(100), 121(18), 249(12), 77(76), 210(14), 175(9), 42(43), 150(12),	19.87	72.96
5b	3278 1650 1605	68.5	CH_3		209-10	3.40(s, 3H, CH_3); 4.50(s, 2H, OCH_2CO); 6.80-7.00(m, 3H, aryl); 7.20-7.40(m, 8H, aryl); 7.70-7.80(m, 2H, aryl); 9.80 (bs, 1H, NH).	413(100), 342(3), 295(4), 263(9), 189(9), 150(7), 121(11), 77(36), 43(25)	14.50	61.53
5c	3294 1669 1559	59.5	H		224-25	2.30(s, 3H, CH_3); 4.50(s, 2H, OCH_2CO); 6.70-7.00(m, 2H, aryl); 7.00-7.20(m, 2H, aryl); 7.20-7.40(m, 6H, aryl); 7.40-7.50(m, 2H, aryl); 9.90(bs, 1H, NH); 10.70(bs, 1H, NH).	—	31.23	56.17
5d	2841 1690 1584	72.1	CH_3		214-15	2.30(s, 3H, CH_3); 3.40(s, 3H, CH_3); 4.50 (s, 2H, OCH_2CO); 6.60-6.70 (m, 2H, aryl); 6.85-7.00(m, 2H, aryl); 7.10-7.30(m, 6H, aryl); 7.40-7.50 (m, 2H, aryl); 9.80 (bs, 1H, NH).	—	27.58	47.31
5e	3312 1640 1608	69.2	H		242-45	3.80(s, 3H, OCH_3); 4.50(s, 2H, OCH_2CO); 6.65-6.70 (d, 2H, aryl); 6.70-6.90(m, 3H, aryl); 7.20-7.30(bs, 5H, aryl); 7.40-7.50 (m, 2H, aryl); 9.70(bs, 1H, NH); 10.60 (bs, 1H, NH).	429(100), 414(22), 266(3), 215(9), 193(11), 121(15), 77(13), 44(25)	50.10	—
5f	2841 1688 1574	70.2	CH_3		195-98	3.40(s, 3H, CH_3); 3.80(s, 3H, OCH_3); 4.50 (s, 2H, OCH_2CO); 6.70-6.80(m, 5H, aryl); 7.00-7.20(m, 5H, aryl); 7.40-7.50(m, 2H, aryl); 9. 70 (bs, 1H, NH).	—	53.54	2.68
5g	3288 1666 1603	5806	H		258-60	4.50(s, 2H, OCH_2CO); 6.60-6.70(m, 2H, aryl); 6.80-6.90(m, 2H, aryl); 7.20-7.40(m, 6H, aryl); 7.60-7.70(m, 2H, aryl); 10.10(bs, 1H, NH); 10.60 (bs, 1H, NH).		24.44	33.56
5h	2707 1690 1602	59.0	CH_3		262-64	3.40(s, 3H, CH_3); 4.50(s, 2H, OCH_2CO); 6.65-6.75 (m, 2H, aryl); 6.85-6.95(m, 2H, aryl); 7.20-7.45 (m, 6H, aryl); 7.60-7.70(m, 2H, aryl); 9.80 (bs, 1H, NH).	—	—	—
5i	3302 1683 1602	61.2	H		242-45	1.40(t, 3H, CH_3); 4.00(q, 2H, CH_2); 4.50 (s, 2H, OCH_2CO); 6.70-7.00(m, 5H, aryl); 7.20-7.30(m, 5H, aryl); 7.45-7.55(m, 2H, aryl); 9.50(bs, 1H, NH); 10.50 (bs, 1H, NH).	—	19.06	68.99

— *Contd*

Table I — Spectral data for compound **5** — *Contd*

Compd	IR (KBr) cm^{-1}	Yield (%)	R	R^1	m.p. (°C)	^{1}H NMR (δ ppm)	Mass (% abundance)	% Inhibition COX-1	COX-2
5j	2820 1679 1564	62.3	CH_3		215-18	1.40(t, 3H, CH_3); 3.40(s, 3H, CH_3); 4.10 (q, 2H, CH_2); 4.50(s, 2H, OCH_2CO); 6.60-6.70(m, 2H, aryl); 6.70-6.85(m, 3H, aryl); 7.20-7.30(m, 5H, aryl); 7.40-7.50(m, 2H, aryl); 9.60 (bs, 1H, NH).	457(100), 428(42), 295(4), 261(5), 229(6), 165(9), 121(6), 44(8)	25.86	51.74
5k	3402 1690 1610	65.2	H		274-75	4.50(s, 2H, OCH_2CO); 6.75-6.80 (m, 1H, aryl); 6.85-7.00(m, 1H, aryl); 7.00-7.20(m, 1H, aryl); 7.20(s, 1H, aryl); 7.25-7.32(m, 5H, aryl); 7.65-7.75(m, 1H, aryl); 7.75-7.85(m, 1H, aryl); 10.10 (bs, 1H, NH). 10.60 (bs, 1H, NH).	451(100), 417(20), 380(5), 281(4), 249(7), 198(8), 176(9), 44(29)	19.77	57.57
5l	2912 1665 1604	68.6	CH_3		269-71	3.60(s, 3H, CH_3); 4.50(s, 2H, OCH_2CO); 6.80-7.90 (m, 2H, aryl); 7.00-7.10(m, 1H, aryl); 7.12-7.20(m, 1H, aryl); 7.22-7.30(m, 5H, aryl); 7.35-7.45(m, 2H, aryl); 9.90 (bs, 1H, NH).	—	43.20	63.86

at 25-30°C for 1 hr. After completion of the reaction (as monitored by TLC), the mixture was poured into cold water. The suspended solid was filtered, washed with water (25 mL) and recrystallised from methanol to give pure **3a (R=H)**. Yield: 5.0 g, (77%) m.p. 134-136°C. IR (KBr): 3060, 2888, 1685, 1601 cm^{-1}. ^{1}H NMR: (DMSO-d_6) δ 4.50 (s, 2H, CH_2); 6.70 (s, 1H, CH); 6.80 (m, 1H, aryl); 7.30 (m, 5H, aryl); 7.50–7.70 (m, 2H, aryl); 10.90 (br s, 1H, NH).

Preparation of 3b(R=CH_3): The above procedure was followed here. The crude product was purified by column chromatography (silica gel 100-200 mesh: chloroform : methanol, 100:1) to obtain **3b** (R=CH_3) as viscous oil (78%). IR (KBr): 2890, 1679, 1607 cm^{-1}; ^{1}H NMR: (DMSO-d_6): δ 3.30 (s, 3H, CH_3); 4.60 (s, 2H, CH_2); 6.20 (s, 1H, CH); 6.90–7.00 (m, 1H, aryl); 7.20–7.70 (m, 7H, aryl).

General Procedure for the Preparation of 5 (Method A): A mixture of **3** (2.8 mmoles) and phenyl thiourea **4** (2.8 mmoles) in ethanol (50 mL) was refluxed for 4-5 hr. At the end of the reaction (as monitored by TLC), the solvent was removed *in vacuum* and the crude product was basified with aq.NH_4OH solution. The separated solid was filtered, washed with water and dried. The crude product was recrystallised from ethanol to give pure **5** (**Table I**).

Method B: A mixture of **3** (2.8 mmoles) and potassium thiocyanate (2.8 mmoles) in ethanol was refluxed for 2-3 hr. Aniline (2.8 mmoles) was added to the reaction mixture after cooling to room temperature and it was refluxed again for 3-4 hr. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature. The precipitated solid was filtered, washed with water followed by ethanol and recrystallised from alcohol to give pure compound **5**.

Acknowledgement

One of the authors (TVK) is grateful to the Managing Director, M/s. Venkar Chemicals (P) Ltd., Hyderabad for providing facilities to carry out the research work. One of the authors (PKD) is indebted to UGC, New Delhi, for financial support.

References

1 Christopher B C, Richard C M B, David C E, Peter L M, Alan G R, Colin F C S, Michael R S & Ian F T, *J Med Chem,* 32, **1989**,1627.
2 Reddy Sastry C V, Srinivasa Rao K, Rastogi K, Jain M L & Reddi G S, *Indian J Chem,* 28B, **1989**, 1096.
3 Hoga T, Nagano H, Sato M & Morita K, *Japan Pat*, 6251685, **1987**; *Chem Abstr,* 108, **1987**, 56115g.
4 a) Donald W C, Marianne S R, Stanley C B, Dieter H K, Alfonso J T, Robert F, Barbara H, Constance L W & John B M, *J Med Chem,* 33, **1990**, 380.
b) Reddy Sastry C V, Srinivasa Rao K, Krishnan V S H, Rastogi K, Jain M L, Verma R K, Tripathi R M, Singh P P, Seshagiri Rao C & Kanshal R, *Indian J Chem,* 28B, **1989**, 882.

5 Alesandro D D & Pedro M, *Comprehensive Heterocyclic Chemistry,* 3, **1982**, 373.

6 a) Talley J J, Bertenshaw S R, Brown D L, Carter J, Graneto M J, Kellogg M S, Koboldt C M, Yuan J, Zhang Y Y & Seibort K, *J Med Chem,* 43, **2000**,1661.
b) Talley J J, Brown D L, Carter J S, Graneto M J, Koboldt C M, Masferrer J L, Perkins W E, Rogers R S, Shaffer A F, Zhang Y Y, Zweifel B S & Seibert K, *J Med Chem,* 43, **2000**, 775.

7 Venkateshwar Kumar T, Srinivasa Rao K, Laxminarayana V, Aparna V & Dubey P K, *Heterocycl. Commun*, 9, **2003**, 51.

8 Frank R L & Smith P V, *Org Syn Coll Vol. III*, **1955**, 735.

9 Copeland R A, Williams J M, Giannars J, Nurnberg S, Covington M, Pinto D, Pick S & Trzaskos J M, *Proc Natl Acad Sci,* USA, 91, **1994**, 11202.