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Synthesis of [2-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazin-6- carbonyl)-1*H*-indol-3-yl]acetic acids as potential COX-2 inhibitors

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The synthesis of potential COX-2 inhibitors by means of internal Michael addition of ortho toluenesulfonylaminophenyl acrylic acid methyl esters **5a-d** with 6-(chloroacetyl)-2*H*-1,4-bezoxazin-4*H*-ones **6a-c** followed by hydrolysis resulting in the formation of [2-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazin-6-carbonyl)-1*H*-indol-3-yl]acetic acids **1a-k** are reported.

Keywords: COX-2 inihibitors, inflammation, acrylic acid methylester, indole acetic acid, benzoxazinone
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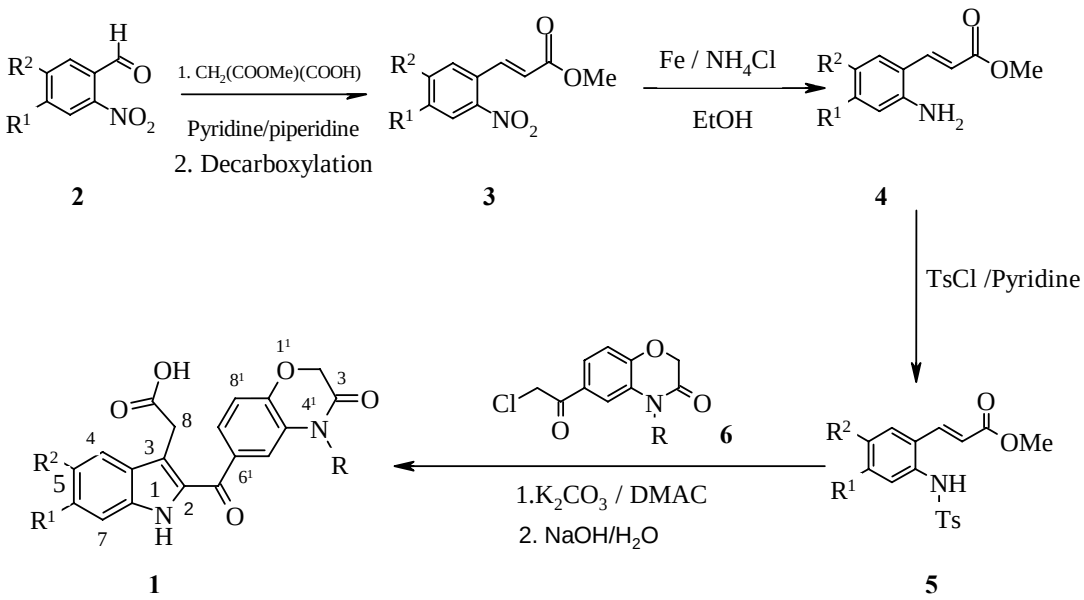
Treatment of inflammation with steroids (i.e., glucocorticoids) is associated with severe side effects leading, at times, to heart, liver and kidney damages[1]. Presently, nonsteroidal anti-inflammatory drugs (NSAIDS) are the preferred agents for the treatment of pain and inflammation, particularly arthritis. Currently available NSAIDS have been characterized as dual COX-1 and COX-2 inhibitors[2]. Even these NSAIDS are found to have some side-effects, due to their inhibitory activity against COX-1[3]. Celecoxib[4], Rofecoxib[5], Valdecoxib[6] and Etoricoxib[7] have been some of the selective COX-2 inhibitors available in the market for the treatment of pain due to Osteoarthritis and rheumatoid arthritis. Some 1,4-benzoxazinones containing dihydrofuranone moiety were synthesized in our laboratory and tested for selective COX-2 inhibitory activity and the results showed marginal activity[8]. Recently, 6-chloro-2-(4′-chlorobenzoyl)-1*H*-indol-3-ylacetic acid was identified as a selective COX-2 inhibitor for the potential treatment of pain and inflammation[9]. In view of these data, it was considered worthwhile to synthesize new chemical entities containing 1,4-benzoxazinone and indolacetic acid moieties **1** and test their COX-2 inhibitory activities. The results of these studies are presented in this paper.

Results and Discussion

The synthesis of target molecule **1**, is outlined in **Scheme I**. Thus *o*-nitrobenzaldehydes **2** were transformed to *o*-tolylaminocinnamic acid esters **5** through the intermediacy of **3** and **4** using literature procedures[9]. The compound **5** on condensation with the benzoxazinone derivatives **6** gave the target molecules **1**. All the products **1a-1k** were obtained after suitable work-up and were purified by column chromatography. The structures of the all the derivatives of **1** have been established by their spectral and analytical data **Table I**. The benzoxazinone derivatives **6** required in the present work have been synthesized using reported procedure[10]. In the ^{1}H NMR spectra of **1**, the C-8 protons of indoleacetic acid moiety appeared as a singlet at δ 3.70 integrating for the two methylene protons. Bezoxazinone's C-2 methylene protons were observed as a singlet at δ 4.60. This signal is a common feature in the ^{1}H NMR of 2*H*-1,4-benzoxazin-3(4*H*)-ones. The aromatic protons of the indole moiety and benzoxazinone moiety appeared as a complex multiplet in the region δ 6.90–7.60. Two broad singlets appearing at δ 10.70 and 11.20 have been assigned to benzoxazinone –NH- and indole–NH-protons respectively based on precedences.

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 is measured using chromogenic assay based



Scheme I

on oxidation of N,N,N′,N′-tetramethyl-*p*-phenylenediamine (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 is 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 seconds 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. However, none of the compounds studied here exhibited significant inhibitory activity when compared to standard inhibitors indomethacin (for COX-1) and celecoxib (for COX-2).

Experimental Section

Melting points are uncorrected. The IR spectra were recorded on a Perkin-Elmer FT-IR 240-c spectrometer. The ^{1}H NMR spectra were recorded on Varian-Gemini 200 MHz spectrometer in DMSO-d_6 using TMS as an internal standard and mass spectra were recorded on a Shimadzu QP 5050A spectrometer.

General Procedure for the Preparation of 4. To a solution of **3** (ref. 12; 24 mmoles) in ethanol (50 mL) was added water (20 mL) and ammonium chloride (1.0 g) followed by iron powder (84 mmoles) at room temperature. The reaction mixture was heated to reflux for 2 hr. After completion of the reaction, as monitored by TLC, the mixture was cooled to 40°C and filtered to remove the inorganic materials present. From the clear filtrate, the solvent was distilled off completely under reduced pressure. To the resulting crude product, hexane (30 mL) was added, the mixture stirred for 30 min and the precipitated solid was filtered. yield = 75-85 (molar %).

4a, R^1=R^2=H, m.p. 60-62°C, **4b,** R^1=R^2= OMe, m.p. 123-24°C, **4c,** R^1= OMe, R^2=OEt, m.p. 122-25°C, **4d,** R^1= OEt, R^2= OMe, m.p. 128-30°C.

General Procedure for the Preparation of 5. To a solution of **4** (28 mmoles) in dry dichloromethane (50 mL), was added pyridine (2.5 mL) at room temp. To this was added 4-toluenesulfonyl chloride (29 mmoles) slowly at 25°C and stirred overnight at room temperature. The reaction mixture was quenched with 1*N* HCl (60 mL). From the resulting two layers, the top organic layer was separated and washed with 50 mL of water. The organic layer was dried over anhydrous sodium sulphate and the solvent was

Table I—Physical and spectral data of **1a-k**

Compd	R	R^1	R^2	Yield (%)	m.p. °C	^{1}H NMR (δ, ppm)	Mass (% of abundance)	% of Inhibition	
								COX-1	COX-2
1a	H	H	H	52.30	223–25	3.80(2H,s,C_8-H), 4.60(2H,s,C_2-H), 6.90-7.30(3H,m,aryl-H), 7.35–7.60 (4H,m,aryl-H), 10.70(1H,s,NH), 11.20(1H,s,NH)	350(2), 306(100), 289(16), 248(17), 220(7), 176(17), 157(12), 130(19), 77(10), 44(44)	27.20	12.69
1b	CH_3	H	H	51.20	210–12	3.30(3H,s,-N-CH_3), 3.70(2H,s,C_8-H), 4.60 (2H,s,C_2-H), 6.90–7.20(3H,m,aryl-H), 7.40–7.60(4H,m,aryl-H), 11.20(1H,s,NH)	364(7), 346(7), 320(100), 303(19), 262(19), 220(8), 190(10), 145(14), 118(14), 91(17), 44(51)	23.19	19.69
1c	C_2H_5	H	H	53.00	224–27	1.20(3H,t,-N-CH_2CH_3), 3.60(2H,s,C_8-H), 3.90(2H,q,-N-CH_2), 4.60(2H,s,C_2-H), 6.80-7.10(3H,m,aryl-H), 7.40(4H,m,aryl-H), 11.00(1H,s,NH)	—	39.49	—
1d	CH_3	OCH_3	OCH_3	52.63	226–27	3.30(3H,s,-N-CH_3), 3.70(2H,s,C_8-H), 3.80 (3H,s,-OCH_3), 3.85(3H,s,-OCH_3), 4.60 (2H,s,C_2-H), 6.80–7.10(3H,m,aryl-H), 7.40 (2H,d,aryl-H), 11.20(1H,s,NH)	424(8), 394(17), 380(100), 365(14), 280(1), 217(6), 202(8), 190(29), 174(10), 146(7), 118(2), 77(3), 44(17)	19.09	—
1e	C_2H_5	OCH_3	OCH_3	58.20	219–21	1.20(3H,t,-N-CH_2CH_3), 3.60(2H,s,C_8-H), 3.80(6H,s,$C_{5,6}$-OCH_3), 3.90(2H,q,-N-CH_2), 4.60(2H,s,C_2-H), 6.80-7.10(3H,m,aryl-H), 7.40(2H,d,aryl-H), 11.00(1H,s,NH)	438(2), 394(100), 379(11), 263(2), 217(7), 204(13), 176(13), 146(8), 77(4), 44(22)	30.95	2.40
1f	H	OC_2H_5	OCH_3	57.50	23–24	1.40(3H,t,-C_6-O-CH_2CH_3), 3.60(2H,s,C_8-H), 3.80(3H,s,-OCH_3), 4.00(2H,q,-C_6-O-CH_2CH_3), 4.60(2H,s,C_2-H), 6.80-7.00 (3H,m,aryl-H), 7.40(2H,d,aryl-H), 10.80(1H,s,NH), 11.00(1H,s,NH)	424(1), 380(100), 365(2), 351(40), 337(5), 291(3), 188(8), 176(32), 148(9), 77(4), 44(92)	27.02	20.13

Contd.

Table I — Physical and spectral data of **1a-k** — *Contd.*

Compd	R	R^1	R^2	Yield (%)	m.p. °C	^{1}H NMR (δ, ppm)	Mass (% of abundance)	% of Inhibition	
								COX – 1	COX - 2
1g	CH_3	OC_2H_5	OCH_3	58.62	224–26	1.40(3H,t,-C_6-O-CH_2CH_3), 3.30(3H,s,-N-CH_3), 3.60(2H,s,C_8-H), 3.80 (3H,s,OCH_3), 3.90 (2H,q,-C_6-O-CH_2CH_3), 4.60(2H,s,C_2-H), 6.80-7.10(3H,m,aryl-H), 7.40(2H,d,aryl-H), 11.00(1H,s,NH)	422(2), 408(7), 394(100), 380(12), 365(31), 280(1), 202(13), 190(21), 120(2), 77(5), 44(39)	31.64	10.72
1h	C_2H_5	OC_2H_5	OCH_3	58.53	223–25	1.20(3H,t,-N-CH_2CH_3), 1.40(3H,t,-C_6- O-CH_2CH_3), 3.60(2H,s,C_8-H), 3.80 (3H,s,OCH_3), 3.90–4.10(4H,m,C_6-O-CH_2CH_3&-N-CH_2), 4.60 (2H,s,C_2-H), 6.80-7.00(3H,m,aryl-H), 7.40 (2H,d,aryl-H), 10.80 (1H,s,NH)	452(2), 408(100), 379(30), 319(3), 204(22), 161(8), 131(4), 105(2), 77(3), 44(21)		
1i	H	OCH_3	OC_2H_5	54.58	238–40	1.40(3H,t,-C_5-O-CH_2CH_3), 3.60(2H,s,C_8-H), 3.80 (3H,s,OCH_3), 4.00(2H,q,-C_5-O-CH_2CH_3), 4.60 (2H,s,C_2-H), 6.80-7.00 (3H,m,aryl-H), 7.40(2H, d,aryl-H), 11.00(1H,s,NH), 11.80(1H,s,NH)	—	33.47	23.85
1j	CH_3	OCH_3	OC_2H_5	57.30	236–37	1.40(3H,t,-C_5-O-CH_2CH_3), 3.10(3H,s, -N-CH_3), 3.20(2H,s,C_8-H), 3.40 (3H,s,OCH_3), 3.60 (2H,q,-C_5-O-CH_2CH_3), 4.60(2H,s,C_2-H), 6.80-7.00 (3H,m,aryl-H), 7.40(2H,d,aryl-H), 11.80 (1H,s,NH)	—	26.67	—
1k	C_2H_5	OCH_3	OC_2H_5	60.91	226–28	1.20(3H,t,-N-CH_2CH_3), 1.40(3H,t,-C_5-O-CH_2CH_3), 3.70(2H,s,C_8-H), 3.90(3H,s,OCH_3), 4.00–4.20(4H,m,-C_5-O-CH_2CH_3 &-N-CH_2CH_3), 4.60(2H,s,C_2-H), 6.90-7.10(3H,m,aryl-H), 7.40 (2H,d,aryl-H), 11.20(1H,s,NH)	452(3), 408(100), 380(32), 321(4), 204(22), 171(8), 136(6), 107(3), 77(4), 44(27)	28.42	—

removed completely under reduced pressure. The resultant crude residue was treated with methanol (30 mL), filtered and dried to get **5**. yield: 70-80 (molar %).

5a, R^1=R^2=H, m.p.174-76°C, **5b,** R^1=R^2= OMe, m.p. 174-76°C, **5c,** R^1= OMe, R^2=OEt, m.p. 169-71°C., **5d,** R^1= OEt, R^2= OMe, m.p.182-86°C.

General Procedure for the Preparation of 1 from 5. To a solution of **5** (3 mmoles) in *N*,*N*-dimethylacetamide (10 mL) were added anh. K_2CO_3 (7 mmoles) followed **6** (3 mmoles) at room temp. The reaction mixture was stirred for about 1hr at 30-35°C, and then 1*N* sodium hydroxide solution (10 mL) was added. The reaction mixture was heated for 6–8 hr at 90-95°C. The resulting mixture was cooled to room temp and extracted with dichloromethane (2 × 50 mL). The desired aqueous layer was acidified with 6*N* HCl and stirred for 30 minutes. The precipitated solid was filtered and the product was washed with 20 mL of acetonitrile. The product was further purified by column chromatography on silica gel Merck 100–200 mesh, chloroform–methanol (8 : 2) to afford the pure **1**. yield: 50-62 (molar %).

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