SYNTHESIS OF 6-(5-OXO-4-PHENYL-2,5-DIHYDROFURAN-3-YL)-2H-1,4-BENZOXAZIN-3(4H)-ONE AS POTENTIAL NON-STEROIDAL ANTI-INFLAMMATORY AGENTS (NSAIDS)

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Abstract: The synthesis and non-steroidal anti-inflammatory activity of a series 6-(5-oxo-4-phenyl-2,5-dihydrofuran-3-yl)-2H-1,4-benzoxazin-3(4H)-one are reported. The results showed that some of the furanones have shown marginal activity with reference to Rofecoxib.

Introduction

Treatment of inflammation with steroids (glucocorticoids) is hampered by severe side-effects[1] leading to heart, liver and kidney damage. Several non-steroidal anti-inflammatory agents (NSAIDS) gained importance for their reduced adverse effects[2]. Mostly NSAIDS act by inhibition of prostaglandin synthesis[3]. Cyclooxygenase enzyme (COX) plays an important role in the synthesis of prostaglandins. Two forms, designated COX-1 and COX-2 have been identified[4]. COX-2 is found in joints and other areas affected by inflammation. Inhibition of COX-2 enzyme reduces the production of compounds associated with inflammation and pain. COX-1 is found primarily in the stomach lining, and inhibition of this enzyme is believed to be associated with the gastric toxicity that occurs with most traditional NSAIDS[5]. Search was on for new chemical entities, which preferably inhibit COX-2 enzyme leaving alone COX-1, thereby the drug is void of gastric toxicity. Recent molecules like Celecoxib[6] and Rofecoxib[7] showed such selection and are already available for treatment. The basic furanone structure is believed to be the important structural part for the COX-2 inhibition activity of Rofecoxib[8].

On the other hand, several 1,4-benzoxazines were synthesized and tested for a wide variety of pharmacological actions such as anti-inflammatory[9], anthelmintic[10-12] and anti-microbial[13] activity.

In view of the above data, it was considered worthwhile to synthesize new chemical entities, which comprises 1,4-benzoxazine and dihydrofuranone moieties and test them for selective COX-2 inhibitory activity, the results of which are presented in this paper.

Results and Discussion

The scheme followed to synthesise the target molecule **6a** is outlined below: -

2-Aminophenol **1** was condensed with chloroacetyl chloride in MIBK in the presence of sodium carbonate. After conventional work-up 2H-1,4-benzoxazin-3-(4H)-one[14] **2** was obtained. Compound **2** on Friedel-Crafts reaction with chloroacetyl chloride in the presence of aluminium chloride gave 6-(2-Chloroacetyl)-2H-1,4-benzoxazin-3(4H)-one[15] **3** which is known in literature. **3** was then converted to the target molecule, 6-(5-oxo-4-phenyl-2,5-dihydrofuran-3-yl)-2H-1,4-benzoxazin-3(4H)-one **6a** by two methods; Either by first preparing the intermediate **5a** and then converting it into **6a** or directly, **3** was converted to **6a** in a single pot. **3** was reacted with phenylacetic acid **4** in dry DMF with diisopropylamine as base to give the 2-Oxo-2-(3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethyl phenylacetate **5a**. In the compound **5a**, IR stretching frequencies at 3208 cm^{-1}, 1732 cm^{-1}, 1707 cm^{-1} and 1678 cm^{-1} may be due to the presence of NH and carbonyl respectively. In the ^1H NMR spectral data, the benzylic protons appeared as a singlet at δ 3.80 ppm may be due to the presence of $-CH_2$ protons. A singlet at δ 5.30 ppm may be the presence of $-CO-CH_2-O-$functionality. The C_2 protons of benzoxazin appeared as a singlet at δ 4.60 ppm which is similar signal in 2H-1,4-benzoxazin-3-(4H)-one. The multiplet at δ 6.8-7.0 ppm may be due to the protons at C_5,C_7 and C_8. The presence of large singlet at δ 7.35 ppm may be due to phenylic protons at $C_{2',3',4',5',6'}$. The NH proton was observed at δ 10.80 as a singlet. All this data confirmed the structure of **5a**. Then, the ester **5a** was once again treated with excess of diisopropylamine at room temperature and stirring at 30°C which effected cyclisation yielding **6a**. In another variation, **3** could be directly converted to **6a** in a single pot by stirring it at 30°C for 36 hours after the addition of the base without isolating the intermediate ester **5a**. The cyclised furanone **6a** after workup was purified by column chromatography. In the compound **6a**, IR stretching frequencies at 3200, 1722 and 1700 cm-1 confirms the presence of -NH and carbonyl groups. In the ^1H NMR spectral data, furanone $-CH_2$ protons appeared at δ 5.12 ppm as a singlet. The $-CH_2$ protons at C_2 were observed at δ 4.50 ppm. A broad multiplet was observed at δ 6.75-6.95 ppm which was attributed to the presence of C_5, C_7 and C_8 protons. The phenylic protons were observed at δ 7.35 ppm. The proton at δ 10.65 ppm may be due to the presence of -NH. The schematic representation of the above method is depicted in **Scheme I**.

In similar way **5b**, **5c**, **5d** and **6b**, **6c**, **6d** were prepared. Their physical, spectral and biological activity data is depicted in **Table-I**.

Biological Activity

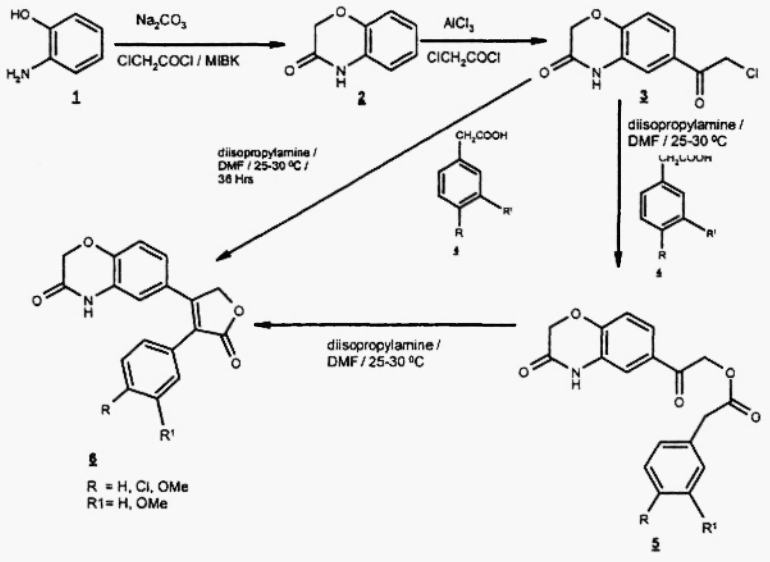
The compounds prepared were tested for cyclooxygenase-1 and cyclooxygenase-2 inhibitory activity. The method of Copeland[16] et al was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of N,N,N',N',-tetramethyl paraphenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX–1 and COX–2 enzymes. COX–1 enzyme is

Table I: Physical, spectral and biological activity data of 2-Oxo-2-(3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethyl phenylacetates and 6-(5-Oxo-4-phenyl-2,5-dihydrofuran-3-yl)-2H-1,4-benzoxazin-3(4H)-ones

S. No.	R	R'	Yield (%)	M.P (0^0 C)	Spectral Data IR (cm^{-1})	Spectral Data ^{1}H NMR δ (ppm)	Mass (% of abundance)	Activity Inhibition COX-I IC_{50} (μm)	Activity Inhibition COX-II IC_{50} (μm)
5a	H	H	80.7	168-9	3208, 1732, 1707	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.35(m, 5H, $C_{2',3',4',5',6'}$-H); 4.6(s, 2H, C_2-H); 5.3(s, 2H, C_{10}-H); 3.8(s, 2H, C_{13}-H); 10.8(s, 1H, N-H)	---	---	---
5b	OMe	H	78.3	164-5	3124, 1749, 1682, 1632	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.5(d, 2H, $C_{3',5'}$-H), 7.25(d, 2H, $C_{2',6'}$-H); 4.6(s, 2H, C_2-H); 5.3(s, 2H, C_{10}-H); 3.75(s, 2H, C_{13}-H); 3.8(s, 3H, $C_{4'}$-OMe); 10.85(s, 1H, N-H)	---	---	---
5c	Cl	H	81.3	174-6	3212, 1747, 1680, 1605	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.2(m, 4H, $C_{2',3',5',6'}$-H); 4.6(s, 2H, C_2-H); 5.25(s, 2H, C_{10}-H); 3.70(s, 2H, C_{13}-H); 10.7(s, 1H, N-H)	---	---	---
5d	OMe	OMe	85.0	178-9	3176, 1738, 1702, 1681	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.7(s, 1H, $C_{2'}$-H), 7.5(m, 2H, $C_{5',6'}$-H); 4.6(s, 2H, C_2-H); 5.3(s, 2H, C_{10}-H); 3.8(s, 3H, $C_{3'}$-OMe); 3.9(s, 3H, $C_{4'}$-OMe); 3.7(s, 2H C_{13}-H); 10.8(s, 1H, N-H)	---	---	---
6a	H	H	31.1	211-13	3200, 1722, 1700	6.75-6.95(m, 3H, $C_{5,7,8}$-H); 7.35(m, 5H, 4'-Ph-H); 4.5(s, 2H, C_2-H); 5.12(s, 2H, $C_{2'}$-H); 10.65(s, 1H, N-H)	307(100%), 278(10%), 250(48%), 176(36%), 97(15%), 69(65%), 57(45%)	9.6	1.4
6b	OMe	H	27.3	145-7	3235, 1720, 1680	6.8-7.0(m,3H, $C_{5,7,8}$-H); 7.25-7.5(m,4H, $C_{2',3',5',6'}$-H); 4.55(s, 2H C_2-H); 5.3(s, 2H, $C_{2'}$-H); 3.85(s, 3H, $C_{4'}$-OMe-H; 10.85(s, 1H, N-H)	---	1.3	0.4
6c	Cl	H	30.3	232-4	3203, 1720 1685	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.4(m, 4H, 4'-Ph-H); 4.50 (s,2H,C_2-H); 5.15(s,2H,$C_{2'}$-H); 10.7(s, 1H, N-H)	341(80%),284(65%) 249(8%),176(85%) 121(50%),119(70%) 78(25%),43(100%).	39.0	43.8
6d	OMe	OMe	23.4	196-8	3331, 1746, 1691	6.8-7.0(m, 3H, $C_{5,7,8}$-H); 7.25-7.5(m 3H, $C_{2',3',6'}$-H); 4.60(s, 2H C_2-H); 5.25(s, 2H, $C_{2'}$-H); 3.9(s, 3H, $C_{4'}$-OMe); 3.8(s, 3H, $C_{3'}$-OMe); 10.8(s, 1H, N-H)	---	26.3	23.0

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 are dissolved in DMSO and stock solution is diluted to required assay concentration. The assay mixture consists of Tris buffer (pH 8.0), EDTA solution, hematin as cofactor. The enzyme and the drug of assay concentration in DMSO. The assay mixture was pre incubated at 25°C and then TMPD in ethanol was added. The enzyme activity is measured by estimating the initial velocity during the first 25 sec. by measuring the 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.



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-577 spectrophotometer. The 1H NMR spectral data of all compounds were recorded on Varian-200 MHz. The mass spectra of the compounds **6a** and **6c** were recorded on a varian MATCH 7A spectrometer at 70ev.

Preparation of 3 from 2 (General Procedure). To a solution of **2** (15g, 0.1005 moles) in methylene dichloride (150 ml) was added chloroacetyl chloride (20g, 0.177 moles) slowly. The reaction mixture was cooled to 0°C and anhydrous aluminium chloride (40.5g, 0.303 moles) was added in 5 equivalent lots at 0-5°C. The temperature was then raised slowly to 20°C and maintained for 12 hours. The

reaction mixture was quenched into ice water containing hydrochloric acid. The separated product was collected by filtration and dried. Yield: 20.1g (87.7%) mp: 227-229°C.

Preparation of 5a from 3 (General Procedure) . 3 (3.0 g, 0.013 moles) was dissolved in dimethylformamide (30 ml) and phenylacetic acid (2.17 g, 0.015 moles) was added. To this mixture diisopropylamine solution (1.73g, 0.017 moles) was added at 20-25°C. The reaction mixture was stirred for 5-6 hours at 25-30°C. The reaction mixture was then quenched by pouring into water. The separated product was collected by filtration and dried. Yield: 3.1g (80.7%) mp: 168-169°C.

Preparation of 6a from 3 (General Procedure). 3 (3.0 g 0.013 moles) dissolved in dimethylformamide (30 ml) and phenylacetic acid (2.17 g, 0.015 moles) was added. To this mixture diisopropylamine solution (8.0 g, 0.079 moles) was added slowly at 20°C. The reaction mixture was then stirred for 36 hours at 30°C. At the end of this period, pH of the reaction mixture was adjusted to 2.0 with 1 N hydrochloric acid and was then poured into water. The separated product was collected by filtration and further purified by column chromatography on silica gel (100-200 mesh) using ethylacetate and benzene (3:7). Yield: 1.2g (31.1%) mp: 211-213°C.

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References

1. K. Parfitt, "Martindale - The complete Drug Reference", Pharmaceutical press, 1999, **32**, 1010.

2. G. Lombardino, "Nonsteroidal Anti-inflammatory Drugs", Wiley Interscience, John Wiley & Sons: NewYork, 1985.

3. J.R. Vane, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-Like Drugs, Nature (New Biol.), 1971, **231**, 232.

4. a) Y. Pirson, C. Van Y persele de strihoy, Am. J. Kidney Dis., **1986**, 8, 337.
   b) C. Richardson, P. Emery, Drug safety 1996, **15**, 249.

5. M.C. Allison, A.G. Howaston, C.J. Torrance, F.D. Lee, R.I.G. Russell, Gastrointestinal Damage associated with the use of NSAIDS, N. Engl. J. Med. 1992, **327**, 749.

6. John J. Talley, Stephen R. Bertenshaw, David L. Brown, Jefferys carter, Mathew J. Graneto, Michael S. Kellogg, Carol M. Koboldt, Jinhua Yuan, Yan Y. Zhang, and Karen seibort, J. Med. Chem. 2000, **43**, 1661.

7. Y. Ducharme, J. Y. Gauthier,P. Prasit, Y. Leblanc, Z. Wang; S. Leger, M. Therien. WO Patent 9518799, 1995, Chem. Abstr, 1996. **124**, 201998.

8. P. Prasit, Z. Wang, C. Bridean, C. C. Chan, S. Charleson, W. Cromlish, D. Ethier, J.F. Evans, A.W. Ford Hutchinson, J. Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G. P. O. Neill, M. Ouellet, M.D. Percival, H. Perrier, D. Riendean, I. Rodger, P. Tagari, M. Therien, P. Vickers, E. Wang. L. J. Xu, R. N. Young, and R. Zamboni, Bio org and Med Chem lett., 1999, **9**, 1773.

9. C.V. Reddy Sastry, K. Srinivasa Rao, V. S. H. Krishnan, K. Rastogi, M. L. Jain & G. K. A. S. S Narayan, and G.S. Reddy, P. P. Singh, C. Sheshagiri Rao and A. Y. Junnarkar, Ind. J Chem., 1990, **29 B**, 396.

10. K. Sirnivasa Rao and C. V. Reddy Sastry, Ind. J. Het. Chem, 1992, **2**, 103.

11. C. V. Reddy Sastry, K. Sirnivasa Rao, V. S. H. Krishnan, K. Rastogi, & M. L. Jain, Ind. J Chem, 1989, **28 B**, 888.

12. C. V. Reddy Sastry, K. Sirnivasa Rao, K. Rastogi, & M. L. Jain, Ind. J Chem., 1988, **27 B**, 290.

13. C. V. Reddy Sastry, K. Sirnivasa Rao, V. S. H. Krishnan, K. Rastogi,, M. L. Jain, & G. K. A. S. S. Narayan, Ind. J Chem., 1989, **28 B**, 48.

14. D. R. Shridhar, M.Jogibhukta and V.S.H.Krishnan, Org. Prep. Proced. Int, 1982, **14**, 195.

15. C. V. Reddy Sastry, O. P. Bansal, K. Srinivasa Rao & M. L. Jain, Ind. J Chem., 1987, **26 B**, 662.

16. Robert A. Copeland, Jean M. Williams, John Giannars, Sherrill Nurnberg, Maryanne Covington, Donald Pinto, Stephen Pick and James M. Trzaskos, Proc. Natl. Acad. Sci. USA 1994, **91**, 11202.

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