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Synthesis of some new pyrazole and isoxazole derivatives

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ABSTRACT

Novel 6-(4-phenyl-1H-pyrazole-3-yl)-2H-1,4-benzoxazin-3(4H)-one (**4**) and 6-(4-phenyl-isoxazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one (**5**) derivatives were prepared starting from 2H-1,4-benzoxazin-3-(4H)-one (**1**) through the intermediacy of (**2**) and (**3**) were identified as possible COX-2 inhibitors.

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KEYWORDS

Pyrazoles and isoxazoles;
Benzoxazinone;
Dimethylformamide
dimethylacetal;
Inflammation;
COX-2 Inhibitors.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed drugs for the treatment of inflammation including pain relief and rheumatoid arthritis^[1]. In the seventies, it was demonstrated that aspirin and other NSAIDs block the formation of the prostaglandins (PGs) produced from arachidonic acid by the enzyme prostaglandin Synthase also called Cyclooxygenase (COX)^[2]. Recently, it was found that there are two isoforms of COX, which differ with each other in their physiological role^[3]. COX-1 is stimulated continuously by normal body physiology and is constitutive^[4]. The COX-2 enzyme is induced upon inflammatory stimuli and is responsible for progression of inflammation. Thus, selective inhibition of COX-2 over COX-1 is useful for the treatment of inflammation and inflammation associated disorders with reduced gastrointestinal toxicities when compared with the traditional NSAIDs.

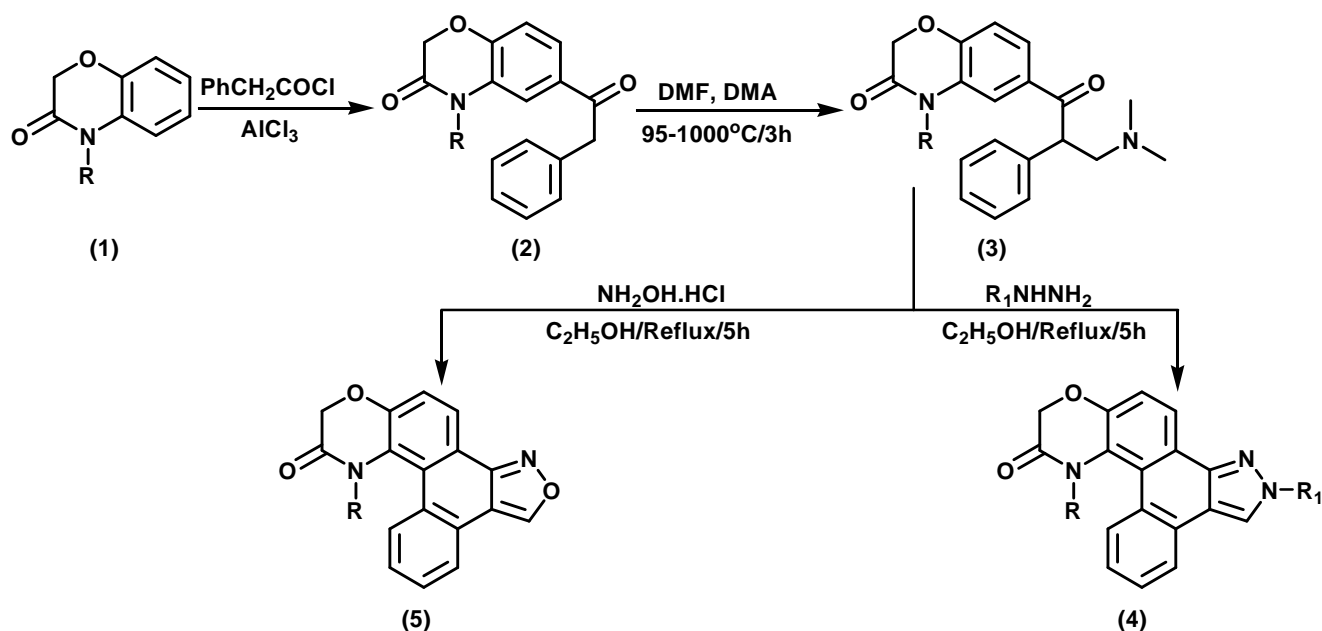
Current research has focused on developing safer NSAIDs which will selectively inhibit COX-2 enzyme.

There are several selective COX-2 inhibitors such as Celecoxib^[5], Rofecoxib^[6,7], Valdecoxib^[8], and Parecoxib sodium^[9] and have been marketed as a new generation NSAIDs structurally featuring with vicinal diaryl heterocycles. Two more COX-2 inhibitors i.e. Etoricoxib^[10] and Lumiracoxib^[11] have been developed which are structurally different from each other. A large number of 2H-1,4-benzoxazin-3(4H)-one derivatives have been reported to possess potent anti-inflammatory and analgesic activities^[12]. Recently some 2H-1,4-benzoxazin-3(4H)-one containing dihydrofuranone moieties were synthesized and tested for selective COX-2 inhibitory activity^[13]. Hence, some new 2H-1,4-benzoxazin-3(4H)-one containing diaryl heterocycle derivatives were prepared in the present study to evaluate their COX-2 inhibitory activity.

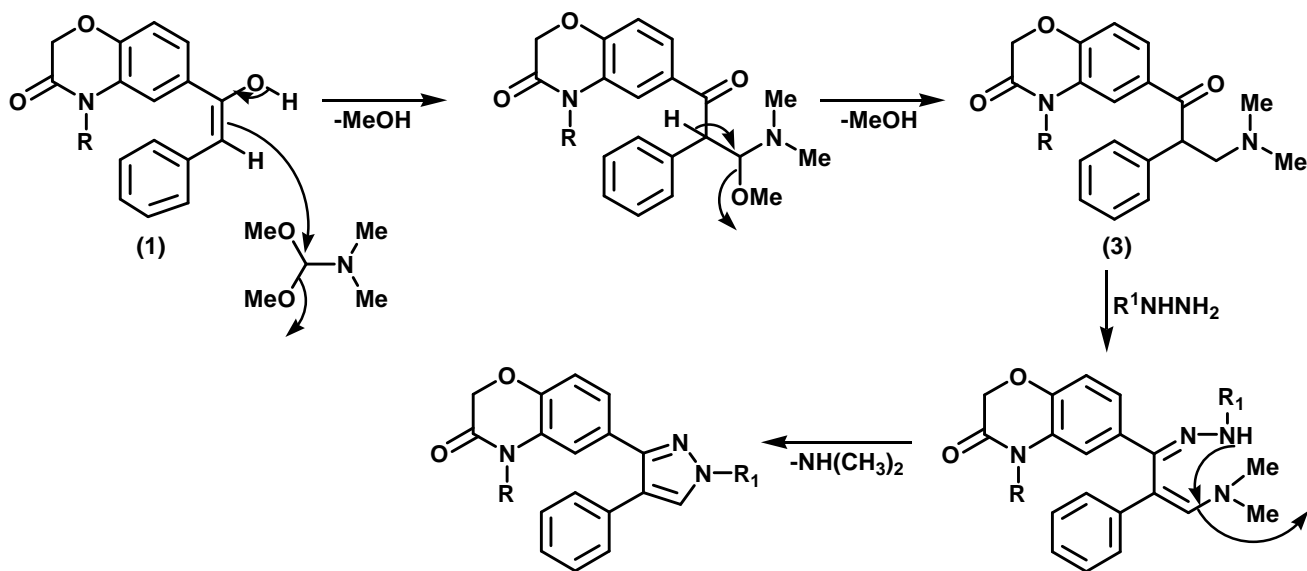
RESULTS AND DISCUSSION

The synthesis of 6-(4-phenyl-1H-pyrazole-3-yl)-2H-1,4-benzoxazine-3(4H)-one (**4**) and 6-(4-phenylisoxazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one

Full Paper

3a, 3b. R = H, CH₃4a, 4b, 4c, 4d. R = H, R₁ = H, C₆H₅, C₆H₄-4-CH₃, C₆H₄-4-OCH₃4e, 4f, 4g, 4h. R = CH₃, R₁ = H, C₆H₅, C₆H₄-4-CH₃, C₆H₄-4-OCH₃5a, 5b, 5c. R = H, CH₃, C₂H₅

Scheme 1



Scheme 2

(5) are outlined in Scheme 1. The synthetic route is based on Friedel-Craft acylation of 2H-1,4-benzoxazin-3(4H)-one derivatives (1)^[13] with phenylacetyl chloride in the presence of anhydrous aluminium chloride using nitrobenzene as a solvent to obtain 6-phenylacetyl-2H-1,4-benzoxazin-3(4H)-one derivatives 2^[14] in 45 to 60% yield. (2) were then reacted with N,N-dimethylformamide dimethylacetal (DMF-DMA) to obtain 6-(3-dimethylamino-2-

phenylacryloyl)-2H-1,4-benzoxazin-3(4H)-one derivatives (3) in good yields. The latter on reaction with substituted hydrazines gave the corresponding substituted pyrazoles (4). (3) were also reacted with hydroxylamine hydrochloride to yield (5). The formation of (3) from (2) seems to follow the mechanism shown in Scheme 2. The latter also depicts the mechanisms for the formation of (4) and (5) respectively from (3).

As a conclusion, we have prepared some novel

pyrazole and isoxazole derivatives and studied the COX-1 and COX-2 activity up to 100 μ M concentration.

Biological activity

The compounds prepared were tested for inhibition of cyclooxygenase-1 and cyclooxygenase-2 activity. The method of Copel and et al.^[15] was followed to determine the IC₅₀ values. The enzyme activity was measured using chromogenic assay based on oxidation of N, N,N'-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandin G₂ to prostaglandin H₂ by COX-1 and COX-2 enzymes. COX-1 enzyme was from Ram seminal vesicles (microsomal fraction) and COX-2 from Recombinant human enzyme purified from SF₉ cells (Baculo virus expression system) were used in the assay.

The compounds were dissolved in DMSO and stock solution is diluted to required assay concentration (10mM). The final concentration of compounds in 1ml reaction mixture was 10 μ M. The assay mixture consists of Tris buffer (pH 8.0), EDTA (3 μ M) and hematin (15 μ M) as cofactor.

The activity of enzymes both COX-1 and COX-2 was checked initially. The assay mixture along with enzyme and test compound was pre-incubated at 25°C for 15 min. The enzyme activity was measured allowing the reaction for 1 min.

Indomethacin was used as the standard inhibitor for COX-1 and Celcoxib for COX-2. All the compounds tested showed no significant inhibition upto 100 μ M concentration.

EXPERIMENTAL

Melting points were determined on a Buchi 540 melting point apparatus and are uncorrected. FT-IR spectra were recorded as KBr pellet on Nicolet 380 FT-IR instrument (Model Thermo Electron Corporation-Spectrum One), ¹H and ¹³C NMR (proton decoupled) spectra were recorded on Varian 400 MHz spectrometer using DMSO-d₆ and CDCl₃ as solvent, and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on Agilent triple quadrupole mass spectrometer equipped with turboion spray interface at 375°C. All the organic extracts were dried over sodium sulfate after work-up.

The dry reactions were carried out under N₂ atmosphere with magnetic/mechanical stirring. Unless otherwise mentioned, all the solvents and reagents used were of LR grade. TLC was performed on pre coated silica-gel plates, which were visualized using UV light and sulphuric acid/ethanol (5:95) charring. Flash column-chromatography was carried out on silica gel (230-400 mesh) unless otherwise stated.

General procedure for the preparation of (3) from (2)

A mixture of N,N-Dimethylformamide dimethylacetal (10mL) and (2) (4 mmoles) was heated to 95-100°C for 3h. The progress of the reaction was monitored by TLC. After completion of reaction, the excess of Dimethylformamide dimethylacetal was distilled off under reduced pressure, the residue cooled to room temperature and poured into ice-water (50mL). The reaction mixture was extracted with ethyl acetate (2 \times 50mL). The organic layer dried over anhydrous sodium sulphate and the ethyl acetate was distilled off completely under reduced pressure. To a resulting residue, methanol (30mL) was added and stirred for 30min. The product was filtered and the cake washed with methanol (10mL) to obtain (3).

6-(3-dimethylamino-2-phenylacryloyl)-2H-1,4-benzoxazine-3(4H)-one, (3a)

R = H; 75% yield; m.p.: 232-236°C; ¹H NMR (DMSO-d₆/TMS): δ 2.80 (s, 6H, -N(CH₃)₂), 4.50 (s, 2H -OCH₂CO), 6.50-7.80 (m, 2H, aryl), 7.00-7.20 (m, 6H, aryl), 7.70 (s, 1H, -CH), 10.40 (s, 1H, -NH); IR (film, KBr) 3125, 1624 cm⁻¹; MS: m/z (M⁺+1) 322.

6-(3-(dimethylamino)-2-phenylacryloyl)-4-methyl-2H-1,4-benzoxazin-3(4H)-one, (3b)

R = CH₃; 85% yield; Dark red coloured viscous oil; ¹H NMR (DMSO-d₆/TMS): δ 2.90 (s, 6H, -N(CH₃)₂), 3.3 (s, 3H, -NCH₃), 4.60 (s, 2H, -OCH₂CO), 6.50-7.80 (m, 2H, aryl), 7.00-7.20 (m, 6H, aryl), 7.70 (s, 1H, -CH); IR (neat) 1624cm⁻¹; MS: m/z (M⁺+1) 336.

General procedure for the preparation of (4) from (3)

To a solution of (3) (1.0mmole) in 98% ethanol (20ml), was added hydrazine hydrate 98% (1.5mmoles)

Full Paper

at 250°C. The reaction mixture was heated to reflux for 3hrs. After completion of the reaction, as monitored by TLC, ethanol was distilled off under reduced pressure, the residue cooled to RT and poured in to ice-water (20mL). The reaction mixture was extracted with ethyl acetate (2 × 25mL), dried over anhydrous sodium sulphate and ethyl acetate distilled off completely under reduced pressure. The crude product was purified by column chromatography (silica gel 100-200mesh, chloroform: methanol 10:0.2ml) to obtain pure (4).

6-(4-phenyl-1H-pyrazole-3(5)-yl)-2H-benzo[b][1,4]oxazine-3(4H)-one, (4a)

R = H, and R¹ = H; 65% yield; m.p.: 216-218°C; ¹H NMR (DMSO-d₆/TMS): δ 4.55 (s, 2H, -OCH₂CO), 6.75-7.40 (m, 8H, aryl), 7.75 (s, 1H, = CH), 8.90 (s, 1H, -NH); IR (film, KBr) 1685cm⁻¹; MS: m/z (M⁺+1) 292.

6-(1,4-diphenyl-1H-pyrazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one, (4b)

R = H, and R¹ = C₆H₅; 62% yield; m.p.: 297-299°C; ¹H NMR (DMSO-d₆/TMS): δ 4.50 (s, 2H, -OCH₂CO), 6.50-7.80 (m, 3H, aryl), 7.10-7.30 (m, 10H, aryl), 7.70 (s, 1H, = CH), 10.60 (s, 1H, -NH); IR (film, KBr) 1671cm⁻¹; MS: m/z (M⁺+1) 306.

6-(4-phenyl-1-p-tolyl-1H-pyrazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one, (4c)

R = H, and R¹ = C₆H₄-4-CH₃; 64.2% yield; m.p.: 277-279°C; ¹H NMR (DMSO-d₆/TMS): δ 2.30 (s, 3H, -CH₃), 4.50 (s, 2H, -OCH₂CO), 6.60-6.90 (m, 2H, aryl), 7.10-7.30 (m, 10H, aryl), 7.80 (s, 1H, = CH), 10.60 (s, 1H, -NH); IR (film, KBr) 1653cm⁻¹; MS: m/z (M⁺+1) 381.

6-(1-(4-methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one, (4d)

R = H, and R¹ = C₆H₄-4-OCH₃; 63.4% yield; m.p.: 238-241°C; ¹H NMR (DMSO-d₆/TMS): δ 3.80 (s, 3H, -OCH₃), 4.50 (s, 2H, -OCH₂CO), 6.60-6.90 (m, 5H, aryl), 7.10-7.30 (m, 7H, aryl), 7.80 (s, 1H, = CH), 10.60 (s, 1H, -NH); IR (film, KBr) 1682cm⁻¹; MS: m/z (M⁺+1) 396.

4-methyl-6-(4-phenyl-1H-pyrazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one, (4e)

R = CH₃, and R¹ = H; 68% yield; m.p.: 216-

218°C; ¹H NMR (DMSO-d₆/TMS): δ 3.20 (s, 3H, -NCH₃), 4.60 (s, 2H, -OCH₂CO), 6.90-7.60 (m, 8H, aryl), 7.70 (s, 1H, = CH); IR (film, KBr) 1671cm⁻¹; MS: m/z (M⁺+1) 306.

6-(1,4-diphenyl-1H-pyrazol-3-yl)-4-methyl-2H-1,4-benzoxazin-3(4H)-one, (4f)

R = CH₃, and R¹ = C₆H₅; 61.2% yield; m.p.: 179-190°C; ¹H NMR (DMSO-d₆/TMS): δ 3.20 (s, 3H, -NCH₃), 4.60 (s, 2H, -OCH₂CO), 6.60-7.00 (m, 3H, aryl), 7.20-7.40 (m, 10H, aryl), 7.90 (s, 1H, = CH); IR (film, KBr) 1682cm⁻¹; MS: m/z (M⁺+1) 381.

4-methyl-6-(4-phenyl-1-p-tolyl-1H-pyrazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one, (4g)

R = CH₃, and R¹ = C₆H₄-4-CH₃; 71.6% yield; m.p.: 171-174°C; ¹H NMR (DMSO-d₆/TMS): δ 2.30 (s, 3H, -CH₃), 2.90 (s, 3H, -NCH₃), 4.60 (s, 2H, -OCH₂CO), 6.70-6.90 (m, 2H, aryl), 7.10-7.30 (m, 9H, aryl), 7.80 (s, 1H, = CH); IR (film, KBr) 1686cm⁻¹; MS: m/z (M⁺+1) 396.

6-(1-(4-methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl)-4-methyl-2H-1,4-benzoxazin-3(4H)-one, (4h)

R = CH₃, and R¹ = C₆H₄-4-OCH₃; 62.7% yield; m.p.: 166-168°C; ¹H NMR (DMSO-d₆/TMS): δ 3.00 (s, 3H, -CH₃), 3.80 (s, 3H, -OCH₃), 4.60 (s, 2H, -OCH₂CO), 6.60-6.90 (m, 5H, aryl), 7.10-7.30 (m, 7H, aryl), 7.80 (s, 1H, = CH); IR (film, KBr) 1682cm⁻¹; MS: m/z (M⁺+1) 411.

General procedure for the preparation of (5) from (4)

To a solution of (3) (1.0mmole) in 98% ethanol (20mL), was added hydroxylamine hydrochloride (1.7mmole) and the reaction mixture was refluxed for 5-6hrs. After completion of the reaction, as monitored by TLC, the mixture was cooled to room temperature and the separated solid was filtered to obtain (5).

6-(4-phenylisoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one, (5a)

R = H; 63.4% yield; m.p.: 181-183°C; ¹H NMR (DMSO-d₆/TMS): δ 4.70 (s, 2H, -OCH₂CO), 6.90-7.00 (d, 1H, aryl), 7.05-7.15 (m, 2H, aryl), 7.30-7.50 (m, 5H, aryl), 8.40 (s, 1H, = CH); IR (film, KBr) 1667cm⁻¹; MS: m/z (M⁺+1) 292.

4-methyl-6-(4-phenylisoxazol-3-yl)-2H-1,4-benzoxazin-3(4H)-one, (5b)

R = CH₃; 60.0% yield; m.p.: 135-139°C; ¹H NMR (DMSO-d₆/TMS): δ 3.20 (s, 3H, -CH₃), 4.60 (s, 2H, -OCH₂CO), 6.90-7.00 (d, 1H, aryl), 7.20-7.35 (m, 2H, aryl), 7.40-7.50 (m, 5H, aryl), 8.40 (s, 1H, =CH); IR (film, KBr) 1667cm⁻¹; MS: m/z (M⁺+1) 306.

4-ethyl-6-(4-phenylisoxazol-3-yl)- 2H-1,4-benzoxazin-3(4H)-one, (5c)

R = C₂H₅; 56.7% yield; m.p.: 149-152°C; ¹H NMR (DMSO-d₆/TMS): δ 1.00-1.15 (s, 3H, -CH₃), 3.72-3.90 (q, 2H, -CH₂), 4.60 (s, 2H, -OCH₂CO), 6.90-7.00 (d, 1H, aryl), 7.20-7.50 (m, 7H, aryl), 8.40 (s, 1H, =CH); IR (film, KBr) 1667cm⁻¹; MS: m/z (M⁺+1) 320.

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