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Design and Synthesis of Coumarin Substituted Oxathiadiazolone Derivatives Having Anti-Inflammatory Activity Possibly through p38 MAP Kinase Inhibition

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Compounds containing oxathiadiazolone nucleus bearing substituted coumarin ring were designed and synthesized while retaining the pharmacophores required for binding with p38 MAP kinase. A four-step synthetic scheme was employed for the synthesis of 7-methoxy-4-(3′-substituted-2′-oxo-1′,2′,3′,5′-oxathiadiazol-4′-yl)-coumarin. The reactions were monitored by TLC and structures of the intermediates and the target compounds were ascertained by IR, NMR, Mass spectral data. The compounds were found to possess anti-inflammatory activity comparable to indomethacin. Superimposition studies of the target compounds with the lead p38 kinase inhibitors suggested that anti-inflammatory activity of the target compounds may be due to p38 MAP kinase inhibition. It was also suggested that methoxy group on coumarin nucleus may improve the binding profile with p38 MAP kinase.

Keywords: Oxathiadiazolone, Coumarin, MAP kinase, Superimposition, Isoxazolone, Pyrazolone

INTRODUCTION

Release and action of pro-inflammatory mediators like tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) is regulated by enzyme p38, which is an intracellular mitogen activated protein (MAP) kinase [1]. The inhibitors of p38 MAP kinase significantly reduce the release of these mediators from human monocytes [2] and also synergize with anti-IL-1β and anti-TNF-α therapy [3]. Moreover, biosynthetic inhibition of these mediators *via* p38 MAP kinase blockade has emerged as a novel approach towards treatment of chronic inflammation and autoimmune diseases [1]. Numerous heterocyclic systems bearing varied functional groups have been reported to inhibit TNF-α production through inhibition of p38 kinase [4-14]. More than 234 patents on small molecules claiming to be p38 kinase inhibitors have been published since 1996 and 17 specific inhibitors are selected for further development [5]. A detailed structure activity relationship studies of these structurally varied inhibitors revealed that the central heterocyclic system must be a five-membered ring and should bear two aryl groups at vicinal positions. Compounds containing pyrazolone **1** and isoxazolone **2** systems (Fig. 1) are highly selective and potent inhibitiors of p38 kinase. The mode of binding of these inhibitors is also well documented [13,14]. However, the amide and ester linkages in these heterocyclic systems make them susceptible to hydrolytic cleavage due to nucleophillic attack on carbonyl carbons [15]. Isosteric replacement of oxo group (C=O) with thioxo group (S=O) increase stability of these heterocyclic systems because nucleophillic attack on

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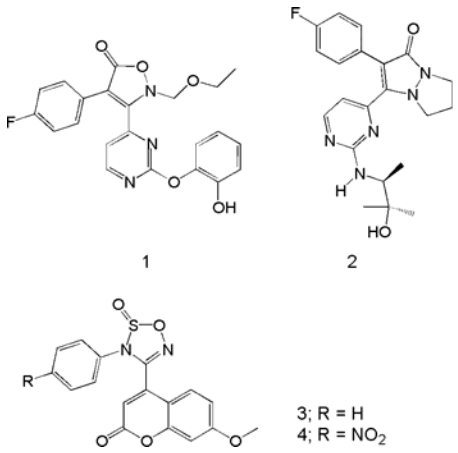

Fig. 1. Chemical structures of the lead and target compounds.

S=O is less favored than on C=O [16]. Therefore, oxathiadiazolone nucleus containing S=O represents a good isosteric replacement for C=O containing pyrazolone and isoxazolone systems.

The present study describes the design and synthesis of target compounds (**3** and **4**; Fig. 1) with oxathiadiazolone nucleus as the central core bearing (un) substituted phenyl and 7-methoxy-4-coumarinyl groups at the vicinal positions. The anti-inflammatory activity is evaluated in carageenan induced rat paw edema model.

EXPERIMENTAL

Chemistry

The reactions were monitored by TLC composed of Silica gel G using varied mobile phases. The melting points were recorded in open sulfuric acid bath and uncorrected. IR spectra were recorded on Spectrum One Series FTIR spectrophotometer (Perkin Elmer, Wellesley, USA). ^{1}H NMR spectra were recorded on Avance II 400 spectrophotometer (Bruker, Fallanden, Switzerland). The mass spectra were recorded using electron ionization on Bruker Ion Trap Esquire 3000 spectrometer (Bruker, Fallanden, Switzerland). In ^{1}H NMR, chemical shifts were reported in δ values using tetramethylsilane as internal standard with multiplicities (br-broad, s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd-double doublet) and number of protons in the solvent indicated. IR spectra were recorded as KBr pellets. When necessary, solvents and reagents were dried prior to use over potassium hydroxide, anhydrous sodium sulphate or calcium chloride (fused).

7-Methoxy coumarin-4-aldoxime (II). A mixture of I (5 mM), hydroxylamine hydrochloride (7.5 mM) and fused sodium acetate (7.5 mM) was refluxed in ethanol (50 ml) for 3 h. Ethanol was then partially removed, the residue was diluted with water and the separated solid was recrystallized from benzene-acetone to give product II as pale yellow colored needles. Yield: 0.800 g (78%), m.p.: 240-242 ºC. υ_{max} (KBr)/cm^{-1} 3197, 3086, 2937, 1699, 1640, 1614, 1486, 1213, 1068, 868, 745. δ_H (400 MHz-[d_6]DMSO) 12.16 (1H, s, NOH), 8.32 (1H, s, CHNO), 8.28 (1H, d, J = 8.5, ArH), 6.91 (1H, dd, J = 8.5 and 2.5, ArH), 6.42 (1H, s, ArH), 6.87 (1H, d, J = 2.51), 3.89 (3H, s, OCH_3). m/z (%intensity) 219 (100%, $M^{+\cdot}$), 201 (14), 191 (29), 176 (49), 173 (20), 158 (26), 120 (12), 148 (16), 119 (18), 108 (13), 77 (10), 51 (17), 44 (7).

7-Methoxycoumarin-4-nitriloxide (III). Sodium hypochlorite solution (5.2 ml) and triethylamine (6 drops) were added to the vigorously stirred solution of II (1 mM) in dichloromethane (30 ml), previously cooled to -5 ºC. The reaction mixture was allowed to stir for 30 min, diluted with water (30 ml) and extracted with dichloromethane (30 ml). The extracts were washed with water, dried over anhydrous sodium sulphate, concentrated in vacuum and the residue was triturated with ether to give product III. Yield: 0.110 g (50%), m.p.: 175-178 °C. υ_{max} (KBr)/cm^{-1} 3078, 2941, 2250, 1721, 1609, 1569, 1480, 1215, 1027, 854, 744. δ_H (400 MHz-[d_6]DMSO) 8.24 (1H, d, J = 9, ArH), 6.98 (1H, dd, J = 9 and 2.5, ArH), 6.86 (1H, d, J = 2.5, ArH), 6.42 (1H, s, ArH), 3.89 (3H, s, OCH_3). m/z (%intensity) 217 (6%, $M^{+\cdot}$), 202 (10), 189 (6), 175 (85), 173 (100), 159 (12), 158 (93), 149 (11), 133 (9), 120 (10), 119 (37), 76 (38), 44 (68).

7-Methoxy-N-phenyl-coumarin-4-carboxamide oxime (IVa). Aniline (3 mM) was added to a dispersion of 3 mM of III in 50 ml of diethyl ether and the mixture was stirred for 2 h at room temperature. The solvent was evaporated to afford the product. Yield: 0.414 g (56%), m.p.: 210-213 °C. υ_{max} (KBr)/cm^{-1} 3300, 3210, 3087, 2935, 1723, 1660, 1601, 1586, 1474, 1230, 1042, 868, 745. δ_H (400 MHz-[d_6]DMSO) 9.15 (1H, s, NOH), 8.16 (1H, d, J = 8.5, ArH), 7.01 (5H, m, ArH), 6.50 (3H, m), 4.23 (1H, s, NH), 3.98 (3H, s, OCH_3). m/z (%intensity) 310 (5%, $M^{+\cdot}$), 295 (2), 293 (11), 282 (36), 168 (11), 150 (42), 149 (5), 120 (18), 119 (29), 76 (16), 44 (100).

7-Methoxy-N-(p-nitrophenyl)-coumarin-4-carboxamide oxime (IVb). The product was prepared as above by using 3 mM of 4-nitroaniline and stirring the reaction mixture for 4 h. Yield: 0.515 g (61%), m.p.: 189-192 ºC. υ_{max} (KBr)/cm^{-1} 3360, 3217, 3069, 2847, 1726, 1628, 1604, 1535, 1468, 1315, 1286, 1214, 1068, 854, 794. δ_H (400 MHz-[d_6]DMSO) 9.26 (1H, s, NOH), 8.30 (1H, d, J = 8.5, ArH), 7.92 (2H, dd, J = 8.5 and 2.4, ArH), 6.98 (1H, d, J = 8.5, ArH), 6.87 (1H, d, J = 2.4, ArH), 6.61 (2H, dd, J = 8.6 and 2.4), 6.35 (1H, s, ArH), 4.30 (1H, s, NH), 3.89 (3H, s, OCH_3). m/z (%intensity) 355 (7%, $M^{+\cdot}$), 340 (1), 338 (4), 327 (16), 309 (20), 283 (9), 244 (6), 150 (38), 149 (7), 120 (8), 119 (29), 76 (20), 44 (100).

7-Methoxy-4-(3-phenyl-2´-oxo-1´,2´,3´,5´-oxathiadiazol-4-yl)-coumarin (3). A solution of pyridine (0.124 g, 1.57 mM) in 1 ml dichloromethane was added to an ice-cooled dispersion of 0.5 mM of IVa in 20 ml of dichloromethane. Thionylchloride (1.22 mM) in 10 ml of dichloromethane was then added dropwise during 30 min. The mixture was stirred for 1 h after which 20 ml of water was added. The organic layer was washed with water (2 × 20 ml), dried over sodium sulphate and evaporated under vacuum on rotary evaporator. The residue was then treated with diethyl ether to give the product **3**. Yield 67%, m.p.: 182 °C. υ_{max} (KBr)/cm^{-1} 3087-2842, 1727, 1625, 1624, 1217, 1072, 1043. δ_H (400 MHz-[d_6]DMSO) 8.2 (1H, d, ArH), 6.92 (5H, m, ArH) 6.35 (3H, m, ArH), 3.98 (3H, s, OCH_3) m/z (%intensity) 356 (18%, $M^{+\cdot}$), 328 (12), 292 (70), 249 (20), 149 (34), 119 (20), 76 (40), 44 (100).

7-Methoxy-4-(3-p-nitrophenyl-2´-oxo-1´,2´,3´,5´-oxa-thiadiazol-4-yl)-coumarin (4). It was prepared similar to **3** by using 5 mM of IVb and reaction mixture was stirred after dropwise addition of 10 ml of dichloromethane for 4 h. The reaction mixture was processed in a similar manner as mentioned above to produce compound **4**. Yield: 64%, m.p.: 161 °C. υ_{max} (KBr)/cm^{-1} 3087-2842, 1700, 1635, 1620, 1542, 1275, 1220, 1086 1058. δ_H (400 MHz-[d_6]DMSO) 8.2 (1H, d, ArH), 7.7 (2H, dd, ArH), 7.0 (1H, d, ArH), 6.8 (1H, d, ArH), 6.6 (2H, d, ArH), 6.3 (1H, s, ArH), 3.89 (3H, s, OCH_3). m/z (%intensity) 401 (10%, $M^{+\cdot}$), 373 (20), 355 (33), 337 (66), 309 (10), 149 (30), 119 (20), 76 (40), 44 (100).

Pharmacology

The anti-inflammatory activity of the test compounds was assessed by means of their ability to inhibit paw edema induced by carrageenan in rats taking indomethacin as reference compound. Edema in the hind paw of each rat was induced by the method as reported by Winter *et al.* [17]. Albino rats of both sexes (150-200 g) were used to assess anti-inflammatory activity of reference and test compounds. The animals were kept at r.t. allowing food (Purina Chow) and water *ad libitum* and were exposed to normal day and night light cycles. The animals were randomly allocated into four groups (six animals in each group). The test compounds and indomethacin suspended in 0.5% carboxymethylcellulose (CMC) solution were administered orally at a dose of 10 mg/kg. The CMC solution was administered to animals of the control group. After 1 h of administration of the compounds or CMC solution 0.1 ml of carrageenan solution (1.0% in sterile 0.9% NaCl solution) was injected subcutaneously into subplantar region of the right hind paw. Paw volume was measured with a glass plethysmometer coupled to a peristaltic pump, immediately before the carrageenan injection and subsequently 3 h after the injection. The data for activity was evaluated statistically using Student's *t*-test. A level of $P < 0.05$ was adopted for the test of significance.

RESULTS AND DISCUSSION

The target compounds were synthesized by a four-step synthetic scheme (Fig. 2). The starting material (compound I) is a known compound [18]. The 4-formyl group in I was converted to the corresponding aldoxime (II) with hydroxylamine hydrochloride which was subsequently subjected to oxidation with NaOCl in the presence of triethylamine to afford the intermediate compound III. The key intermediate compound IV was synthesized by stirring III with equimolar quantity of aniline or nitroaniline in diethyl ether at room temperature. Finally, the target compounds (**3** and **4**) were synthesized by stirring IV with SO_2Cl_2 in dichloromethane in the presence of pyridine. Compound **3** showed anti-inflammatory activity comparable to indomethacin whereas compound **4** had less activity than indomethacin (Table 1).

The coumarin moiety was selected as one of the aromatic residues to confer anti-inflammatory activity on the target compounds. This rationale was based on emergence of 4,7-

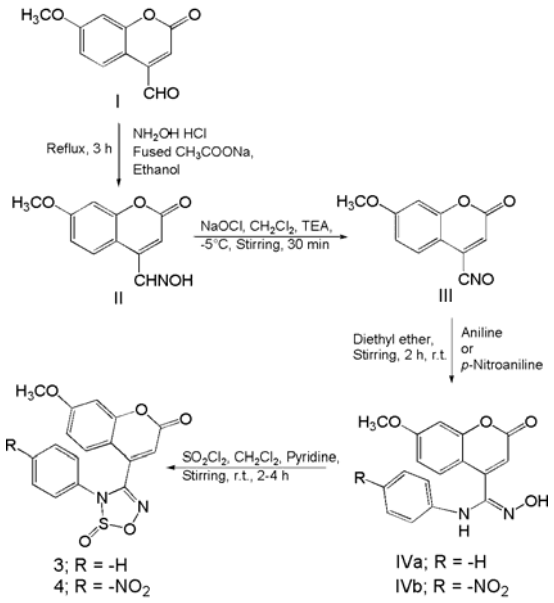

Fig. 2. Synthetic scheme for the target compounds **3** and **4**.

Table 1. Anti-Inflammatory Activity of Test and Reference Compounds

Compound	Paw volume (ml)	%Inhibition
Caregenan control	0.729 ± 0.065	-
3	0.326 ± 0.041	55.28[a]
4	0.395 ± 0.046	45.82[a]
Indomethacin	0.317 ± 0.027	56.52

[a]$p < 0.05$ Vs indomethacin.

disubstituted coumarin based compounds as potent anti-inflammatory and TNF-α inhibitors [19-21]. The superimposition studies of the target compounds (**3** and **4**) and lead molecules (**1** and **2**) in their energy minimized conformations revealed that functional groups in **3** and **4** are disposed very similar to that in **1** and **2** (Fig. 3). Based on these similarities in the superimposition studies, the target

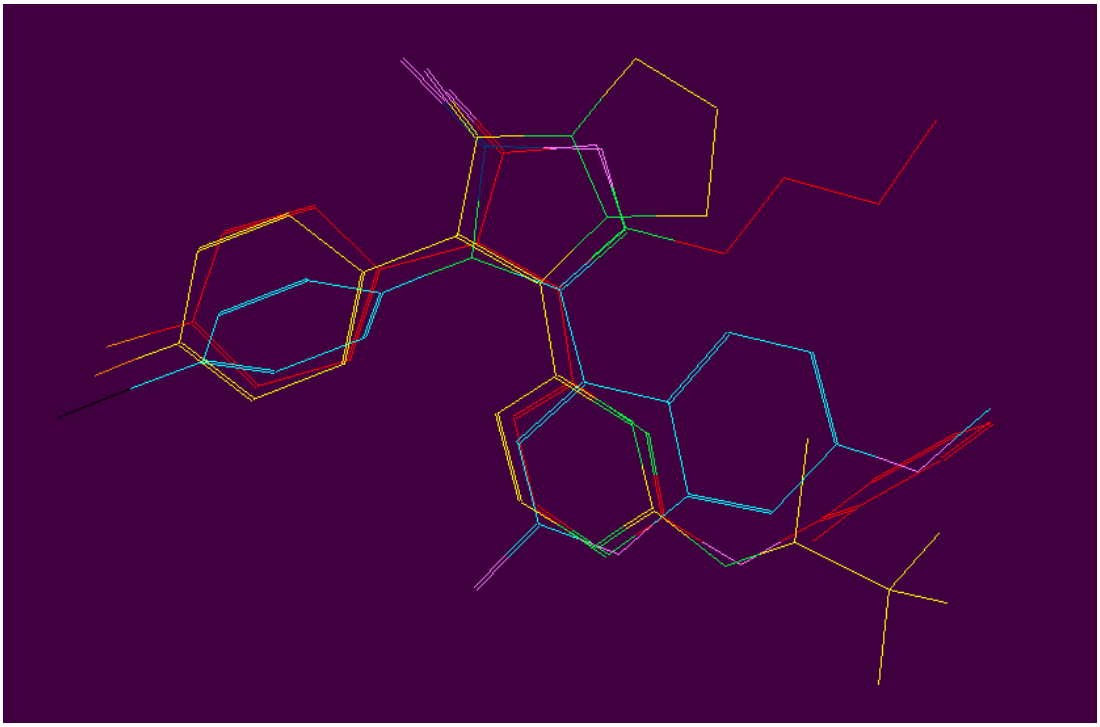

Fig. 3. Superimposition studies of 1 (yellow), 2 (red) and 4 (blue) showing overlay of oxygens (pink) of carbonyls of 1 and 2 with that of sulfonyl of 4, overlay of the aromatic residues of three compounds and oxygen of coumarin nucleus of 4 with nitrogen of the pyrimidines of 1 and 2.

compounds may be proposed to act through p38 kinase inhibition. The known receptor ligand binding interactions of the lead molecules suggest that S=O at 2 position of oxathiadiazolone can form H-bond with Lys-53 in the binding site similar to oxo group of the leads. The phenyl ring at 3 position of the oxathiadiazolone can occupy the same hydrophobic pocket as that by phenyl ring in the lead compounds. Ring oxygen of the coumarin nucleus, superimposing over the pyrimidine nitrogens in the leads, can interact with the main chain -NH- of Met-109 through H-bonding. Introduction of the $-NO_2$ group on phenyl ring was expected to increase activity based on the reported increase in activity by a strongly electron withdrawing substituents at that position [14]. However, compound **4** was found to be less potent than **3** which suggested that $-NO_2$ group at 4-position of phenyl ring may be responsible for decreased receptor ligand interactions. Moreover the strategic positioning of 7-methoxy coumarin ring can offer additional binding interactions resulting in probably better binding profile of **3**. Based on above discussion, the anti-inflammatory activity of test compounds may be attributed to the presence of coumarin nucleus as such or their ability to inhibit p38 MAP kinase. Hence, these compounds can further serve as leads for development of more potent anti-inflammatory agents which may act through p38 kinase inhibition which is currently under investigation.

CONCLUSIONS

Oxathiadiazolone based compounds were designed by isosteric replacement by C=O in pyrazolone and isoxazolone based p38 MAP kinase inhibitors. The designed compounds were synthesized and anti-inflammatory activity of one compound is found equivalent to indomethacin. Based on superimposition studies of the target compounds with the lead molecules, anti-inflammatory activity of the former may be due to p38 MAP kinase inhibition.

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