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Synthesis and Biological Evaluation of 1,3,4-Oxadiazole Derivatives as Novel Analgesic and Antiinflammatory Agents

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A novel series of 4-(2-(4-(5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl)phenylamino)ethoxy)-2*H*-chromen-2-one (**7a-j**) have been synthesized from 4-(5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl)benzenamine (**4a-j**) and 4-(2-bromoethoxy)-2*H*-chromen-2-one (**5**). The synthesized compounds were characterized on the basis of their spectral (IR, ^{1}H NMR) data and evaluated for the antiinflammatory and analgesic activity by using different pharmacological models. The most active compounds were subjected to acute ulcerogenesis activity and were found to be less ulcerogenic than the standard.

Key Words: 1,3,4-Oxadiazole, Coumarin, Antiinflammatory, Analgesic.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli. The process of inflammation is not undesirable; it is a protective mechanism essential for survival.[1]

NSAIDs have the potential to relieve pain and inflammation without the immunosuppressive and metabolic side effects associated with corticosteroids. However, all NSAIDs have the potential for other adverse effects that should be considered in the overall management of the inflammatory process[2]. The good number of reports shows that synthetic 1,3,4-oxadiazole and coumarin derivatives possess potent antiinflammatory and analgesic activity[3-5]. This led us to undertake research where in two active moieties are incorporated in a single molecule with the anticipation that the newly synthesized compounds may exhibit better analgesic and antiinflammatory activity with lower incidences of GI side effects. With the aid of literature survey, we planned to synthesize some novel compounds having coumarin nucleus and 1,3,4-oxadiazole rings linked to each other with ether bridge.

EXPERIMENTAL

All the chemicals used in the synthesis were of laboratory grade. Melting points were determined in open capillary on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide or chloroform. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO using NMR varian-mercury 300 MHz spectrophotometer and chemical shifts are given in units as parts per million, downfield from tetra methyl silane (TMS) as an internal standard. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin Layer Chromatography was performed on microscopic slides (2 × 7.5 cm) coated with silica gel-G, using appropriate solvent systems and the spots were visualized under ultra-violet light or by exposure to iodine vapours.

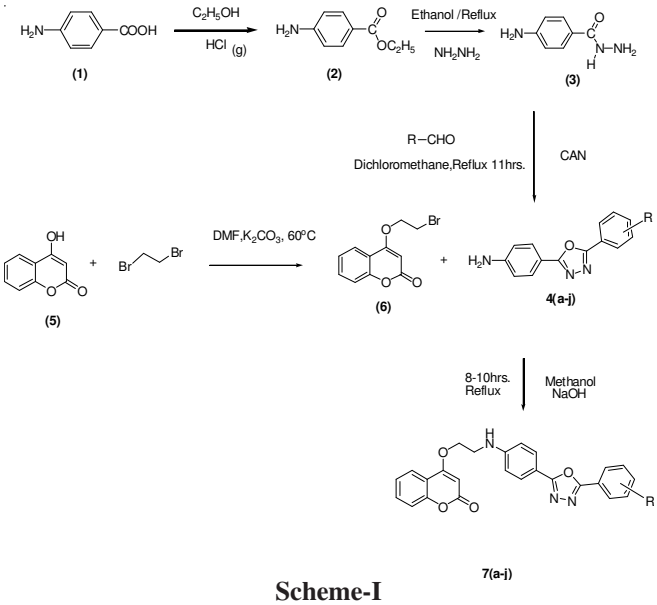
Ethyl 4-aminobenzoate (**1**) and 4-hydroxy coumarin (**5**) were obtained by the method reported in the literature.

Synthesis of 4-aminobenzohydrazide (3)[6]: 1 mL (0.01468 mol) of hydrazine hydrate was taken in a round bottom flask fitted with a short reflux condenser. 1 g (0.006053 mol) of ester was added and mixture was heated gently under reflux for 15 min. Then just enough ethanol was added through the condenser to produce a clear solution. The resulting solution was refluxed for further 2-3 h. Ethanol was distilled off and solution was cooled and crystals of acid hydrazide were filtered and recrystallized from ethanol.

Synthesis of 4-(2-bromoethoxy)-2*H*-chromen-2-one (6)[7]**:** 4-hydroxy coumarin (**5**) (6 mm) and excess of 1,2-dibromo-ethane (58 mm) were dissolved in the DMF (25 mL), anhydrous potassium carbonate (59 mm) was added and the suspension was stirred at 60 ºC overnight (**Scheme-I**). The solution was neutralized to pH 6 using HCl aq. (1N); the precipitate of product was filtered and dried. The product was purified by column chromatography using dichloromethane as mobile phase.

Synthesis of 4-(5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl)benzenamine (4a-j)[8]: 4-aminobenzohyd-razide (**3**) (1 mm)

and substituted aromatic aldehyde (1 mm) was taken in dry dichloromethane, cerric ammonium nitrate (CAN) (1 mm) was added in the solution and the mixture was refluxed for 11 h (**Scheme-I**) and reaction was monitored by the TLC, water was added in the reaction mixture and was extracted with the ethyl acetate. The organic layer was dried over the sodium sulphate, the product was obtained by evaporating ethyl acetate layer.



Scheme-I

Synthesis of 4-(2-(4-(5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl)phenylamino)ethoxy)-2*H*-chromen-2-one (7a-j)[9]: 4-(2-Bromoethoxy)-2*H*-chromen-2-one (**5**) and different 1,3,4-oxadiazole derivatives (**4a-i**) were taken in methanol containing 5 % NaOH solution. The solution was refluxed for 8-10 h on heating mantle, reaction was monitored by the TLC. After complete conversion the solution was filtered and solvent was evaporated from the filtrate. The product was isolated, extracted with chloroform: water mixture. Chloroform layer was collected and dried over sodium sulphate bed. The product was obtained by evaporating chloroform layer and recrystallized using ethanol (**Scheme-I**).

Antiinflammatory activity: The suspensions of test compounds were prepared in sterile 0.9 % NaCl solution. In all cases control received the same quantity of sterile 0.9 % NaCl solution as vehicle. Antiinflammatory activity was evaluated by carrageenin induced rat paw edema method of Winter *et al.*[10]. Sprague dawley rats of either sex weighing between 150-250 g were randomly distributed in control and experimental group of six animals. At 0 h the target compounds (**7a-j**) (20 mg/kg) and standard (indomethacin) (20 mg/kg) doses were administered orally. 1 h after compounds and standard were administered orally; 0.1 mL of 1 % (w/v) suspension of carrageenin in distilled water was injected into the planter tissue of right paw of rat by using 27 gauge needles. The paw was marked with ink at the level of the tibia-tarsal junction and the initial volume of paw was measured by plathysmometer within 30 s. of injection. The relative increase in paw volume was found by remeasuring the paw volume after 3 h of carrageenin injection (Table-1).

Analgesic activity

Acetic acid induced writhing in mice: Analgesic activity was determined in vivo by calculating total number of writhings, following intraperitonial (I.P) administration of 0.6 % (0.1 mL/10 g) acetic acid in mice[11]. Albino mice of either sex (25-30 g) were used. Target compounds (**7a-j**) were administered orally (20 mg/kg) as a suspension in sterile 0.9 % NaCl solution as vehicle.

Diclofenac sodium (20 mg/kg) was used as the standard drug under the same conditions. Acetic acid solution was administered intraperitonialy 0.5 h after the administration of the compounds. 10 min after intraperitonial injection of the acetic acid solution, the number of writhings per animal were recorded for 20 min. Control animals received an equal volume of vehicle.

Analgesic activity was expressed as percentage of inhibition of number of writhings, when compared with the vehicle control group. Results of percentage analgesic activity of compounds are calculated and shown in (Table-1).

Formalin induced licking and biting in mice: The analgesic activity was determined by calculating total number of licking and biting in mice, following administration of 0.1 % (0.1 mL/10 g) formalin into the sub planter area of right hind paw of mice. Albino mice of either sex (25-30 g) were used. Target compounds (**7a-j**) were administered intraperitonialy (20 mg/kg) as a suspension in sterile 0.9 % NaCl solution as vehicle. Diclofenac sodium (20 mg/kg) was used as the standard drug under the same conditions. After 0.5 h, the administration of the compounds, number of paw licking, an index of nociception, was measured at 0-5 min (first phase, which indicates central analgesic activity) and 15-25 min (second phase, which indicates peripheral analgesic activity) after formalin administration. Control animals received an equal volume of vehicle. Analgesic activity was expressed as percentage of inhibition of duration of paw licking, when compared with the vehicle control group and the results are shown in Table-1.

Acute ulcerogenesis: Acute ulcerogenesis test was performed according to the method of Cioli *et al*[12]. The study was carried out on healthy rats (150-200 g) at a dose thrice the anti-inflammatory dose. The animals were divided into three groups of six animals each, group I served as control and received vehicle only and group II received pure indomethacin (60 mg/kg). III group were administered test compounds in dose of 150 mg/kg. The animals were fasted 8 h prior to a single dose of each of the vehicle, standard and test compounds respectively and sacrificed 17 h later during which period food and water were available. The gastric mucosa of the rats was examined by means of a 4 X binocular magnifier. For each stomach the severity of mucosal damage was assessed according to the following scoring system: 0-no lesions of up to five punctiform lesions; 1-more than five punctiform lesions; 2-one to five small ulcers; 3-more than five small ulcers of one large ulcer; 4-more than one large ulcer. The mean score of each treated group minus the mean score of the control group was considered as the 'severity index' of gastric damage (Table-1).

RESULTS AND DISCUSSION

Synthesized target compounds were screened for antiinflammatory activity by paw edema method. Among the ten synthesized derivatives, compounds **7a**, **7c**, **7e** and **7i** exhibited promising activity as compared to the Indomethacin. Compounds **7b**, **7d**, **7f** and **7g** possess the moderate antiinflammatory activity comparable with that of Indomethacin.

Synthesized target compounds were screened for analgesic activity by acetic acid induced writhing and formalin induced licking and biting in mice. Among the ten synthesized derivatives, compounds **7a**, **7c** and **7e** exhibited promising analgesic activity as compared to the diclofenac sodium by acetic acid induced writhing method, other compounds showed moderate activity as compared to standard. The most of the compounds showed less or insignificance activity in first phase for formalin induced licking and biting model, but showed significant activity in second phase i.e. synthesized compounds were peripherally active. Among ten **7a**, **7c**, **7e**, **7g** and **7i** exhibited promising analgesic activity as compared to the diclofenac sodium.

The most active compounds (**7a** and **7i**) were screened for the gastric irritation study on rat stomach and were found to be showing less gastric irritation than the standard drug indomethacin.

Spectral data of target compounds (7a-j)

4-(2-(4-(5-(4-Phenyl)-1,3,4-oxadiazol-2-yl)phenylamino)-ethoxy)-2*H*-chromen-2-one (7a): IR (KBr ν_{max}, cm^{-1}): 3501 (N-H), 3088 (-C-H), 1701 (-C=O), 1624 (-C=N), 1276 (Ar-O-) ^{1}H NMR (δ):4.32(1H,s,N-H), 5.68(1H, s, coumarin), 3.74 and 4.43 (t, each, 2H,-CH_2-CH_2), 6.69-6.71 (2H, d, Ar-H), 7.30-7.35(1H, t, Ar-H), 7.47-7.89 (5H, m, Ar-H), 8.11-8.13(2H, d, Ar-H).

4-(2-(4-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-phenylamino)ethoxy)-2*H*-chromen-2-one (7b): IR (KBr, ν_{max}, cm^{-1}): 3450 (N-H), 3059 (-CH), 1718 (-C=O), 1624 (-C=N), 1566 (-C=C), 1249 (Ar-O-), 767 (-C-Cl). ^{1}H NMR (δ): 4.06 (1H, s, N-H), 5.66 (1H, s, coumarin), 3.74 and 4.43 (t, each, 2H, -CH_2-CH_2), 6.67-6.70 (2H, d, Ar-H), 7.88-7.89 (2H, d, Ar-H), 7.86-7.86 (2H, d, Ar-H), 7.76-7.74 (2H, d, Ar-H), 7.60-7.65 (1H, t, Ar-H).

4-(2-(4-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)-phenylamino)ethoxy)-2*H*-chromen-2-one (7c): IR (KBr, ν_{max}, cm^{-1}): 3446 (N-H), 2920 (C-H), 1724 (-C=O), 1635 (-C=N), 1541 (-C=C), 1280 (Ar-O-), 763 (-C-Br). ^{1}H NMR (δ): 4.05 (1H, s, N-H), 5.67 (1H, s, coumarin), 3.74 and 4.43 (t, each, 2H, -CH_2-CH_2), 6.68-6.71 (2H, d, Ar-H), 8.09-8.15 (2H, d, Ar-H), 7.86-7.89 (2H, d, Ar-H), 7.76-7.77 (2H, d, Ar-H), 7.51-7.57 (1H, t, Ar-H).

TABLE-1
BIOLOGICAL ACTIVITIES OF TARGET COMPOUNDS 7(a-j)

Compounds	Anti-inflammatory activity (Inhibition, %) (Std-Indomethacin)	Analgesic activity (Protection, %)(std-Diclofenac sodium)		Ulcerogenic activity (severity index) (Std-Indomethacin)
		Acetic acid induced writhing response in mice	Formalin induced licking and biting response in mice	
CONTROL (Saline)	0.0	0.0	0.0	0.0
STD	73.77***	84.05***	77.68***	3.5
7a	60.65***	47.25*	63.88**	2
7b	39.34**	41.36*	58.42**	-
7c	47.54**	55.30**	68.31**	-
7d	27.86*	26.55*	53.42**	-
7e	44.26**	51.18**	70.08**	-
7f	34.42**	32.25*	50.33*	-
7g	31.14*	43.83*	70.66***	-
7h	14.75^{ns}	21.86*	45.76*	-
7i	50.81**	39.22*	75.12***	2.5
7j	6.55^{ns}	29.34*	35.42*	-

n = 6, ***P < 0.001, P** - P < 0.01, *P < 0.05 ^{ns}-non significant, compared with control (saline) group,
Data expressed as Mean ± SEM.
Data was analyzed by one-way ANNOVA followed by Dunnett's test

TABLE-2
PHYSICAL DATA OF TARGET COMPOUNDS (**7a-j**)

Comp. No.	R	m.f.	m.w.	m.p. (·C)	R_f value*	Yield (%)
7a	-H	$C_{25}H_{19}N_3O_4$	425.0	160-162	0.50	79
7b	4-Cl	$C_{25}H_{18}N_3O_4Cl$	459.5	110-112	0.70	67
7c	4-Br	$C_{25}H_{18}N_3O_4Br$	504.0	115-117	0.71	68
7d	4-NO_2	$C_{25}H_{18}N_4O_6$	470.0	178-180	0.59	68
7e	3-Cl	$C_{25}H_{18}N_3O_4Cl$	459.5	120-122	0.76	50
7f	3-Br	$C_{25}H_{18}N_3O_4Br$	504.0	157-159	0.55	65
7g	2-NO_2	$C_{25}H_{18}N_4O_6$	470.0	130-132	0.50	79
7h	4-OCH_3	$C_{26}H_{21}N_3O_5$	455.0	134-136	0.54	59
7i	3,4,5 $(OCH_3)_3$	$C_{28}H_{25}N_3O_7$	515.0	138-140	0.59	52
7j	4-N $(CH_3)_2$	$C_{27}H_{24}N_4O_4$	468.0	140-142	0.62	62

* Mobile Phase- pet. ether: ethyl acetate = 8:2

4-(2-(4-(5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl) phenylamino)ethoxy)-2*H*-chromen-2-one (7d): IR (KBr, ν_{max}, cm^{-1}): 3450 (N-H), 3173 (-CH), 1716 (-C=O), 1624 (-C=N), 1566 (C=C), 1249 (Ar-O-). ^{1}H NMR (δ): 4.26 (1H, s, N-H), 5.66 (1H, s, coumarin) 3.75 and 4.43 (t, each, 2H, $-CH_2-CH_2$), 6.67-6.70 (2H, d,Ar-H), 7.55-7.60(1H, t, Ar-H), 7.74-7.76 (2H, d, Ar-H), 7.79-7.84 (2H, d, Ar-H), 8.41-8.60 (2H, d, Ar-H).

4-(2-(4-(5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-phenylamino)ethoxy)-2*H*-chromen-2-one (7e): IR (KBr, ν_{max}, cm^{-1}): 3415 (N-H), 3092 (-CH), 1701 (-C=O), 1624 (-C=N), 1564 (-C=C), 1259 (Ar-O-), 752 (C-Cl). ^{1}H NMR (δ): 4.09 (1H, s, N-H), 5.67 (1H, s, coumarin), 3.74 and 4.43 (t, each, 2H, $-CH_2-CH_2$), 6.71-6.75 (2H, d, Ar-H), 8.09 (1H, s, Ar-H), 8.00-8.02 (2H, d, Ar-H), 7.86-7.94 (4H, m, Ar-H), 7.51-7.57(1H, t, Ar-H).

4-(2-(4-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-phenylamino)ethoxy)-2*H*-chromen-2-one (7f): IR (KBr, ν_{max}, cm^{-1}): 3483 (N-H), 2914 (C-H), 1726 (-C=O), 1635 (-C=N), 1539 (-C=C), 1280 (Ar-O-), 754 (C-Br). ^{1}H NMR (δ): 4.10(1H, s, N-H), 5.66(1H, s, coumarin), 3.74 and 4.43 (t, each, 2H, $-CH_2-CH_2$), 6.68-6.71 (2H, d, Ar-H), 8.11(1H, s, Ar-H), 7.86-7.94 (1H, t, Ar-H), 7.54-7.79 (4H, m, Ar-H), 7.49-7.51 (1H, t, Ar-H).

4-(2-(4-(5-(2-Nitrophenyl)-1,3,4-oxadiazol-2-yl)-phenylamino)ethoxy)-2*H*-chromen-2-one (7g): IR (KBr, ν_{max}, cm^{-1}): 3510 (N-H), 3174 (-CH), 1724 (-C=O), 1629 (-C=N), 1533 (-C=C), 1247 (Ar-O-). ^{1}H NMR (δ): 4.04 (1H, s, N-H), 5.70 (1H, s, coumarin), 3.73 and 4.78 (t, each, 2H, $-CH_2-CH_2$), 6.67-6.70 (2H, d, Ar-H), 8.13-8.20 (2H, d, Ar-H), 7.5-7.8(2H, dd, Ar-H), 7.52-7.55 (1H, t, Ar-H), 7.51-7.53 (2H, d, Ar-H).

4-(2-(4-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl) phenylamino) ethoxy)-2*H*-chromen-2-one (7h): IR (KBr, ν_{max}, cm^{-1}): 3543 (N-H), 3034 (-CH), 1718 (-C=O), 1608 (-C=N), 1494 (-C=C), 1251 (Ar-O-). ^{1}H NMR(δ): 4.07 (1H, s, NH), 5.67(1H, s, coumarin), 3.88 (3H, s, OCH_3), 3.74 and 4.43(t, each, 2H, $-CH_2-CH_2$), 6.74-6.77(2H, d, Ar-H), 7.00-7.03(2H, d, Ar-H), 8.02-8.05 (2H, dd, Ar-H), 7.86-7.89(2H, d, Ar-H), 7.57-7.60 (1H,t,Ar-H).

4-(2-(4-(5-(3, 4, 5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)phenylamino)ethoxy)-2*H*-chromen-2-one (7i): IR (KBr, ν_{max}, cm^{-1}): 3543 (N-H), 3105 (-CH), 1718 (-C=O), 1608.69 (-C=N), 1496 (-C=C), 1234 (Ar-O-). ^{1}H NMR (δ): 4.09 (1H, s, NH), 5.67(1H, s, coumarin), 3.97(9H, s, OCH_3), 3.74 and 4.43 (t, each, 2H, $-CH_2-CH_2$), 6.75-6.78 (2H, d, Ar-H), 6.87-6.90 (2H, d, Ar-H), 7.30-7.55 (1H, t, Ar-H), 7.91-7.94 (2H, d, Ar-H).

4-(2-(4-(5-(4-(Dimethylamino)phenyl)-1,3,4-oxadiazol-2-yl)phenylamino)ethoxy)-2*H*-chromen-2-one (7j): IR (KBr, ν_{max}, cm^{-1}): 3520 (N-H), 3048 (-CH), 1722 (-C=O), 1630 (-C=N), 1521 (-C=C), 1245 (Ar-O-). ^{1}H NMR (δ): 4.15(1H, s, NH), 5.67(1H, s, coumarin), 3.74 and 4.43 (t, each, 2H, $-CH_2-CH_2$), 3.06(6H, s, NCH_3), 6.69-6.71(2H, d, Ar-H), 6.74-6.77(2H, d, Ar-H), 7.86-7.89 (2H, d, Ar-H), 7.92-7.94(2H, d, Ar-H), 7.55-7.60 (1H,t,Ar-H).

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