

other constituents of formulations were observed. Table I shows the inter and intra assay variations results. Different formulations and bulk drug samples of 87/132 were analysed by this method and the results are given in table No. 2.

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Synthesis, Pharmacological Evaluation and QSAR Studies of 4,5-Dihydro-4-[(substituted Phenyl) Methylene]-5-oxo-2-Phenyl/methyl-1H-Imidazole-1-Acetic Acids

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A series of (Z)-4,5-dihydro-4-[(substituted phenyl)methylene]-5-oxo-2-phenyl-1H-imidazole-1-acetic acids (1-11) and a few of (Z)-4,5-dihydro-4-[(substituted phenyl)methylene]-2-methyl-5-oxo-1H-imidazole-1-acetic acids (12-14) were synthesized and evaluated for antiinflammatory activity. Ten compounds showed significant antiinflammatory activity. Compound 2 exhibited activity comparable to phenylbutazone. It also showed significant antiarthritic activity and was less ulcerogenic than phenylbutazone. Five compounds exhibited significant analgesic activity. Several compounds showed good activity in scavenging the stable free radical DPPH. QSAR studies suggested that none of the physicochemical parameters studied showed good correlation to the antiinflammatory activity.

We have previously reported the antiinflammatory activity of a number of compounds containing styryl carbonyl moiety namely, phenylbutenones¹, chalcones², cinnamic acids³, 3-(benzylideneamino) coumarins⁴, styryl sydnone⁵ and so on. The present study describes the synthesis and antiinflammatory, antiarthritic and analgesic activities, ability to scavenge DPPH free radical and gastric ulcerogenicity of the title compounds. QSAR studies were carried out on the antiinflammatory activity of

the compounds. The title compounds (1-14) were synthesized by the reaction of substituted oxazolones with glycine in fused sodium acetate and glacial acetic acid⁶. The intermediate (Z)-4-(substituted benzylidene)-2-phenyl/methyl-oxazol-5(4H)-ones were synthesized by condensing ring substituted aromatic aldehydes with benzoylglycine or acetylglycine respectively, in presence of acetic anhydride and anhydrous sodium acetate⁷. The synthesis and antiinflammatory activity of compound 12 was reported earlier⁸.

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Melting points were determined in open capillaries on a Toshniwal melting point apparatus and are uncorrected. Elemental analysis (C,H and N) was carried out on a Carlo Erba strum DP 200. UV spectra were recorded on a HP 8452 Diode Array spectrometer, IR (KBr) spectra on Perkin-Elmer 781 and PMR (CDCl₃) spectra on Perkin Elmer R-32 using TMS as internal standard. Progress of the reactions and purity of the products were analyzed by TLC using silica gel G, and were detected by iodine vapour. Male albino rats (Charles-Foster strain) and Swiss albino mice of either sex were used. The compounds were administered, p.o. as homogenised suspension in 0.5% sodium carboxymethylcellulose.

The compounds were tested for anti-inflammatory activity by carrageenan-induced edema model⁸ in rats, antiarthritic activity by adjuvant-induced arthritis⁹ in rats for analgesic activity by acetic acid-induced writhing assay¹⁰ in mice. The compounds were also tested for gastric ulceration¹¹, for acute toxicity and for the ability of test compounds to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical¹². QSAR studies were carried out on IBM PC-286 using a QSAR-PC:PAR package by Biosoft, U.K. The biological activity, percent inhibition of edema volume, was converted to their logit values¹³.

$$B_A = \frac{MW}{d} \log \frac{P}{100 - P}$$

where B_A is biological activity in mM⁻¹, MW, the molecular weight of the compound, d, the dose in mg/kg body weight of rat, and P, the percent inhibition of edema volume.

All compounds were tested for antiinflammatory activity at a dose of 100 mg/kg, p.o. in carrageenan-induced edema assay in rats (table 1). Ten compounds showed significant (P<0.05) activity. Compounds containing electron donating groups at para position such as methyl, dimethylamino and diethylamino showed appreciable activity. The methyl derivative (**2**) exhibited highest activity (67.5%) which is comparable to phenylbutazone (66.6%). A number of vanillinyl derivatives also showed significant activity. In these compounds (**6-9, 11** and **14**), the phenolic hydroxyl is not free as it was acetylated in the preparation of oxazolones in presence of acetic anhydride. In case of compound **10**, the hydroxyl group

was not acetylated but it is sterically hindered by tertiary butyl groups at 3 and 5 positions.

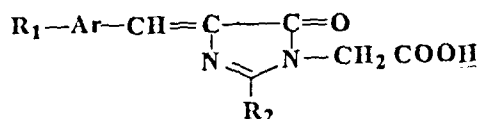
Compounds **2** and **4** which showed more than 50% inhibition in carrageenan-induced paw edema assay at 100 mg/kg dose were further tested at lower doses (50 mg/kg and 10 mg/kg, p.o.). At 10 mg/kg dose, compound **2** showed significant activity (31.5%) which appears to be comparable to phenylbutazone (34.2%). At other lower doses tested, these compounds were less active than phenylbutazone. These two compounds were also tested for antiarthritic activity in adjuvant-induced arthritis in rats. Compound **2** showed significant antiarthritic activity (31.4%). However, the activity of this compound was less than phenylbutazone (42.8%) and indomethacin (45.7%).

All compounds were tested for analgesic activity at 100 mg/kg dose in acetic acid-induced writhing assay in mice. Five compounds showed significant activity, but none exhibited activity comparable to aspirin. Compound **2** was tested for gastric ulcerogenicity and was found to be less ulcerogenic (lesion index, 12.36) than phenylbutazone (lesion index, 45.17). Compounds **2** and **4** were tested for acute toxicity in mice. No deaths were seen over a period of seven days following doses of up to 1000 mg/kg.

Reactive oxygen species are thought to play an important role in the development of inflammatory disorders¹⁴. A radical scavenging antioxidant react with 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical and reduces it to 1,1-diphenyl-2-picryl hydrazine. Vanillinyl derivatives showed good scavenging of DPPH free radical. Compounds **6, 8** and **11** showed higher activity than α-tocopherol.

Quantitative structure-activity relationship (QSAR) studies were carried out on the antiinflammatory activity of the title compounds (**1-11**) in carrageenan-induced edema assay at 100 mg/kg dose. The physicochemical parameters that were considered to study their influence on activity were Hansch hydrophobic constant (π), Hammett electronic constant (σ), Molar refractivity (MR), Steriomol parameters for length (L), minimum width (B1) and van der Waals volume (V_w) for the substituents on the phenyl ring. Regression analysis showed that Hammett constant of the substituents at para position (σ_p) gives the highest correlation (r=0.46). When two parameters

Table I - Physical Data and Pharmacological Activities of Compounds 1-14



Compound	R ₁	m.p.*(°)	Formula ^b	Antiinflammatory activity(%) ^d	Analgesic activity(%)*	% Reduction of DPPH ^e
1	H	253-54	C ₁₈ H ₁₄ O ₃ N ₂	29.7	13.6	NA
2	4-CH ₃	276-78	C ₁₉ H ₁₆ O ₃ N ₂	67.5*	41.9*	12.0
3	4-N(CH ₃) ₂	251-53	C ₂₀ H ₁₉ O ₃ N ₃	41.6*	22.5	NA
4	4-N(C ₂ H ₅) ₂	170-72	C ₂₂ H ₂₃ O ₃ N ₃	55.5*	25.8	30.2
5	4-Cl	202-04	C ₁₈ H ₁₃ O ₃ N ₂ Cl	16.6	NT	23.5
6	4-OCOCH ₃ 3-OCH ₃	268-70	C ₂₁ H ₁₈ O ₆ N ₂	45.9*	33.3*	60.2
7	4-OCOCH ₃ 3-OC ₂ H ₅	304-05	C ₂₂ H ₂₀ O ₆ N ₂	41.6*	39.3*	37.1
8	4-OCOCH ₃ 3-OCH ₃ , 5-I	208-10	C ₂₁ H ₁₇ O ₆ N ₂	40.0*	22.7	73.9
9	4-OCOCH ₃ , 3,5-(CH ₃) ₂	165-66	C ₂₂ H ₂₀ O ₅ N ₂	30.0	NT	30.5
10	3,5-[C(CH ₃) ₃] ₂ , 4-OH	247-48	C ₂₈ H ₃₂ O ₅ N ₂	41.6*	27.2	49.5
11	4-OCOCH ₃ , 3,5-(OCH ₃) ₂	151-52	C ₂₂ H ₂₀ O ₇ N ₂	40.0*	NT	55.2
12	H	244-45	C ₁₃ H ₁₂ O ₃ N ₂	27.0	16.1	NA
13	4-N(CH ₃) ₂	246-47	C ₁₅ H ₁₇ O ₃ N ₃	45.9*	48.3*	NA
14	4-OCOCH ₃ , 3-OC ₂ H ₅	234-36	C ₁₇ H ₁₈ O ₆ N ₂	35.1*	32.2*	42.0
	Phenylbutazone			66.6*	—	—
	Ibuprofen			70.2*	—	—
	Aspirin			—	60.6*	—
	α-tocopherol			—	—	51.2

Compounds 1-11 : C₆H₅ (R₁); Compounds 12-14: CH₃ (R₂); Yields were between 22-55%; * purification from repeated methanol/water system crystallization; ^ball of the compounds were analyzed for C, H, and N, and the results agreed to ±0.4% of the theoretical values; ^cR₁ (2:1 ethylacetate-methanol), 0.48; UV (ethanol): 376 nm (ε 19, 840), 252 (13, 440); IR (KBr) : 2980 (O-H), 1705, 1690 (C=O), 1635, 1610 cm⁻¹ (C=C, C=N); PMR (DMSO-d₆): δ 2.3(s, 3H Ar-CH₃), 3.4 (s, 2H, CH₂), 7.1-8.3(m, 10H, Ar-H and olefinic).

^dat 100 mg/kg, p.o. antiinflammatory activity was calculated by comparing edema volume at 3 h after carrageenan injection with that of the respective vehicle treated control animals; ^eat 100 mg/kg, p.o. analgesic activity was calculated by comparing number of writhes with the vehicle treated control animals; ^freduction of stable free radical, 1,1-diphenyl-2-picrylhydrazyl (100 μM) was estimated in alcoholic solution at 517 nm: NA: Not active (activity <10%); NT: Not tested; *Statistically significant, p<0.05 (Mann-Whitney test or Anova).

were considered, highest correlation was obtained with σ_4 and σ_3 . Equation-1 gives the complete regression analysis.

$$B_A = -1.2488 (\pm 0.618) \sigma_4 + 4.6687 (\pm 3.71) \sigma_3 - 0.8353 (\pm 0.268) \quad \text{Eq. 1}$$

(t-value=2.0) (t-value=1.2)
 n=11 r=0.59 s=0.805 $F_{2,8} = 2.10$
 (Table t-value (95%) = 2.306; Table $F_{2,8}$ (0.01) = 8.65)

In the above equation B_A is biological activity, σ_4 and σ_3 are Hammett constants for substituents at position 4 and 3 on the phenyl ring; n, r, s and F are number of compounds considered, correlation coefficient, standard error and F-test of significance respectively. The equation is not statistically significant with low F-value and low correlation coefficient. Perusal of the data shows that 4-CH₃ derivative is the outlier. When this compound was deleted from regression analysis, it resulted in equation 2.

$$B_A = -1.1936 (\pm 0.428) \sigma_4 + 5.3124 (\pm 2.574) \sigma_3 - 1.0166 (\pm 0.194) \quad \text{Eq. 2}$$

(t-value = 2.8)* (t-value = 2.1)
 n=10 r=0.74 s=0.5561 $F_{2,7} = 4.26$
 (Table t-value (95%)=2.37; Table $F_{2,7}$ (0.01)= 9.55)

Equation 2 is an improvement over equation 1. The equation explains 55% of data ($r^2=0.55$). Contribution of σ_4 to the equation is statistically significant. The correlation analysis has shown that none of the physicochemical parameters studied showed good correlation to the antiinflammatory activity. Only the Hammett constant of the substituents at position 4 on the phenyl ring appears

to play some role in influencing the activity.

Thus, compound 2 showed good antiinflammatory activity and significant antiarthritic and analgesic activity and was found to be less ulcerogenic than phenylbutazone. Number of vanillinyl derivatives showed significant scavenging of DPPH free radical.

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