Synthesis and Pharmacological Evaluation of 2-(4-Halosubstituted phenyl)-4,5-diphenyl-1H-imidazoles

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Abstract: In the present study, 2-(4-halosubstituted phenyl)-4,5-diphenyl-1H-imidazoles have been synthesized by refluxing benzil and ammonium acetate with different 4-halosubstituted aromatic aldehydes in glacial acetic acid. The structural assignment of this compound has been made on the basis of elemental analysis, UV, IR, ¹H NMR and Mass spectral data. Toxicity of the compound has been determined. The synthesized compound was evaluated for anti-inflammatory activity against carrageenan induced paw oedema and anticonvulsant activity against maximal electro-shock-induced convulsions in rats.

Keywords: Imidazoles, 4,5-Diphenylimidazoles, Anti-inflammatory activity, Anticonvulsant activity.

Introduction

Imidazole provide one of the most fascinating class of compounds recognized for various pharmacological activities¹ like anti-HIV², anti-convulsant², calcium antagonist and inhibitors of thromboxane A₂ synthase³, antihistaminic⁴, tranquillizer⁵, anti-parkinsonism⁶ and MAO inhibitor⁷. 4,5-Diphenylimidazoles have been reported to possess antisenescence⁸, anti-muscarinic⁹, antiarthritic¹⁰, non sedative anxiolytic¹¹, cardiogenic¹², inhibitors of Acyl CoA Cholesterol *o*-acyl transferase (ACAT)¹³, HMG CoA reductase (HMGR)¹⁴ and HIV-1 protease¹⁵.

Encouraged by these observations, we have synthesized various 2-(4-halosubstituted phenyl)-4,5-diphenyl-1H-imidazoles (3_{a-e}) obtained by the condensation of benzil and ammonium acetate with various 4-halosubstituted aromatic aldehydes (2_{a-e}) in glacial acetic acid and evaluate their anti-inflammatory and anticonvulsant activity against carrageenan induced paw oedema and maximal electro-shock-induced convulsions in rats.

Experimental

The identification and purity of the products were checked by TLC (Merck Silica-60F₂₅₄) with hexane: ethanol: acetic acid (65:30:5) using iodine vapours and UV light as detecting agents and the R_f value were given below. Melting points were measured on open capillaries in a

liquid paraffin bath and are uncorrected. UV ($\lambda_{\max}$) was determined on an Elico SL-164 UV-Visible double beam spectrophotometer in methanol. IR Spectra were taken on a Perkin Elmer 1600, FTIR Spectrophotometer using potassium bromide pellets. ^1H NMR spectra were recorded in $\text{DMSO}-d_6$ on Bruker DRX - 300 (300 MHz) spectrometer using TMS as an internal standard (Chemical shift in δ ppm). FAB mass spectra were taken out on a JEOL SX102/DA-6000 mass spectrometer using Argon/ Xenon (6 kv, 10 mA) as the FAB gas. Elemental analysis was obtained on a Carlo Erba 1108 Heraeus elemental analyzer. All the chemicals used were of LR and AR grade and was procured from Lancaster Ltd., England and Merck, Mumbai, India.

General procedure for the synthesis of 2-(4-halosubstituted phenyl)-4,5-diphenyl-1H-imidazole (3a-e)

A mixture of benzil (0.025 mol), ammonium acetate (0.129 mol) and 4-halosubstituted benzaldehyde(s) (0.025 mmol) (2_{a-e}) in glacial acetic acid (50 mL) was heated under reflux for 5-6 h. After refluxing, the reaction mixture was allowed to stand to attain room temperature. The solid that appeared after the addition of water (150 mL) was filtered. The filtrate was neutralized with ammonium hydroxide to give solid and was filtered. The solid mass obtained from first and second crop was dried in vacuum and recrystallized from absolute ethanol. Yield and melting point of the product(s) were determined.

Compound name and spectral data: 2,4,5-triphenyl-1H-imidazole (3_a)

Yield: 88 %; colourless solid; melting point: 249-251°C; R_f value: 0.82; UV ($\lambda_{\max}$, nm): 299.72; IR (KBr, cm^{-1}): 3416.19 (N-H), 1460.45, 1488.16 (C=C), 1600.12 (C=N), 3037.48 (aromatic C-H); ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 12.80 (1H, s, (N-H)), 8.12 (2H, d, (C_3 and C_5 H of 2-phenyl)), 8.04 (2H, d, (C_2 and C_6 H of 2-phenyl)) and 7.20-7.76 (10H, m, (4&5-diphenyl)); MS (FAB): m/z 296 (M^+ , 50%), 297 ($\text{M}+1$, 100%), 295 ($\text{M}-1$, 12%), 298 (28), 297 (base peak).

2-(4-Fluorophenyl)-4,5-diphenyl-1H-imidazole (3_b)

Yield: 81 %; colourless crystalline solid; melting point: 239-241°C; R_f value: 0.79; UV ($\lambda_{\max}$, nm): 298.4; IR (KBr, cm^{-1}): 3410.0 (N-H), 1492.79, 1450.79 (C=C), 1608.24 (C=N), 3028.30 (aromatic C-H), 1409.94, 1072.25 (C-F); ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 12.82 (1H, s, (N-H)), 8.14 (2H, d, (C_3 and C_5 H of 2-phenyl)), 7.95 (2H, d, (C_2 and C_6 H of 2-phenyl)) and 7.83-7.20 (10H, m, (4&5-diphenyl)); MS (FAB): m/z 314 (M^+ , 48 %), 315 ($\text{M}+1$, 100%), 313 ($\text{M}-1$, 8 %), 316 (25), 315 (base peak).

2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazole (3_c)

Yield: 83%; colourless solid; melting point: 246-248°C; R_f value: 0.85; UV ($\lambda_{\max}$, nm): 310.0; IR (KBr, cm^{-1}): 3390.12 (N-H), 1486.11 (C=C), 1599.74 (C=N), 3026.85 (aromatic C-H), 833.86, 764.48, 672.64 (C-Cl); ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 12.80 (1H, s, (N-H)), 8.12 (2H, d, (C_3 and C_5 H of 2-phenyl)), 8.04 (2H, d, (C_2 and C_6 H of 2-phenyl)) and 7.84-7.20 (10H, m, (4&5-diphenyl)); MS (FAB): m/z 331 (M^+ , 100%), 332 ($\text{M}+1$, 36 %), 330 ($\text{M}-1$, 45%), 333 (32), 331 (base peak).

2-(4-Bromophenyl)-4,5-diphenyl-1H-imidazole (3_d)

Yield: 72%; pale brown solid; melting point: 221-223°C; R_f value: 0.72; UV ($\lambda_{\max}$, nm): 310.15; IR (KBr, cm^{-1}): 3415.82 (N-H), 1599.46, 1483.15 (C=C), 1638.13 (C=N), 3030.0 (aromatic C-H stretch), 715.13, 671.82 (C-Br); ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 12.80 (1H, s, (N-H)), 8.11 (2H, d, (C_3 and C_5 H of 2-phenyl)), 7.83 (2H, d, (C_2 and C_6 H of 2-phenyl)) and 7.81-7.20 (10H, m, (4&5-diphenyl)); MS (FAB): m/z 375 (M^+ , 100%), 376 ($\text{M}+1$, 61%), 374 ($\text{M}-1$, 42%), 377 (88), 375 (base peak).

2-(4-Iodophenyl)-4,5-diphenyl-1H-imidazole (3_e)

Yield: 65 %; brown solid; melting point: 197-199°C; R_f value: 0.62; UV (λ_{max} , nm): 313.13; IR (KBr, cm⁻¹): 3416.07 (N-H), 1594, 1578.61, 1481.50 (C=C), 1659.29 (C=N), 3000.0 (aromatic C-H stretch), 642.28 (C-I); ¹H NMR (DMSO-d₆, δ ppm): 12.76 (1H, s, (N-H)), 8.12 (2H, d, (C₃ and C₅ H of 2-phenyl)), 7.95 (2H, d, (C₂ and C₆ H of 2-phenyl)) and 7.64-7.21 (10H, m, (4&5-diphenyl)). MS (FAB): *m/z* 422 (M⁺, 58%), 423 (M+1, 100%), 421 (M-1, 8%), 424 (28), 423 (base peak).

Acute Toxicity Studies

Albino mice of either sex were divided into twenty-five groups containing ten animals of each and treated *i.p.* with the dose ranges from 40-220 mg/ kg test drugs. The animals were observed for mortality till 72h and the LD₅₀ was calculated using graphical method¹⁶.

Anti-inflammatory activity

Anti-inflammatory activity was determined by carrageenan induced rat paw oedema method¹⁷. Swiss albino rats (120-150 g) were used in this study and worked-up as above method. The positive control used here was indomethacin (10 mg/kg *i.p.*). The test groups received between 105.64 and 121.81 mg/kg of compounds (3_{a-e}) *i.p.* After the carrageenan injection the paw volume was measured immediately and at 1, 2, and 3hr by plethysmometer (UGO-BASILE, Italy). The difference between the left and right paw was taken as a measure of oedema. Any significant reduction in the volume of the paw compared to the control group was considered as anti-inflammatory response.

Antiepileptic activity

Electro shock method^{18,19} was followed to study the antiepileptic activity. Swiss albino rats (120-150g) were used in this study and worked-up as above method. The positive control used here was phenytoin (25mg/kg *i.p.*). The test groups received 105.64 and 121.81mg/kg of compounds (3_{a-e}) *i.p.* Supramaximal electroshock of 150mA for 0.25sec by a techno convulsimeter was given to the rats. Animals which showed positive hind limb tonic extensor response during pre-screening were selected and the test drugs were injected *i.p.* half an hour before supramaximal shock. The protective index was defined as the abolition or reduction of the hind limb tonic extension component of the seizure.

Results and Discussion

2-(4-Halosubstituted phenyl)-4,5-diphenyl-1H-imidazoles (3_{a-e}) was synthesized by condensation reaction involving three reagents such as benzil, 4-halosubstituted benzaldehydes(s) and ammonium acetate in glacial acetic acid (Scheme 1). The physical data of the compounds (3_{a-e}) were collected and are presented under compound name and spectral data. The yields of (3_{a-e}) fall in the range of 65-88%. Most of them are colorless crystalline solids. The spectral (IR, ¹H NMR and MS) and analytical data are in good agreement with their structures. The analytical data of the compounds (3_{a-e}) are presented in Table 1.

Scheme 1. Synthesis of 2-(4-halosubstituted phenyl)-4,5-diphenyl-1H-imidazoles (3_{a-e})

The infrared spectra of (3_{a-e}) showed the characteristic absorption bands of imidazole moiety (ν N-H) at 3416.19-3390.12 and (ν C=N) 1659.29-1599.74 cm^{-1} . The presence of *p*-halosubstituted phenyl at 2-position of (3_{a-e}) has exhibited their C-X (X= F, Cl, Br and I) characteristic stretching bands at 1072.25, 764.48, 642.28 cm^{-1} .

In the NMR spectra, the proton on N-1 position of (3_{a-e}) showed a sharp singlet at δ 12.82-12.76 ppm. The sharp singlet may be due to the rapid exchange of the proton on nitrogen atom which may be decoupling from it. The influence of -I effect contributed by $-\text{C}_6\text{H}_4\text{-X}$ (p) (X= F, Cl, Br, I) at 2-position of imidazole moiety is felt on the chemical shift values of N-H proton. As a result, the NH signal goes to downfield compared to the parent moiety. Electron withdrawing groups F, Cl, Br and I (3_{a-e}) caused the C₃ and C₅-H for downfield shift compared to C₂ and C₆-H. The mass spectra of these compounds show the molecular ion peak as base peak (100%).

Table 1. Analytical data of compounds (3_{a-e})

Compound No.	Molecular Formula	Elemental Analysis Found (Calcd.), %		
		C	H	N
3a	C ₂₁ H ₁₆ N ₂	84.66 (85.10)	5.52 (5.54)	9.15 (9.45)
3b	C ₂₁ H ₁₅ N ₂ F	80.17 (80.23)	4.81 (4.80)	8.59 (8.91)
3c	C ₂₁ H ₁₅ N ₂ Cl	77.16 (76.24)	4.65 (4.57)	8.52 (8.46)
3d	C ₂₁ H ₁₅ N ₂ Br	67.36 (67.21)	4.05 (4.02)	7.49 (7.47)
3e	C ₂₁ H ₁₅ N ₂ I	59.26 (59.73)	4.05 (3.58)	6.23 (6.63)

The toxicity study of compounds (3_{a-e}) indicates the LD₅₀ values lie in between 105.64 and 121.81mg/kg, body weight. The therapeutic dose of the drug is considered as 1/10 of LD₅₀ value.

The results in Table 2 and Figure 1 indicate that the compounds 3_b and 3_c are equipotent active ($P < 0.001$) with the standard. Moreover, compounds 3_a, 3_d and 3_e show less significant anti-inflammatory activity ($P < 0.01$). Carageenan induced paw oedema was taken as a prototype of exudative phase of inflammation. The development of oedema has been described as biphasic²⁰. The initial phase is due to the release of histamine, serotonin and kinins in the first hour, after injection of carrageenan. More pronounced second phase is related to the release of prostaglandin like substances in 2-3 h. Hence, the significant anti-inflammatory effect may be due to an inhibitory effect exerted predominantly on the mediators of inflammation induced by phlogogenic stimuli. The compounds 3_b, 3_c and 3_d exhibit significant antiepileptic activity ($P < 0.001$) and the compounds 3_a and 3_e show the less significant activity ($P < 0.01$) (Table 3, Figure 2). Drugs like phenytoin, methoin and ethotoin have nucleus of reduced form of imidazole. Hence the antiepileptic activity of the compounds may be attributed to the fact that these drugs may either block the initiation of electric impulses from the focal area or spread of abnormal electric discharge to adjacent brain tissues. This may cause for decrease in post tinitic potentiation which may be responsible for the spread of seizure discharge²¹.

Table 2. Anti-inflammatory activity of compounds (3_{a-e})

Compound No	Dose, mg/kg	Increase in paw volume after 3h. (mean $\pm$ SEM)	Decrease in paw volume, % (mean $\pm$ SEM)
3a	107.96	0.17 $\pm$ 0.01*	22.99 $\pm$ 0.21
3b	106.45	0.01 $\pm$ 0.00**	44.13 $\pm$ 0.12
3c	121.81	0.01 $\pm$ 0.00**	48.28 $\pm$ 0.18
3d	105.18	0.04 $\pm$ 0.00**	40.23 $\pm$ 0.12
3e	106.29	0.09 $\pm$ 0.01**	33.33 $\pm$ 0.31
Control	5mL/kg	-----	-----
Standard	25mL/kg	0.02 $\pm$ 0.00	48.28 $\pm$ 0.34

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.02$ P Vs Standard

Table 3 Anticonvulsant activity of compounds (3_{a-e})

Compounds	Flexor	Extensor	Clonus	Stupor
3a	2.33 ± 0.7	8.16 ± 0.06	2.33 ± 0.07	114.5 ± 1.06
3b	2.33 ± 0.07	0.6 ± 0.01**	2.16 ± 0.11	102 ± 1.46
3c	2.33 ± 0.07	0.16 ± 0.06**	2.16 ± 0.11	98.5 ± 1.061
3d	2.83 ± 0.11	0.5 ± 0.08**	3.0 ± 0.13	101.33 ± 2.08
3e	2.16 ± 0.11	3.66 ± 0.157*	2.66 ± 0.12	98.66 ± 1.79
Control	2.86 ± 0.07	9.5 ± 0.15	2.5 ± 0.15	117.5 ± 0.48
Standard	2.66 ± 0.07	1.16 ± 0.17**	42 ± 0.36	102 ± 2.17

*P<0.01, **P<0.01

Figure 1. Anti-inflammatory activity of compounds (3_{a-e}) **Figure 2.** Anticonvulsant activity of compounds (3_{a-e})

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