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DRUG SYNTHESIS

SYNTHESIS AND BIOLOGICAL SCREENING OF DI- AND TRISUBSTITUTED IMIDAZOLES

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Abstract: Disubstituted imidazoles were prepared by reacting appropriate phenylglyoxal with different aryl aldehydes in the presence of ammonium acetate. Trisubstituted imidazoles were prepared by reacting disubstituted imidazoles with chlorobenzene in the presence of catalytic amount of triethylamine (TEA). The synthesized compounds were characterized on the basis of IR, ^{1}H-NMR and mass spectral data and elemental analysis results. They were tested for their antiinflammatory and antimicrobial actions. Two compounds showed good antiinflammatory activity in carrageenan induced rat paw edema test with very low ulcerogenic activity. Fair number of compounds were found to have significant antimicrobial activity especially against fungal species.

Keywords: substituted imidazoles, phenylglyoxal, antiinflammatory, ulcerogenic, antibacterial, antifungal

The chemistry of nitrogen heterocyclic compounds especially imidazoles has attracted more attention during recent years due to their reactivity and novel biological activities. Compounds bearing imidazole nucleus are known to show unique antiedema and antiinflammatory activities (1, 2). Differently substituted imidazoles have also been found to have other important activities such as anthelmintic (3, 4), analgesic (5), anti-bacterial (6), anti-fungal (7), antiviral (8), antitubercular (9), anti-cancer (10) and COX-2/LOX inhibitor (2, 11) and they are also known as melanocortin-4 receptor (MC4-R) antagonists (12). Besides their pharmacological actions they also function as dyestuffs catalysts and polymerizing agents (13). 2-Nitroimidazole (azomycin) and 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole) are good antimicrobial agents with particular applications as trichomonacides. Along with metronidazole other nitroimidazoles (misonidazole, metrazole and clotrimazole) are important anticancer drugs. Two imidazolines priscol and privine are valuable vasodilating and vasoconstricting drugs. In view of these observations, it was considered worthwhile to study various di- and trisubstituted imidazoles.

EXPERIMENTAL

The melting points were determined in open glass capillary tubes using Kjeldahl flask containing liquid paraffin and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates and spots were visualized by exposure to iodine vapors. Microanalysis was done on Perkin-Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. ^{1}H-NMR spectra were recorded on DPX-300 NMR spectrometer and BRUKER-400 Ultra Shield™ spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS). Mass spectra were recorded on a Jeol JMS-D 300 instrument. The physical constants and spectral data of the synthesized compounds are presented in Table 1.

Synthesis of phenylglyoxals (**1a,b**)

A mixture of pure selenium dioxide (0.5 mol), water (10 mL), and dioxane (300 ml) was heated in a round bottom flask (500 mL) at 50-55°C with stirring till clear solution was obtained. To the solution acetophenone/4-chloroacetophenone (0.5 mol) was added in one lot. The mixture was further refluxed with stirring for 4 h, during refluxing small amount of selenium was precipitated. The reaction mixture was decanted to remove the precipitate. The clear solution so obtained was distilled to remove excess dioxane and water. A yellow liquid was found pure on TLC examination (toluene : ethyl acetate : formic acid, 5:4:1, v/v/v).

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Synthesis of 2,4-disubstituted-1*H*-imidazoles **(2a-i)**

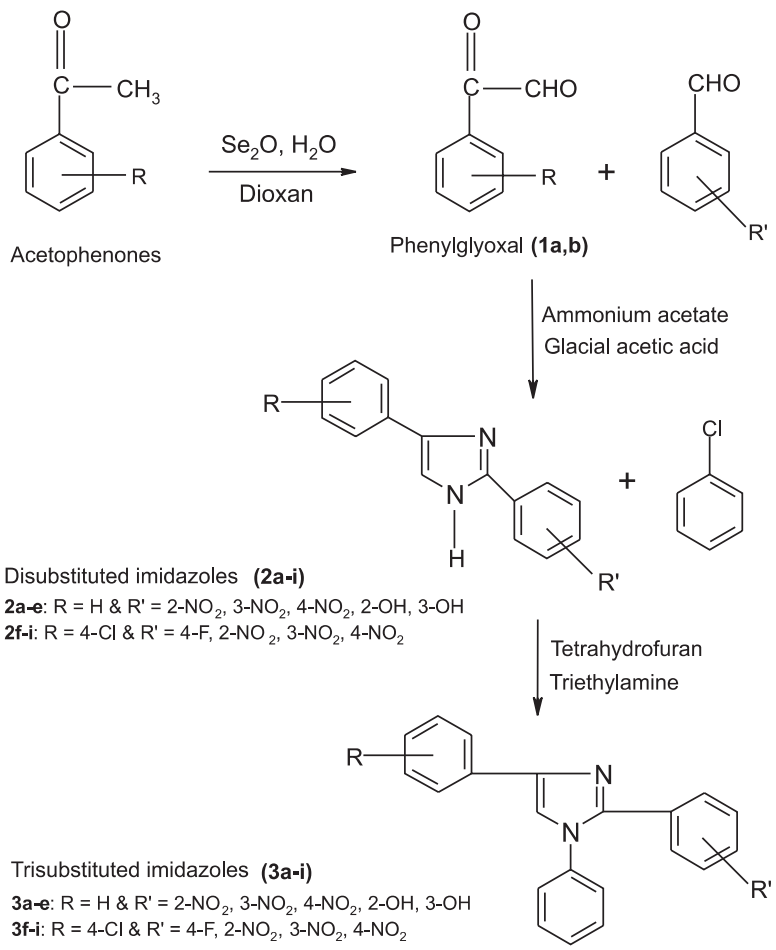
A mixture of compound **1a,b** (0.025 mol), aryl aldehyde (0.025 mol) and ammonium acetate (10 g) in glacial acetic acid (50 mL) was refluxed in round bottom flask for 5 h. After refluxing, the mixture was cooled to room temperature and then poured into cold water (200 mL). A precipitate was separated out, which was filtered, washed, dried and crystallized from acetone. The compound was found pure by TLC (toluene : ethyl acetate : formic acid, 5:4:1, v/v/v).

Synthesis of 1,2,4-trisubstituted-1*H*-imidazoles (**3a-i**)

Compound **2a-h** (0.01 mol) was suspended in tetrahydrofuran (20 mL) and was refluxed with excess of chlorobenzene (2 mL) in the presence of 2 to 3 drops of triethylamine, for 8 h. The completion of reaction was determined by TLC. After refluxing, acetone was added to the reaction mixture and it was kept at room temperature overnight. Later a precipitate formed which was filtered and recrystallized from ethanol. The compound was found pure on TLC examination (benzene : acetone, 8 : 2, v/v).

Biological evaluation

Wistar rats used in this study were housed and treated in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC). The protocol of animal experiments has been approved by the IAEC. Wistar rats of either sex (Hamdard University, Animal House, New Delhi, India), weighing 180-200 g were used. Pregnant females were excluded. The animals were housed in groups of six and acclimatized to room conditions for at least 2 days before the experiments. Food and water



Scheme 1. Protocol for synthesis of substituted imidazoles (**2a-i, 3a-i**)

Table 1. Physical and analytical data of the title compounds.

Compd. no.	R	R'	M.p. (°C)	Yield (%)	Mol. Form.#/ Mol. Wt.	†¹H NMR / Mass spectral data
1a	H	-	54-56	72	$C_8H_6O_2$ 134.13	7.42-7.51 (m, 3H, H-3,4,5), 7.61-7.66 (m, 2H, H-2,6), 9.06 (s, 1H, CHO).
1b	4-Cl	-	64	78	$C_8H_5ClO_2$ 168.57	7.39 (d, 2H, H-3,5), 7.57 (d, 2H, H-2,6), 9.72 (s, 1H, CHO).
2a	H	2-NO_2	174	40	$C_{15}H_{11}N_3O_2$ 265.27	7.41-7.47 (m, 3H, H-4',2,6), 7.50 (s, 1H, H-4), 7.59-7.67 (m, 2H, H-5' + 1H imidazole), 7.88 (s, 1H, H-3'), 7.93 (d, 2H, H-3, 5), 8.02 (s, 1H, H-6'), 10.26 (s, 1H, NH). 265 (M^+), 219, 143, 116, 67, 53.
2b	H	3-NO_2	198-200	42	$C_{15}H_{11}N_3O_2$ 265.27	7.51-7.59 (m, 3H, H-2,4,6), 7.61 (s,1H, imidazole), 7.94-8.01 (m, 3H, H- 4',3,5), 8.06 (s, 1H, H-5'), 8.52 (d, 2H, H-2',6'), 9.23 (s, 1H, NH). 265 (M^+), 204, 128, 67.
2c	H	4-NO_2	163-65	64	$C_{15}H_{11}N_3O_2$ 265.27	7.42 (d, 2H, H-2,6), 7.51 (s, 1H, H-4), 7.60 (s,1H, imidazole), 8.13 (d, 2H, H-3,5), 8.19 (d, 2H, H-3',5'), 8.43 (d, 2H, H-2',6'), 10.16 (s, 1H, NH). 265 (M^+), 219, 204, 188, 143, 128, 67, 53.
2d	H	2-OH	178	48	$C_{15}H_{12}N_2O$ 236.27	4.46 (s, 1H, OH), 6.81-7.14 (m, 3H, H-3',4',5'), 7.42-7.51 (m, 3H, H-2,4,6), 7.53 (s, 1H, imidazole), 7.95 (d, 2H, H-3, 5), 8.43 (s, 1H, H-6'), 10.33 (s, 1H, NH). 236 (M^+), 218, 219, 143, 116.
2e	H	3-OH	169-71	45	$C_{15}H_{12}N_2O$ 236.27	4.59 (s, 1H, OH), 7.33-7.52 (m, 5H, H-4',5',2,4,6), 7.61 (s, 1H, imidazole), 7.95-8.03 (m, 4H, H-2', 6',3,5), 10.36 (s, 1H, NH). 236 (M^+), 218, 143, 67, 53.
2f	4-Cl	4-F	180	74	$C_{15}H_{10}ClFN_2$ 272.71	7.58 (s, 1H, imidazole), 7.61 (d, 2H, H-2,6), 7.90-8.01 (m, 4H, H-2',6',3,5), 8.16 (d, 2H, H-3',5'), 9.63 (s, 1H, NH). 272 (M^+), 273 (M+1), 204, 143, 128.
2g	4-Cl	2-NO_2	174	43	$C_{15}H_{10}ClN_3O_2$ 299.71	7.19 (d, 2H, H-3,5), 7.44-7.86 (m, 6H, H-3',4',5',2,6 + imidazole), 7.92 (d, 1H, H-6'), 10.86 (s, 1H, NH). 299 (M^+), 300 (M+1), 219, 143, 128, 67.
2h	4-Cl	3-NO_2	189-91	62	$C_{15}H_{10}ClN_3O_2$ 299.71	7.36-7.52 (m, 4H, H-5',6',2,6), 7.60 (s,1H,, imidazole) 7.91-8.03 (m, 4H, H-2',4',3,5), 10.35 (s, 1H, NH). 299 (M^+), 300 (M+1), 204, 128. 7.50 (d, 2H, H-3,5), 7.65 (s, 1H, imidazole),
2i	4-Cl	4-NO_2	190-92	60	$C_{15}H_{10}ClN_3O_2$ 299.71	7.66 (d, 2H, H-2,6), 8.20 (d, 2H, H-3',5'), 8.61 (d, 2H, H-2',6'), 13.73 (s, 1H, NH). 299(M^+), 300 (M+1), 204, 144, 128, 67, 52.
3a	H	2-NO_2	250	40	$C_{21}H_{15}N_3O_2$ 341.37	7.18-7.64 (complex m, 14H, 3 × phenyl), 7.89 (s, 1H, imidazole). 341 (M^+), 204, 67, 53.
3b	H	3-NO_2	210-12	48	$C_{21}H_{15}N_3O_2$ 341.37	7.20-7.27 (m, 3H, H-3,4,5), 7.42-7.45 (m, 4H, H-2,6,2",6"), 7.50 (s, 1H, H-4"), 7.58 (d, 2H, H-2',6'), 7.86-7.94 (m, 3H, H-4',3",5"), 8.28 (d, 2H, H-5' + imidazole). 341 (M^+), 204, 77, 53.
3c	H	4-NO_2	229-31	63	$C_{21}H_{15}N_3O_2$ 341.37	7.05-7.58 (complex m, 14H, 3 x phenyl), 7.97 (s, 1H, imidazole). 341 (M^+), 204, 77, 53.
3d	H	2-OH	196	40	$C_{21}H_{16}N_2O$ 312.37	4.51 (s, 1H, OH), 6.87-7.49 (complex m, 14H, 3 x phenyl), 7.82 (s, 1H, imidazole). 312 (M^+), 204, 77, 53
3e	H	3-OH	182	41	$C_{21}H_{16}N_2O$ 312.37	4.66 (s, 1H, OH), 7.24-7.49 (complex m, 14H, 3 x phenyl) 7.93 (s, 1H, imidazole). 312 (M^+), 77, 53.
3f	4-Cl	4-F	240-42	53	$C_{21}H_{14}ClFN_2$ 348.81	7.18-7.91 (complex m, 13H, 3 x phenyl), 7.95 (s, 1H, imidazole). 348 (M^+), 349 (M+1), 77, 53.

Table 1. cont.

Compd. no.	R	R'	M.p. (°C)	Yield (%)	Mol. Form.#/ Mol. Wt.	^{1}H NMR / Mass spectral data
3g	4-Cl	2-NO_2	210-12	51	$C_{21}H_{14}ClN_3O_2$ 375.81	7.24-7.93 (complex m, 13H, 3 x phenyl), 8.16 (s, 1H, imidazole). 375(M^+), 376(M+1), 204, 77, 53.
3h	4-Cl	3-NO_2	234	54	$C_{21}H_{14}ClN_3O_2$ 375.81	7.31-7.89 (complex m, 13H, 3 x phenyl), 8.06 (s, 1H, imidazole). 375(M^+), 376(M+1), 77, 53.
3i	4-Cl	4-NO_2	250-52	58	$C_{21}H_{14}ClN_3O_2$ 375.81	7.33-7.37 (m, 5H, H-2**?**,3**?**,4**?**,5**?**, 6**?**), 7.45-7.48 (m, 4H, H-2,6,2**'**,6**'**), 7.57-7.79 (m, 4H, H-3,5,3**'**,5**'**), 8.13 (s, 1H, imidazole). 375(M^+), 376(M+1), 204, 77, 53.

#Elemental analysis for C, H, N were within ± 0.4% of the theoretical value; †s = singlet; d = doublet; m = multiplet.

were freely available up to the time of experiments. The food was withdrawn on the day before the experiment, but free access to water was allowed.

Antiinflammatory activity

Antiinflammatory activity of the synthesized compounds was evaluated by carrageenan induced rat paw edema method (14) on groups of six animals each. A freshly prepared suspension of carrageenan (1% w/v solution in 0.9% saline, 0.1 mL) was injected in the plantar region of the right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs (10 mg/kg body weight) suspended in 1.0% carboxymethylcellulose (CMC) given orally 1 h before the carrageenan treatment. The volume was measured before and after 3 h of carrageenan treatment using a plethysmometer. The activity was calculated according to the following formula:

$$\text{Antiinflammatory activity (\% inhibition)} = (Vc - Vt) / Vc \times 100$$

where Vc and Vt are the edema volumes in the control group and drug-treated groups. Indomethacin was used as the standard drug for comparison.

Ulcerogenic activity

Two most active compounds in antiinflammatory assays, namely **3c**, and **3g**, were also tested for their ulcerogenic activity in rats (15). The rats were divided into groups consisting of six animals in each group. Ulcerogenic activity evaluated after *p.o.* administration of test compounds or indomethacin at the dose of 60 mg/kg body weight. Control group rats received vehicle (suspension of 1% CMC). After the drug treatment, the rats were fed normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The gastric mucosa of the rats were examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following system: 0.5 = redness; 1.0 = spot ulcers; 1.5 = hemorrhagic streaks; 2.0 = ulcers > 3, but ≤ 5; 3.0 = ulcers > 5. The mean score of each treated group was regarded as severity index of gastric mucosal damage.

Table 2. Antiinflammatory activity of synthesized compounds.

Compound no.	% Inhibition ± SEM (after 3 h) #
2c	21.87 ± 1.33
2d	25.00 ± 0.80*
2f	28.90 ± 1.36*
2g	35.15 ± 0.90*
2h	31.25 ± 1.22*
2i	38.28 ± 0.92*
3c	58.59 ± 1.70*
3d	24.21 ± 0.60*
3f	35.93 ± 0.75*
3g	51.56 ± 1.79*
3h	28.90 ± 0.72*
3i	23.43 ± 0.40*
Control - Indomethacin	68.48 ± 1.18*

#Relative to standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n = 6; *p value < 0.01.

Antibacterial and antifungal study

All the synthesized compounds were screened for *in vitro* antibacterial and antifungal activities at the concentration of 100 and 200 µg/mL by cup plate method (16). The bacterial strains Gram positive (*Bacillus subtilis*, MTCC-441), Gram negative (*Escherichia coli,* MTCC-40) and fungal strains (*Candida albicans*; MTCC-183 and *Aspergillus flavus*; MTCC-871) were used. DMF was used as a

Table 3. Antibacterial and antifungal activities of the synthesized imidazoles.

Compound no.	Conc. (μg/mL)	Diameter of zone of inhibition [mm]			
		E. coli	*B. subtilis*	*C. albicans*	*A. flavus*
Voriconazole	20	—	—	27	31
Ofloxacin	20	27	29	—	—
2a	100 200	12 16	10 15	10 14	12 18
2b	100 200	14 20	15 18	13 15	18 22
2c	100 200	16 18	12 12	12 14	18 22
2d	100 200	12 18	11 15	12 15	19 25
2e	100 200	10 12	08 12	10 12	12 19
2f	100 200	14 18	12 14	14 16	25 31
2g	100 200	12 16	09 12	11 14	16 20
2h	100 200	13 19	13 15	14 18	19 25
2i	100 200	18 22	12 14	12 15	18 24
3a	100 200	11 18	11 17	11 18	12 18
3b	100 200	16 24	14 16	17 20	18 25
3c	100 200	15 20	12 12	12 14	19 24
3d	100 200	12 19	10 15	13 15	21 27
3e	100 200	10 14	08 12	12 16	18 22
3f	100 200	15 20	13 16	16 18	24 29
3g	100 200	12 16	11 15	13 18	15 19
3h	100 200	13 20	14 16	16 21	21 27
3i	100 200	17 22	12 15	12 16	17 23

solvent. Plates were incubated for 24 h and 48 h at 37°C for antibacterial and antifungal activity, respectively, and zone of inhibition was measured in mm. Ofloxacin and voriconazole were used as reference drugs for comparison.

RESULTS AND DISCUSSION

Chemistry

The di- and trisubstituted imidazoles described in this study are shown in Table 1 and the reaction sequence for the synthesis is outlined in Scheme 1. Disubstituted imidazoles (**2a-i**) were prepared by reacting appropriate phenylglyoxal (**1a,b**) with different aryl aldehydes in the presence of ammonium acetate. The required phenylglyoxals (starting material) were prepared by refluxing *via* stirring acetophenone/4-chloroacetophenone in dioxane with selenium dioxide. Trisubstituted imidazoles (**3a-i**) were prepared by reacting disubstituted imidazole (**2a-i**) with chlorobenzene in the presence of catalytic amount of triethylamine (TEA). The purity of the

compounds was checked by TLC and elemental analyses. The structures were confirmed on the basis of spectral data (MS and ^{1}H-NMR) (Table 1).

Antiinflammatory activity

Antiinflammatory activity was carried out at a dose of 10 mg/kg *p.o*., using indomethacin as reference drug at the same dose level. The compounds tested showed activity in the range of 21.87-58.59% inhibition. Two compounds (**3c** and **3g)** out of twelve, showed significant antiinflammatory activity (58.59 and 51.56% inhibition, respectively), while the standard drug indomethacin showed 68.48% inhibition. The rest of compounds was moderate in their action (Table 2).

Ulcerogenic activity

The ulcerogenic liability of two most active compounds in antiinflammatory assays, namely **3c**, and **3g,** and indomethacin was evaluated after oral administration at the dose of 60 mg/kg in rats (15). The tested compounds showed low ulcerogenic activity (severity index = 0.666 and 0.833, respectively), whereas the standard drug indomethacin showed high ulcerogenic activity (severity index = 2.66).

Antibacterial and antifungal study

Among the tested compounds, **2f, 3d, 3f,** and **3h** exhibited very good activity against *A. flavus*. Compound **2i, 3b,** and **3i** showed good activity against *E. coli*, compounds **3b** and **3h** were also good in their action against *C. albicans*. Moderate activity was shown by all the tested compounds against *B. subtilis*. An analysis of the results showed that these compounds were having better activity against fungal species especially *Aspergillus flavus* in comparison to bacterial species, *Bacillus subtilis* and *Escherichia coli*. (Table 3).

CONCLUSIONS

In conclusion, the present study reveals the antiinflammatory and antimicrobial potential of di- and trisubstituted imidazoles. The results indicated that compounds **3c** and **3g** showed significant antiinflammatory activity with very low ulcerogenicity. Some compounds like **2f**, **2i**, **3d**, **3f**, **3h** and **3i** also showed significant antimicrobial activity. An analysis of antimicrobial results showed that the synthesized compounds had better activity against fungal species than bacterial species. Thus, the above mentioned compounds due to their higher degree of activity obviously may have future commitment for the development of safer antiinflammatory and antifungal moieties.

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