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Synthesis, antibacterial, anti-inflammatory and anti-allergy activities of 2,4-pyridinedicarbohydrazide and its related derivatives

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ABSTRACT

2,4-Pyridinedicarbohydrazide **(3)** was used in synthesizing several new heterocyclic compounds with expected potential biological activity, *via* its reaction with various chemical reagents. A series of bis-(arylmethylidene)pyridine-2,4-dicarbohydrazides **(4a-e)** were synthesized by condensation of **(3)** with various aldehydes. Further, replacement of the dicarbxylic acid functionality of **(1)** with five membered heterocycles, in the hope of obtaining additional pharmacological activity, encouraged us to synthesize some 2,4-bis-{pyrazoly1 **(5)**; pyrazolony1 **(6)**; 1,3,4-oxadiazolyl **(7)**, **(9)**, **(11)**, **(14)**; 1,2,4-triazolyl **(15)** and triazolo[3,4-*b*][1,3,4]thiadiazinyl **(17)**}pyridines. The structures of the synthesized products were identified by elemental analysis, IR, ^{1}H NMR and EIMS spectroscopy. The synthesized compounds were screened for their *in vitro* growth inhibiting activity against different strains of bacteria and compared with the standard antibiotic (Chloramphenicol) at triplicate concentration (5, 2.5,1 μg/ml). Also, they were screend for their *in vivo* anti-inflammatory activity [% Inhibition of oedema and % inhibition of plasma prostaglandin (PGE_2)] and anti-allergic activity (anti-histamine). Their toxicity (ALD_{50} mg kg^{-1} p.o.) and ulcerogenic (UD_{50} mg kg^{-1} i.p.) activities were measured and promising results were obtained.

KEYWORDS

Pyridine;
Antibacterial;
Anti-inflammatory;
Anti-allergy.

INTRODUCTION

The extensive use of antibiotics has led to the appearance of multi-drug resistant microbial pathogens[1]. This highlights the incessant need for the development of new classes of antimicrobial agents and alteration of known drugs in such way that would allow them to retain their physiological action, but reducing their resistance to the pathogens. The design of novel chemotherapeutic agents is particularly beneficial due to their dissimilar mode of action which can avoid cross resistance to known drugs[2].

Asthma, inflammation and allergic diseases are of current interest[3] because there are no selective drugs for the treatment of most of the diseases like rheumatoid arthritis[4], allergic rhinitis, psoriasis[5,6], ulcerative colitis and asthma[7].

Pyridine is one of the most popular *N*-heteroaromatics incorporated into the structure of many pharmaceuticals. Also, many naturally occurring as well

as synthetic compounds containing the pyridine scaffold exhibit interesting pharmacological properties[8,9]. Among these pyridine derivatives were found to have antimicrobial[10], anti-hypertensive[11], cardiovascular[12], anti-inflammatory, analgesic, antipyretic properties[13] as well as 1KK-inhibitor properties[14].

In the present work, as part of our ongoing development of efficient protocols for the preparation of biologically active heterocycles from common intermediates, keeping in mind the biological activity of the pyridine derivatives and our continuing interest in the preparation and pharmacological evaluation of pyridine derivatives[15-17], pyridinedicarboxylic acids[18-22] and 2,4-disubstitutedpyridines[23,24], we report the synthesis, characterization, antibacterial, anti-inflammatory and anti-allergic activities of a new series of 2,4-disbstitutedpyridines.

RESULTS AND DISCUSSION

2,4-Pyridinedicarbohydrazide **(3)**, the key intermediate for this work, was readily available, in 90% yield, *via* esterification of **(1)** to get 2,4-dimethoxycarbonylpyridine **(2)**, followed by the treatment of **(2)** with hydrazine hydrate in methanol. The N^2,N^4-bis-(arylmethylidene)pyridine-2,4-dicarbohydrazide derivatives **(4a-e)** were prepared by acid catalyzed condensation of **(3)** with the appropriate aromatic aldehydes in ethanol[25]. The structures of **(4a-e)** were confirmed by their elemental analyses and spectral (MS, IR and ^{1}H NMR) data. For example their ^{1}H NMR revealed, in each case, a characteristic signal in the region δ 7.98-8.07 ppm assignable to the -N=CH protons. Their IR spectra showed the characteristic band for the N-H stretch of the hydrazone group in the region 3291-3257 cm^{-1} (scheme 1).

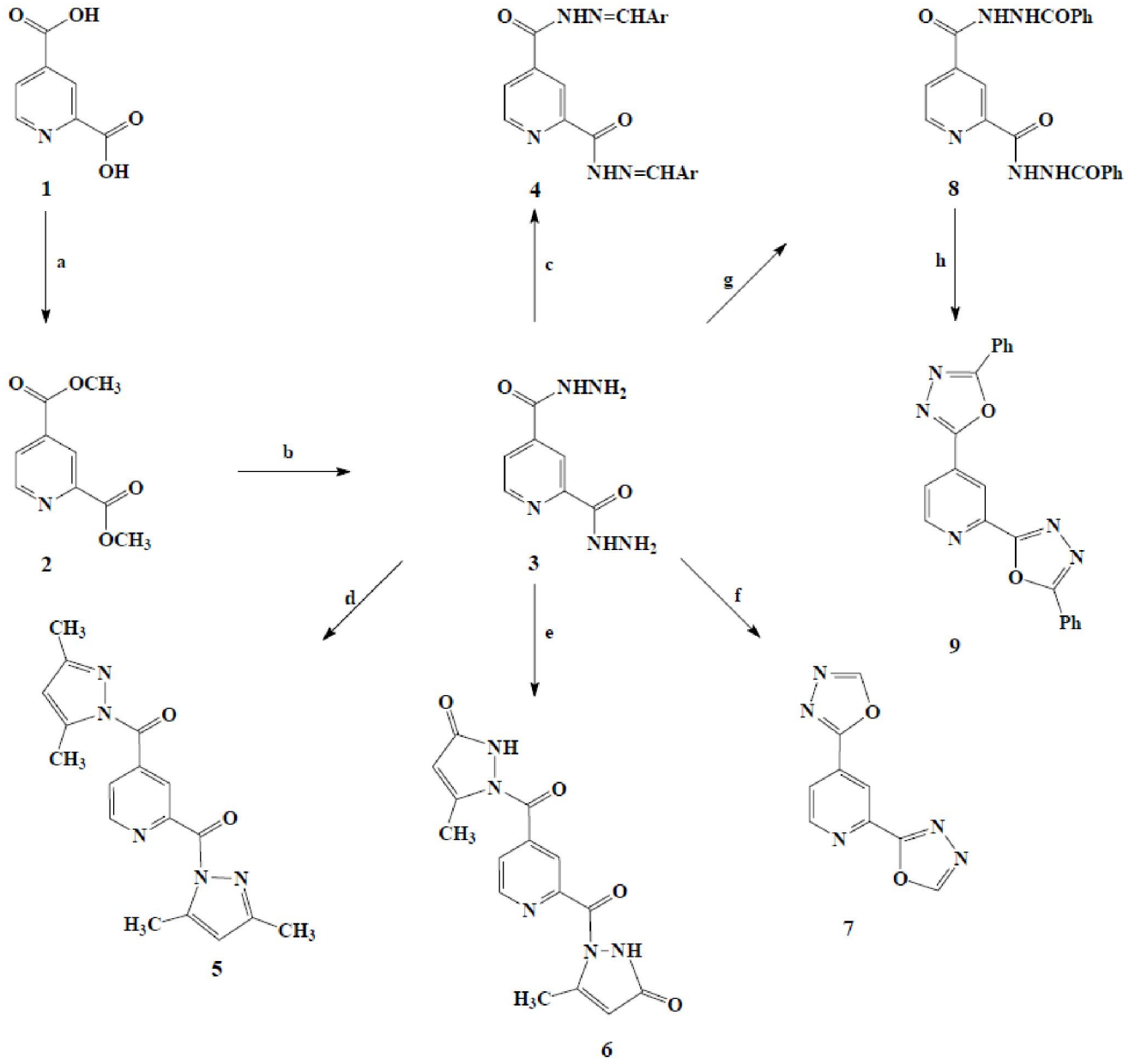
Pyrazoles belong to an important class of compounds which possess a wide variety of pharmaceutical and agrochemical properties[26,27]. The main synthetic method used to prepare pyrazoles involves a [3+2] cyclization such as the classical 1,3-diketone with hydrazines[28,29]. In the present investigation, 2,4-pyridinedicarbohydrazide **(3)** was condensed with 2,4-pentanedione and ethyl acetoacetate readily, on heating without solvent, to afford the corresponding 2,4-bis-[(3,5-dimethyl-1*H*-pyrazol-1-yl)-*N*-carbonyl]pyridine **(5)** and 2,4-bis-[(3-methyl-1*H*,2*H*-pyrazol-5-onyl)-*N*-carbonyl]-pyridine **(6)**, respectively.

A number of 1,3,4-oxadiazole derivatives have been reported to exert most notable antimicrobial and anti-inflammatory activities[30]. Moreover 1,3,4-oxadiazolones have fungicidal, herbicidal, ant-inflammatory, analgesic and antipyretic activities[31]. In addition 1,2,4-triazole derivatives exhibited pronounced antibacterial, antifungal, analgesic and anti-inflammatory activities[32]. These observations prompted us to synthesize some 1,3,4-oxadiazoles and their bioisosteric analogues 1,3,4-thiadiazoles as well as 1,2,4-triazole derivatives. Thus, the 2,4-bis-(1,3,4- oxadiazol-2-yl)pyridine **(7)** was synthesized by condensing 2,4-pyridinedicarbohydrazide **(3)** with triethyl orthformate in DMF. Reaction of **(3)** with benzoyl chloride in dry pyridine gave **(8)**, which was cyclized to 2,4-bis-(5-phenyl-1,3,4-oxadiazol-2-yl)pyridine **(9)** by the action of PPA in 54% yield. The IR spectra of **(7)** and **(9)** are devoid of both amide and amino bands of **(3)**; also their ^{1}H NMR spectra revealed the absence of signals corresponding to NH protons in addition to the expected signals at δ= 6.92 ppm for oxadiazolyl protons of **(7)** and phenyl protons at δ= 7.22-7.54 ppm for **(9)** beside those representing the pyridine moiety.

The reaction of dicarbohydrazide **(3)** with carbon disulfide in the presence of potassium hydroxide gave different products depending on the reaction medium and temperature. That, performing the reaction in ether at ambient temperature led to the formation of the potassium salt **(10)**, meanwhile in refluxing ethanol the 2,4-bis-[(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)]pyridine **(11)**, which was readily obtained by refluxing **(10)** in ethanolic potassium hydroxide solution (scheme 2). The IR and ^{1}H- NMR spectra of **(11)** showed its presence as tautomeric thione- and thiol-forms. The IR spectrum (KBr) included C=S band at 1357 cm^{-1} as well as NH stretching vibrations at 3294 cm^{-1}, which is compatible with its presence in the thione-form structure. The ^{1}H NMR spectrum showed the thiol-protons (SH) as a singlet (2H) at 3.56 ppm which was disappeared on deuteration. Reaction of the potassium salt **(10)** with phenacyl bromide in refluxed ethanol proceeded through intermediate **(12)**, which underwent intramolecular cyclization to afford **(13)** by elimi-

nation of water molecule rather than the expected intramolecular cyclization by elimination of H_2S molecule to give **(14)**[33]. The formation of **(14)** was achieved by reaction of **(11)** with phenacyl bromide in the presence of anhydrous K_2CO_3.

The 1*H*-1,2,4-triazole compounds are considered interesting heterocycles since they possess important pharmacological activities *e.g. N*-aminotriazoles are useful intermediates for the preparation of compounds with anti-inflammatory, antihypertensive and herbicidal properties[34,35]. Treatment of the dipotassium salt **(10)** with hydrazine hydrate in ethanol afforded 2,4-bis-[4-amino-3-thioxo-4*H*-1,2,4-triazol-5-yl]pyridine **(15)**. The structure of **(15)** was based on analogy[36], and its spectral data *e.g.* ^{1}H NMR spectrum showed two singlets at δ 5.34 (4H) and 8.57 ppm (2H), which were disappeared on deuteration, assignable to the amino and imino protons. Furthermore, the structure of **(15)**

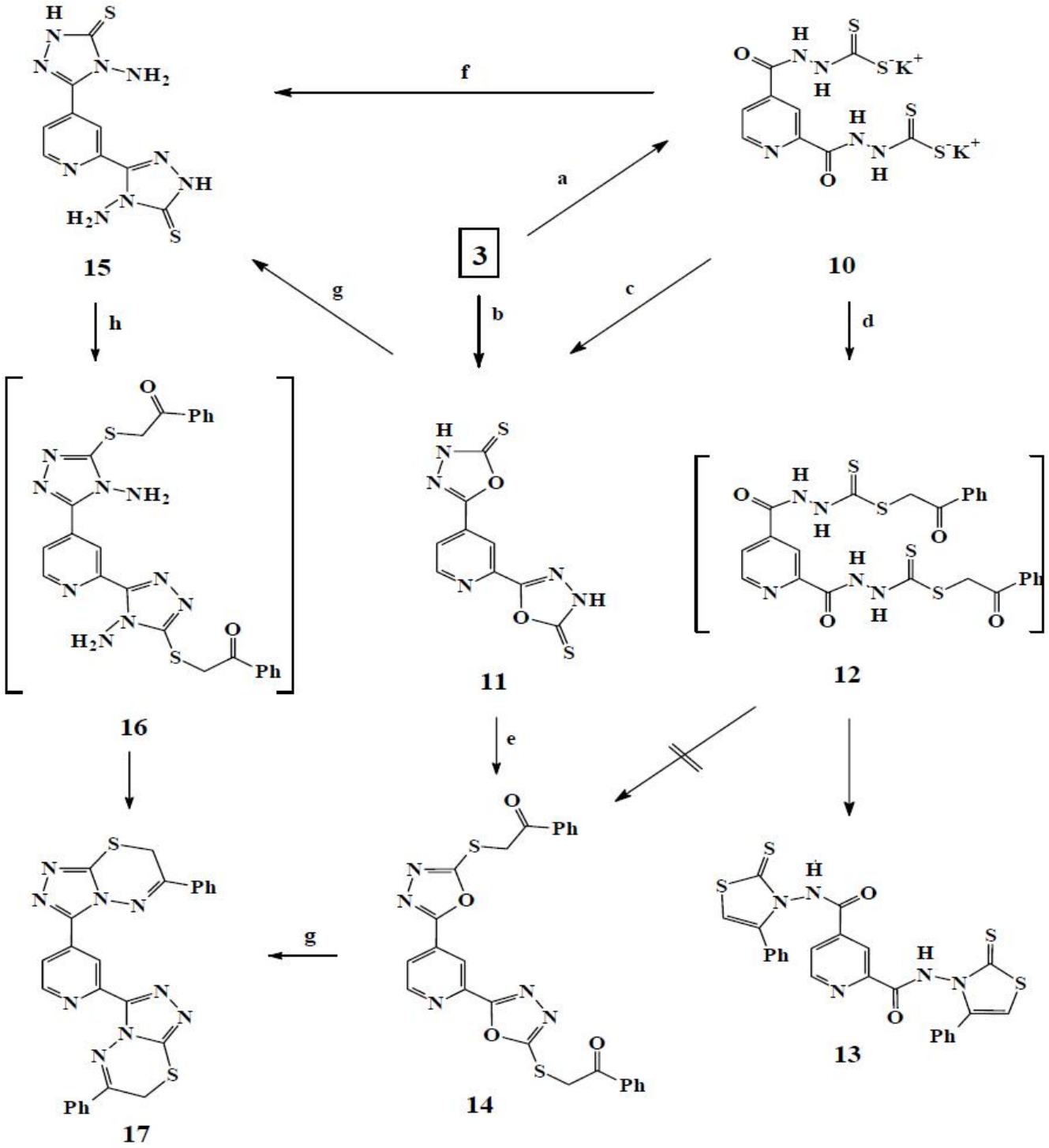


a, CH_3OH, HCl (g); b, $NH_2NH_2.H_2O$; c, ArCHO, AcOH; d, 2,4-pentanedione; e, ethyl acetoacetate; f, $HC(OC_2H_5)_3$; g, C_6H_5COCl; h, polyphsphoric acid (PPA).

Scheme 1

was also confirmed by its independent synthesize via the condensation of **(11)** with hydrazine hydrate in refluxed acetic acid.

In accordance with previous findings by Sasaki *et al*[37], condensation of **(14)** with hydrazine hydrate in refluxed acetic acid gave rise to 2,4-bis-[6-phenyl-*7H*-



Scheme 2

s-triazolo[3,4-*b*][1,3,4]thiadiazin-5-yl]pyridine **(17)**. The IR spectrum of **(17)** showed the absence of carbonyl band of **(14)**, its ^{1}H NMR spectrum showed a singlet (4H) at δ 4.15 ppm and its mass spectrum showed the molecular ion peak at 507 corresponding to its molecular formula $C_{25}H_{17}N_9S_2$. The product **(17)** was further proved by an independent synthesize by treatment of **(15)** with phenacyl bromide in refluxed ethanol *via* the intermediate **(16)**.

Antibacterial activity

Antibacterial activity of the obtained products were studied against gram *+ve* bacteria (*S.aureus* and *B.subtillus*), and gram *–ve* bacteria (*E.coli* and *P.aureginosa*) at triplicate concentrations (5, 2.5 and 1) mg/ml in DMF as a solvent by single disc method using Chloramphenicol as a standard antibacterial agent. The hydrazone products **(4a-e)** showed to be more active than their relevant hydrazide **(3)**, with a remarkable broad antimicrobial reactivity of the product **(4c)**. Except product **(4c)**, none was particularly effective against the tested organisms, but it is noted that the products inhibited the growth of *B.subtilis* to different extents (even less than the standard). These results are presented in (TABLE 1).

TABLE 1 : The antibacterial activity of the tested products

Sample Concentration	S. aureus			B. subtilis			E. coli			P. aureginosa		
	mg/mL			mg/mL			mg/mL			mg/mL		
	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1
3	+	0	0	++	+	+	+	0	0	+	0	0
4a	0	0	0	++	++	+	+	+	0	0	0	0
4b	+	+	0	++	+	+	+	0	0	+	0	0
4c	+++	++	+	+++	++	+	++	++	+	++	+	+
4d	+	0	0	++	+	+	++	+	+	++	+	+
4e	+	+	0	++	++	+	++	+	0	+	+	0
5	+	0	0	+	+	+	+	+	0	+	+	0
6	++	+	+	++	++	+	0	0	0	0	0	0
7	++	+	+	++	++	+	++	+	0	+	+	0
8	+	0	0	++	++	+	+	0	0	+	0	0
9	0	0	0	++	+	+	++	+	+	0	0	0
11	0	0	0	++	++	+	++	++	+	+	0	0
13	0	0	0	++	+	+	++	+	0	0	0	0
14	+	+	0	++	++	++	++	++	+	+	+	0
15	+	0	0	++	++	+	++	+++	++	+	+	0
17	++	+	0	++	++	++	++	++	+	++	+	+
St.	++	++	++	+++	+++	++	+++	+++	++	++	++	++

St. = Reference standard; Chloramphenicol was used as a standard antibacterial agent
The test was done using the diffusion agar technique.
Well diameter: 0.6 cm (100 µL each concentration was tested).
(1) Inhibition values = 0.1-0.5 cm beyond control = +; Inhibition values = 0.6-1.0 cm beyond control = ++
(2) Inhibition values = 1.1-1.5 cm beyond control = +++; 0= Not detected.

Pharmacology

Random screening of compounds and reference drug, Diclofenac potassium, was performed at two dose levels 2.5 & 5 mg kg^{-1} p.o. All the tested compounds showed potent anti-inflammatory activities with potent prostaglandin inhibition at the two dose levels tested. They are more potent than Diclofenac potassium and the degree of potency in descending order is **(13)**, **(11)**, **(4d)**, **(5)**, **(9)**, **(6)**, **(4e)**, **(4d)**, **(15)**, **(17)**, **(4c)**, **(14)**, **(4a)**, **(7)**, **(4b)**.

The evaluation acute toxicity (LD_{50}) of tested compounds of this series showed values above 987 mg kg^{-1} p.o., with maximum in compound **(4b)** (2457.87 mg kg^{-1} p.o). All the results are depicted in TABLE 2.

The ulcerogenic activity (UD_{50} mg kg^{-1} i.p.) of the tested compounds and Diclofenac potassium are men-

tioned in TABLE 2. All compounds are safer than indomethacin due to high ulcerogenic causing doses that were above 101 mg kg^{-1} i.p. (the standard 66.70 mg kg^{-1} i.p.).

All the tested products have been investigated for their anti-allergy activity. No anti-allergic activity was observed for the tested products except compounds **(17)** and **(5)** that are 33% and 28% compounds relative to the anti-allergic activity of Loratadine [(Clorinix®), (71%)] (TABLE 2).

TABLE 2 : Acute toxicity, ulcerogenic, anti-inflammatory and antiallergic activity results

Compd	Acute toxicity (ALD_{50} mg kg^{-1} p.o.)	Ulcerogenic activity (UD_{50} mg kg^{-1} i.p.)	Anti-inflammatory activity			Antiallergy activity
			Dose mg kg^{-1} p.o.	% Inhibition of oedema	% Inhibition of plasma PGE_2	% Inhibition In contraction
4a	2345.87	188.40	2.5	82.98	76.15	NA
			5	87.98	85.32	
4b	2457.87	199.40	2.5	81.09	75.89	NA
			5	85.44	84.87	
4c	1422.99	208.60	2.5	85.78	80.00	NA
			5	89.67	86.07	
4d	1213.55	201.16	2.5	87.67	82.40	NA
			5	91.22	88.69	
4e	1079.14	211.09	2.5	88.76	83.98	NA
			5	94.98	90.00	
4d	998.98	113.20	2.5	91.16	86.89	NA
			5	97.98	93.00	
5	2135.88	185.88	2.5	90.98	85.31	28
			5	96.89	92.09	
6	1833.44	144.90	2.5	89.67	84.87	NA
			5	95.99	91.08	
7	978.88	103.20	2.5	81.16	76.89	NA
			5	87.98	83.00	
9	1177.64	133.28	2.5	89.14	87.98	NA
			5	95.96	93.00	
11	1079.98	128.90	2.5	92.23	87.44	NA
			5	98.67	93.00	
13	987.67	123.89	2.5	94.20	88.17	NA
			5	99.88	93.60	
14	1613.89	198.70	2.5	83.89	77.98	NA
			5	88.98	86.00	
15	987.86	101.22	2.5	84.16	79.89	NA
			5	90.98	85.00	
17	1513.67	234.00	2.5	86.98	81.67	33
			5	90.00	87.66	
St 1	2345.87	66.70	2.5	77.00	72.00	-
			5	80.12	81.40	
St 2	-	-	-	-	-	71

St 1: Diclofenac Potassium (anti-inflammatory standard); St 2: Loratadine (anti-allergy standard); NA: no activity.

EXPERIMENTAL

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, UK) and are uncorrected. Microanalyses were performed on an Elementar-Vario EL (Elementar-Vario EL, Germany)

(Micro-analytical Unit, Central Services Laboratory, National Research Centre, Cairo; Egypt). The ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, USA). ^{1}H NMR spectra were run at 300 MHz in DMSO-d_6 as solvent. Chemical shifts δ are quoted in ppm and were related to that of the solvents. Mass spectra were recorded on a Shimadzu GCMS-QP 1000EX (EI, 70 eV) (Shimadzu, Japan) and Hewlett-Packard (EI, 70 eV) (Hewlett-Packard, USA). IR spectra were obtained with a Brucker-Vector 22 (Bruker Rhein-Stetten, Germany). The purity of the obtained products and the follow up of the reactions was checked by their chromatographic behavior (TLC) using silica gel aluminum sheets $60F_{254}$ (Merck).

2,4-Dimethoxycarbonylpyridine (2)

A stream of dry hydrogen chloride gas was allowed to pass through a suspension of 2,4-pyridinedicarboxylic acid (10 g; 60 mmol) in absolute methanol (250 ml) till a clear solution was obtained. The resulted solution was refluxed for 5h and the solvent was distilled off. The residue was dissolved in water and neutralized with 1*N* sodium bicarbonate solution. The crude product was extracted with ether and the ether extract was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the obtained residue was crystallized from petroleum ether (40-60°C) to give 2,4-dimethoxycarbonylpyridine **(2)**, as white needles, m.p. 59 - 60°C [Lit.[38] 58°C], yield 11.2 g (95%).

2,4-Pyridinedicarbohydrazide (3)

Hydrazine hydrate (8 ml) was added to a solution of **(2)** (9.75 g, 50 mmol) in hot methanol (150 ml). The reaction mixture was refluxed for 3h and left to cool. The separated solid was collected by filtration and crystallized to afford 8.9 g (90%) of **(3)** as a yellowish white powder, m.p. 251 - 253°C (from DMF/ H_2O) [Lit.[38] 256°C (from ethanol)].

N², N⁴-Bis-(arylmethylidene)pyridine-2,4-dicarbohydrazide (4a-e)

General procedure

To a mixture of 2,4-pyridinedicarbohydrazide **(3)** (0.59 g, 3 mmol) and the appropriate aldehydes (namely; 4-methoxybenzaldehyde, 4-fluorobenzaldehyde, 2,4-dihydroxy-benzaldehyde, 2-thiophenealdehyde and 2-furanaldehyde) (6 mmol) in ethanol (50 ml), few drops of acetic acid were added and the reaction mixture was refluxed for 3h and then cooled. The precipitate was filtered off, washed with water, ethanol and crystallized from the appropriate solvent to give the corresponding hydrazone derivatives **(4a-e)**.

N², N⁴-Bis-(4-methoxyphenylmethylidene) pyridine-2,4-dicarbohydrazide (4a)

As a pale yellow powder; yield 1.2 g (92%), m.p. 240 - 242°C (from DMF/H_2O). IR (KBr) ν cm^{-1}: 3272 (NH), 1638 (CO); ^{1}H NMR (TMS, DMSO-d_6) d (ppm): 3.68 (s, 6H, CH_3), 6.94-7.63 (m, 8H, Ph-H), 8.01 (s, 2H, -N=CH), 8.18 (d, 1H, pyridine-H_5), 8.91 (s, 1H, pyridine-H_3), 9.26 (d, 1H, pyridine-H_6), 10.37 (s, 2H, NH, D_2O-exchange, disappear). Anal. for $C_{23}H_{21}N_5O_4$ (431.44) Calcd.: C, 64.03; H, 4.91; N, 16.23; Found: C, 63.97.88; H, 4.83; N, 16.40. MS (m/z, relative abundance): 352 (M^++1, 54%), 133 (100%).

N², N⁴-Bis-(4-fluorophenylmethylidene)pyridine-2,4-dicarbohydrazide (4b)

As a yellowish white powder; yield 1.1 g (87%), m.p. 261 - 263°C (from dioxane/H_2O). IR (KBr) ν cm^{-1}: 3262 (NH), 1642 (CO); ^{1}H NMR (TMS, DMSO-d_6) d (ppm): 7.31-7.76 (m, 8H, Ph-H), 8.07 (s, 2H, -N=CH), 8.21 (d, 1H, pyridine-H_5), 8.96 (s, 1H, pyridine-H_3), 9.32 (d, 1H, pyridine-H_6), 10.22 (s, 2H, NH, D_2O-exchange, disappear). Anal. for $C_{21}H_{15}F_2N_5O_2$ (407.37) Calcd.: C, 61.91; H, 3.71; N, 17.19; Found: C, 61.84; H, 3.62; N, 17.12. MS (m/z, relative abundance): 408 (M^++1, 9%), 105 (100%).

N²,N⁴-Bis-(2,4-dihydroxyphenylmethylidene) pyridine-2,4-dicarbohydrazide (4c)

As white crystals; yield 0.52 g (40%), m.p. 290 - 292°C (from dioxane/H_2O). IR (KBr) ν cm^{-1}: 3257 (NH), 1662 (CO); ^{1}H NMR (TMS, DMSO-d_6) δ (ppm): 6.87-7.3 (m, 6H, Ph-H), 8.02 (s, 2H, -N=CH), 8.18 (d, 1H, pyridine-H_5), 8.89 (s, 1H, pyridine-H_3), 9.23 (d, 1H, pyridine-H_6), 10.1 (s, 2H, NH, D_2O-ex-

change, disappear), 11.87 (s, 4H, 4OH, D_2O-exchange, disappear). Anal. for $C_{21}H_{17}N_5O_6$ (435.39) Calcd.: C, 57.93; H, 3.94; N, 16.09; Found: C, 57.88; H, 4.01; N, 15.89. MS (m/z, relative abundance): 352 (M^++1, 14%), 105 (100%).

N^2, N^4-Bis-(2-thienylmethylidene)pyridine-2,4-dicarbohydrazide (4d)

As yellow crystals; yield 0.76 g (66%), m.p. 276 - 278°C (from ethanol). IR (KBr) ν cm^{-1}: 3291 (NH), 1639 (CO); ^1H NMR (TMS, DMSO-d_6) δ (ppm): 7.03 (dd, 2H, thiophene –H_4), 7.31 (d, 2H, thiophene-H_3), 7.50 (d, 2H, thiophene-H_5), 7.98 (s, 2H, -N=CH), 8.18 (d, 1H, pyridine-H_5), 8.84 (s, 1H, pyridine-H_3), 9.18 (d, 1H, pyridine-H_6), 10.87 (s, 2H, NH, D_2O-exchange, disappear). Anal. for $C_{17}H_{13}N_5O_2S_2$ (383.45) Calcd.: C, 53.25; H, 3.42; N, 18.26; S, 16.72; Found: C, 53.31; H, 3.49; N, 18.19, S 16.68; MS (m/z, relative abundance): 384 (M^++1, 72%), 133 (100%).

N^2, N^4-Bis(2-furylmethylidene)pyridine-2,4-dicarbohydrazide (4e)

As brownish yellow crystals, yield 0.6 g (58%), m.p. 255 - 257°C (from ethanol). IR (KBr) ν cm^{-1}: 3263 (NH), 1644 (CO); ^1H NMR (DMSO-d_6), δ (ppm): 6.54 (dd, 2H, furan-H_4), 6.72 (d, 2H, furan-H_3), 7.74 (d, 2H, furan-H_5), 8.01 (s, 2H, -N=CH), 8.18 (d, 1H, pyridine-H_5), 8.86 (s, 1H, pyridine-H_3), 9.21 (d, 1H, pyridine-H_6), 10.4 (s, 2H, 2NH, D_2O-exchange, disappear). Anal. for $C_{17}H_{13}N_5O_4$ (351.32) Calcd.: C, 58.12; H, 3.73; N, 19.93; Found: C, 58.20; H, 3.81; N, 20.01. MS (m/z, relative abundance): 352 (M^++1, 34%), 105 (100%).

2,4-Bis-[(3,5-dimethyl-1H-pyrazol-1-yl)-N-carbonyl)]pyridine (5)

A mixture of 2,4-pyridinedicarbohydrazide **(3)** (0.58 g, 3 mmol) and 2,4-pentanedione (0.6 ml, 6 mmol) was heated on a steam bath for 5h. The reaction mixture was cooled, triturated with petroleum ether (60-80°C) then with diluted HCl and water. The formed precipitate was filtered off, dried and crystallized from 2-propanol to give a white powder identified as 5, yield 0.52 g (54%), m.p. 139 - 141°C. IR (KBr) ν cm^{-1}: 1697 (CO); ^1H NMR (TMS, DMSO-d_6) δ (ppm): 2.93 (s, 12H, CH_3), 6.85 (s, 2H, pyrazole-H), 8.24 (d, 1H, pyridine-H_5), 8.98 (s, 1H, pyridine-H_3), 9.34 (d, 1H, pyridine-H_6). Anal. for $C_{17}H_{17}N_5O_2$ (323.35) Calcd.: C, 63.15; H, 5.30; N, 21.66; Found: C, 63.22; H, 5.24; N, 21.73. MS (m/z, relative abundance): 352 (M^++1, 94%), 105 (100%).

2,4-Bis-[(3-methyl-2H-pyrazol-5-onyl)-N-carbonyl)]pyridine (6)

Synthesis of **(6)** was performed as described for the synthesis of **(5)**. Compound **(6)** was obtained from **(3)** (0.58 g, 3 mmol) and ethyl acetoacetate (0.78 g a" 0.76 ml, 6 mmol) as a white powder; yield 0.62 g (63%), m.p. 197 - 199°C (from methanol). IR (KBr) ν cm^{-1}: 3241 (NH), 1691, 1664 (CO); ^1H NMR (TMS, DMSO-d_6) δ (ppm): 1.84 (s, 6H, CH_3), 6.05 (s, 2H, pyrazole-H), 8.12 (d, 1H, pyridine-H_5), 8.82 (s, 1H, pyridine-H_3), 8.97 (d, 1H, pyridine-H_6), 8.86 (s, 2H, NH, D_2O-exchange, disappear). Anal. For $C_{15}H_{13}N_5O_4$ (327.29) Calcd.: C, 55.05; H, 4.00; N, 21.40; Found: C, 55.14; H, 3.96; N, 21.47. MS (m/z, relative abundance): 328 (M^++1, 100%).

2,4-Bis-(1,3,4-oxadiazol-2-yl)pyridine (7)

A mixture of **(3)** (0.58 g, 3 mmol) and ethyl orthoformate (2.2 ml, 15 mmol) in DMF (5 ml) was refluxed for 3h, left to cool and poured onto crushed ice. The separated solid was collected by filtration, dried and crystallized to afford **(7)** as pale yellow powder. Yield 0.43 g (67%), m.p. 287 - 289°C (from ethanol/H_2O). IR (KBr) ν cm^{-1}: 3061 (CH-aromatic), 1623 (C=N); ^1H NMR (TMS, DMSO-d_6) δ (ppm): 6.92 (s, 2H, oxadiazole-H), 7.34 (d, 1H, pyridine-H_5), 8.15 (s, 1H, pyridine-H_3), 8.55 (d, 1H, pyridine-H_6). Anal. for $C_9H_5N_5O_2$ (215.17) Calcd.: C, 50.24; H, 2.34; N, 32.55; Found: C, 50.17; H, 2.29; N, 32.47. MS (m/z, relative abundance): 216 (M^++1, 9%), 78 (100%).

2,4-Bis-(benzoylcarbohydrazido)pyridine (8)

A stirred cold solution (-5 - 0°C) of **(3)** (1.16 g, 6 mmol) in dry pyridine (15 ml) was treated with benzoyl chloride (1.68 ml; 12 mmol) and stirring was maintained for 1hr at same temperature. The reaction mixture was then heated on a water bath for 1hr and poured onto crushed ice. The separated solid, on addition of diluted HCl, was collected by filtration and crystallized to give **(8)** as white crystals. Yield: 2.12 g (92%), m.p. 263 - 265°C (from ethanol/H_2O). IR (KBr) ν cm^{-1}: 3227, 3116 (NH), 1653 (CO). Anal. for $C_{21}H_{17}N_5O_4$

(403.39) Calcd.: C, 62.53; H, 4.25; N, 17.36; Found: C, 62.57; H, 4.37; N, 17.42. MS (m/z, relative abundance): 204 (M^++1, 100%).

2,4-Bis-(5-phenyl-1,3,4-oxadiazol-2-yl)pyridine (9)

Polyphosphoric acid (PPA) (15 g) was stirred and heated at 140-150°C then **(8)** (1.5 g, 3.5 mmol) was added in portions. The reaction mixture was kept at same temperature for 2h, cooled and poured onto ice. The separated solid, on addition of diluted ammonia solution, was filtered off, washed with water and crystallized to afford **(9)** as pale greenish powder. Yield: 0.74 g (54%), m.p. 289 - 291°C (from dioxane/H_2O). IR (KBr) ν cm^{-1}: 3061 (CH-aromatic), 1623 (C=N); ^{1}H NMR (TMS, DMSO-d_6) δ (ppm): 7.22-7.54 (m, 11H, Ph-H + pyridine-H_5), 8.14 (s, 1H, pyridine-H_3), 8.54 (d, 1H, pyridine-H_6). Anal. for $C_{21}H_{13}N_5O_2$ (367.36) Calcd.: C, 68.66; H, 3.57; N, 19.06; Found: C, 68.57; H, 3.45; N, 19.13. MS (m/z, relative abundance): 368 (M^++1, 38%), 77 (100%).

2,4-Pyridinedithiocarbazate dipotassium salt (10)

Potassium hydroxide (2.80 g, 50 mmol) was added in portions to a stirred suspension of 2,4-pyridinedicarbohydrazide **(3)** (4.88 g, 25 mmol) in diethyl ether (200 ml) at 0°C. After stirring for 30 min, carbon disulfide (4.6 g, 60 mmol) was added dropwise at 0-10°C over 1h, an orange solution was obtained. The reaction mixture was stirred at room temperature for 3h and left overnight. The solid thus formed was collected by filtration, washed with diethyl ether and dried under reduced pressure at room temperature. The potassium salt was obtained in 7.9 g (75%) yield and was used without further purification.

2,4-Bis-[(2(3H)-thioxo-1,3,4-oxadiazol-5-yl)]pyridine (11)

Method A

A solution of potassium hydroxide (3.92 g, 70 mmol; in water 20 ml) was added to a well stirred suspension of 2,4-pyridinedicarbohydrazide **(3)** (5.86 g, 30 mmol) in ethanol (200 ml). To the stirred reddish solution, carbon disulfide (15 ml, excess) was added in portions at room temperature. The reaction mixture was refluxed on a water bath for 3h then left to cool. The separated solid was collected by filtration, washed with water, dried and crystallized to afford **(11)**, Yield: 5.9 g (72%), m.p. 257 - 259°C (from dioxane/H_2O). IR (KBr) ί cm^{-1}: 3061 (CH-aromatic), 1623 (C=N); ^{1}H NMR (TMS, DMSO-d_6) d (ppm): 3.56 (s, 2H, SH, D_2O-exchange, disappear), 7.71 (d, 1H, pyridine-H_5), 8.14 (s, 1H, pyridine-H_3), 8.78 (d, 1H, pyridine-H_6). Anal. for $C_9H_5N_5O_2S_2$ (279.3) Calcd.: C, 38.70; H, 1.80; N, 25.07; S, 22.96; Found: C, 35.78; H, 1.84; N, 25.13; S, 22.89. MS (m/z, relative abundance): 279 (M^+, 100%), 280 (M^++1, 15%), 279 (M^++2, 8%).

Method B

To a stirred solution of the potassium salt **(10)** (4.24 g, 10 mmol) in ethanol (96%, 100 ml), a solution of KOH (1.68 g, 30 mmol) in water (10 ml) was added and the reaction mixture was refluxed for 2h. The resulted solution was left to cool, poured onto ice-water and then acidified with 1*N* HCl. The resulted solid was collected by filtration, washed with water, dried and crystallized from aqueous dioxane to give **(11)** as identified by m.p.'s and mixed m.p. and their same chromatographic behavior (TLC).

N^2,N^4-Bis[4-phenyl-2-thioxothiazol-3(2H)-yl]pyridine-2,4-dicarboxamide (13)

Phenacyl bromide (1.2 g, 6 mmol) was added to a solution of the potassium salt **(10)** (1.28 g, 3 mmol) in ethanol (50 ml). The reaction mixture was refluxed for 3h, left to cool and poured onto crushed ice. The obtained solid was filtered off, washed with water, dried and crystallized to give **(13)** as white crystals. Yield: 0.9 g (55%), m.p. 233 - 235°C (from dioxane/H_2O). IR (KBr) ν cm^{-1}: 3356 (NH), 1652 (CO); ^{1}H NMR (TMS, DMSO-d_6) d (ppm): 6.73 (s, 2H, thiazole-H), 7.22-7.38 (m, 10H, Ph-H), 8.18 (d, 1H, pyridine-H_5), 8.79 (s, 1H, pyridine-H_3), 9.15 (d, 1H, pyridine-H_6), 10.12 (s, 2H, NH, D_2O-exchange, disappear). Anal. For $C_{25}H_{17}N_5O_2S_4$ (547.69) Calcd.: C, 54.82; H, 3.13; N, 12.79; S, 23.42; Found: C, 54.85; H, 3.17; N, 12.85; S, 23.48. MS (m/z, relative abundance): 548 (M^++1, 7%), 105 (68%), 77 (100%).

2,4-Bis-[2-(phenylethanon-1-yl)thio-1,3,4-oxadiazol-5-yl]pyridine 14

A mixture of **(11)** (0.84 g, 3 mmol), phenacyl bromide (1.2 g, 6 mmol) and anhydrous K_2CO_3 (1.2 g, 9 mmol) in dry ether (100 ml) was refluxed on a water

bath for 3h. The solvent was distilled off and the obtained residue was treated with water and left overnight at room temperature. The formed solid was filtered off, washed with water, dried and crystallized to give **(14)** as white powder. Yield: 0.9 g (55%), m.p. 161 - 163°C (decomp.) (from aqueous dioxane). IR (KBr) ν cm^{-1}: 1667 (CO); ^{1}H-NMR (TMS, DMSO-d_6) δ (ppm): 4.32 (s, 4H, CH_2), 7.37-7.73 (m, 11H, Ph-H + pyridine-H_5), 8.14 (s, 1H, pyridine-H_3), 8.79 (d, 1H, pyridine-H_6). Anal. For $C_{25}H_{17}N_5O_4S_2$ (515.56) Calcd.: C, 58.24; H, 3.32; N, 13.58; S, 12.44; Found: C, 58.35; H, 3.27; N, 13.64; S, 12.38. MS (m/z, relative abundance): 515 (M^+, 3%), 177 (35%), 119 (23%), 77 (100%).

2,4-Bis-[4-amino-3-thioxo-4H-1,2,4-triazol-5-yl]pyridine (15)

Method A

To a solution of the dipotassium salt **(10)** (2.12 g, 5 mmol) in ethanol (50%, 50 ml) hydrazine hydrate (80%, 3 ml, excess) was added. The reaction mixture was refluxed for 3h, left to cool, poured onto crushed ice. The solid so obtained was collected by filtration, washed with water, dried and crystallized to give **(15)** as a white powder. Yield: 1.15 g (75%), m.p. 212 - 214°C (decomp.) (from aqueous dioxane). IR (KBr) ν cm^{-1}: 3294, 3144 (NH), 1631 (C=N); ^{1}H NMR (TMS, DMSO-d_6) δ (ppm): 6.14 (br s, 4H, NH_2, D_2O-exchange, disappear), 7.73 (d, 1H, pyridine-H_5), 8.17 (s, 1H, pyridine-H_3), 8.81 (d, 1H, pyridine-H_6), 9.21 (s, 2H, NH, D_2O-exchange, disappear). Anal. For $C_9H_9N_9S_2$ (307.36) Calcd.: C, 35.17; H, 2.95; N, 41.01; S, 20.86; Found: C, 35.21; H, 2.89; N, 41.11; S, 20.79. MS (m/z, relative abundance): 307 (M^+, 19%), 308 (M^++1, 5%), 310 (M^++2, 1.2%), 105 (100%).

Method B

Hydrazine hydrate (80%, 3 ml) was added to a solution of **(11)** (0.89 g, 3 mmol) in glacial acetic acid (10 ml) and the reaction mixture was refluxed for 2h, left to cool then poured into a mixture of ice and sodium bicarbonate. The separated solid was collected by filtration, washed with water, dried and crystallized from aqueous dioxane to give **(15)** as identified by m.p.'s and mixed m.p. and their same chromatographic behavior (TLC).

2,4-Bis-[6-phenyl-7H-s-triazolo[3,4-b][1,3,4]thiadiazin-5-yl]pyridine (17)

Method A

A mixture of **(14)** (1.55 g, 3 mmol) and hydrazine hydrate (3 ml, excess) in glacial acetic acid (25 ml) was heated under reflux for 5h. Solvent was distilled off under reduced pressure to its half volume and the reaction mixture was left to cool then poured into a mixture of ice and sodium bicarbonate. The obtained product was separated by filtration, washed with water thoroughly then crystallized from 2-propanol to afford **(17)** as white fine crystals. Yield: 1.05 g (69%), m.p. 107-109°C. IR (KBr) ν cm^{-1}: 1617 (C=N); ^{1}H NMR (TMS, DMSO-d_6) δ (ppm): 4.15 (s, 4H, CH_2), 7.35-7.78 (m, 11H, Ph-H + pyridine-H_5), 8.15 (s, 1H, pyridine-H_3), 8.79 (d, 1H, pyridine-H_6). Anal. For $C_{25}H_{17}N_9S_2$ (507.59) Calcd.: C, 59.16; H, 3.38; N, 24.83; S, 12.63; Found: C, 59.23; H, 3.28; N, 24.94; S, 12.55. MS (m/z, relative abundance): 507 (M^+, 7%), 404 (63), 288 (13), 175 (8), 105 (12), 77 (100%).

Method B

A mixture of **(15)** (1.54 g, 5 mmol) and phenacyl bromide (2 g, 10 mmol) in ethanol was refluxed for 5h. Solvent was distilled off and the residue was triturated with water (25 ml x 3) with scratching and left to stand overnight. The formed solid was treated with water, separated by filtration, dried and crystallized (decolorized charcoal) from 2-propanol to give white fine crystals of **(17)** as identified by m.p.'s and mixed m.p. and their same chromatographic behavior (TLC).

Evaluation of acute toxicity study

The test compounds were administered orally at different dos levels in separate groups of animals. After 24 h of drug administration percent mortality in each group was observed. From the data obtained, Lethal Dose (LD_{50}) (TABLE 2) was calculated by the method of Smith[39].

Evaluation of ulcerogenic activity

This activity was done according to the method of Verma *et al*[40]. In this method, adult albino rats, fasted 24h prior to the administration of drugs, were divided into groups of ten animals each. Water was allowed ad libitum to the animals. The test compounds and stan-

dard drugs were given intraperitoneally and the animals sacrificed 8h after drugs treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) petechial and frank haemorrhage and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity. The results are presented in TABLE 2.

Evaluation of anti-inflammatory activity

The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of winter *et al*[41,42].

Groups of adult male albino rats (150-180 gm), each of 8 animals were orally dosed with the test compounds at a dose level of 2.5 & 5 mg/kg on hour before carrageenan challenge. Foot paw edema was induced by sub planter injection of 0.05 ml of 1% suspension of carrageenin in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animal was decapitated, blood was collected and the paws were rapidly excised.

The average weight of edema was estimated for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated then percentage Protection against edema was estimated (TABLE 2).

Diclofenac potassium (2.5 and 5 mg/kg) was employed as standard reference against which the test compounds were compared.

Estimation of plasma prostaglandin (PGE_2)

Heparinized blood samples were collected from rats (n= 8), plasma was separated by centrifugation at 12000 r/min for 2 min at 4°C and immediately stored frozen 20°C until use.

The designs correlate-EIA prostaglandin in E_2 (PGE_2) kit is a competitive immune assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature the excess reagents were washed away and the substrate was added. After a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro plate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples[43-45]. The percentage inhibition of plasma PGE_2 for each compound was estimated (TABLE 2).

Evaluation of anti-allergic activity

Albino guinea pigs of either sex weighing 300-450 g are sacrificed with an overdose of ether. The chest cavity is opened and the lungs are removed. They were cut into strips of 5 cm and placed into a physiological saline solution. Thereafter, the lung strips are mounted in an organ bath containing a nutritive solution. The bath was bubbled with carbogen and maintained at 37°C under a pre-load of 0.5 g - 3 g; the tissue was left to equilibrate for 30-60 minutes. Prior to testing carbachol is added to the bath to test the lung strips ability of contraction. Twenty minutes later, two values are obtained by adding the spasmogen[46].

- Histamine dihydrochloride 10^{-6} g/ml for 5 minutes,
- Ca-ionophore 5 x 10^{-6} g/ml for 5 minutes, or
- Leukotriene LTC_4 (10^{-9}-10^{-8}) g/ml for 10 minutes,

To the bath and recording the contractile force at its maximal level.

Following 20 minutes, equilibration period resulted, the spasmogen is administrated again, 5 minutes thereafter, and the test compound is added in cumulative doses from 10^{-8}-10^{-4} g/ml at 5 or 10 minutes intervals. The contractile response is determined isometrically. The percentage of inhibition in contraction (due to histamine release) is the anti-allergic potency, which is compared to that of Loratadine (standard reference drug) (TABLE 2).

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