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Note

Synthesis and antiulcer activity study of 1,4-dihydropyridines and their Mannich bases with sulfanilamide

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3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-(substituted)-pyridines **1a-e** are prepared following Hantzsch pyridine synthesis by condensation of ethyl acetoacetate with different aromatic aldehydes in presence of ammonium hydroxide. Compounds **1a-e** on reaction with paraformaldehyde and sulfanilamide yielded 3,5-diethoxycarbonyl-1-[(4′-sulfamoyl-1′-amino methyl) phenyl]-1,4-dihydro-2,6-dimethyl-4-(substituted)-pyridines **2a-e**. The antiulcer activities have been performed by estimating volume of gastric acid, *p*H, free acidity, total acidity and ulcer index. The antiulcer activity of 1,4-dihydropyridines is enhanced significantly on conjunction with sulfanilamide. Substitution of methoxy group increased the antiulcer potential of the compounds.

Keywords: Ethyl acetoacetate, 1,4-dihydropyridines, sulfanilamide, antiulcer activity

Acetylcholine, histamine and gastrin are known to stimulate acid secretion from gastric parietal cells through Ca^{2+} [1,2]. Several calcium channel blockers including nifedipine are reported with antiulcer activity[3-5]. Carbonic anhydrases together with H^+/K^+-ATP-ase are the key enzymes involved in gastric acid secretion[6,7]. It is thus envisageable that structural analogues of nifedipine may possess antiulcer potential that can be enhanced on conjunction with sulfanilamide, a prototype of carbonic anhydrase inhibitor. Prompted by these observations and in continuation of our work on development of antiulcer agents, we report herein the reaction of aromatic aldehydes with ethyl acetoacetate and ammonium hydroxide to give 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-(substituted)-pyridine **1a-e**[8]. Compounds **1a-e** in presence of sulfanilamide and paraformaldehyde furnished the 3,5-diethoxycarbonyl-1-[(4′-sulfamoyl-1′-amino methyl) phenyl]-1,4-dihydro-2,6-dimethyl-4-(substituted)-pyridine **2a-e (Scheme I,** Ref. 9) .

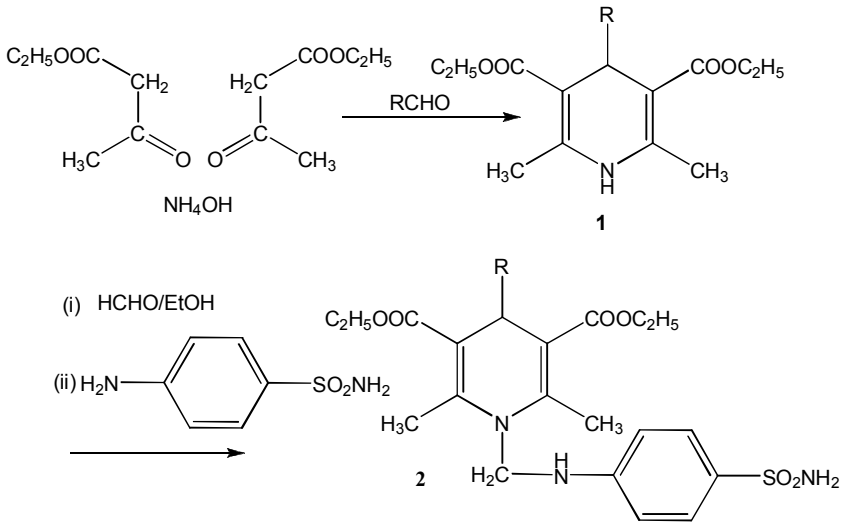
The structures of the synthesized compounds **1-2a-e** have been confirmed by elemental analysis, IR, ^{1}H NMR, mass and physicochemical data **(Table I)**. The antiulcer activity evaluation[10,11] indicated antiulcer potential of 1,4-dihydropyridines **1a-e** that was enhanced on conjunction with sulfanilamide **(Table II)**. Compound **2b-e** reduced the volume of acid secretion significantly ($P < 0.01$). The acid neutralizing capacity of **2d** was comparable with that of omeprazole. The capacity to reduce total acidity and free acidity was significant ($P < 0.01$) for compound **2b-e**. All the compounds exhibited better ulcer index values compared to control ($P < 0.01$). Among the compounds, **1b, 1d, 2b** and **2d** showed better antiulcer potential. The increase in activity could be attributed to the methoxy group substitution on the phenyl ring. Substitution of hydroxyl group does not seem to affect the antiulcer potential of the compounds.

Antiulcer activity

The screening for antiulcer activity was conducted at pharmacology laboratory (Registration no-990/c/06/CPCSEA) of University Department of Pharmaceutical Sciences, Utkal University by pyloric ligation method[10,11]. Albino rats of either sex weighing 200-250 g were kept at RT (25-30ºC) and fasted for overnight. All compounds (10 mg/kg) were given orally (1 mL) 40 minutes prior to pyloric ligation. Control group received normal saline. Under chloroform anesthesia the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted out and ligated, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were kept deprived of

Abbreviations

CPCSEA:	Committee for the Purpose of Control and Supervision of Experiments on Animals
ANOVA:	Analysis of Variance
TMS:	Tetra Methyl Silane
SEM:	Standard Error of Mean
df:	Degrees of Freedom
Ompr:	Omeprazole



Scheme I

Table I — Analytical data of compounds **1a-e** and **2a-e**

Compd	R	m.p. (°C)	Yield (%)	Mol. formula
1a	2-OH-C_6H_4	122	72	$C_{19}H_{23}NO_5$
1b	3-OCH_3-4-OH- C_6H_4	192	68	$C_{20}H_{25}NO_6$
1c	OC_4H_3	165	65	$C_{17}H_{21}NO_5$
1d	4-OCH_3- C_6H_4	169	70	$C_{20}H_{25}NO_5$
1e	C_6H_5	175	75	$C_{19}H_{23}NO_4$
2a	2-OH-C_6H_4	136	62	$C_{26}H_{31}N_3O_7S$
2b	3-OCH_3-4-OH- C_6H_4	198	70	$C_{27}H_{33}N_3O_8S$
2c	OC_4H_3	230	60	$C_{24}H_{29}N_3O_7S$
2d	4-OCH_3- C_6H_4	205	65	$C_{27}H_{33}N_3O_7S$
2e	C_6H_5	184	70	$C_{26}H_{31}N_3O_6S$

food and water during postoperative period and were sacrificed at the end of 4 hr after the operation. The stomach was separated as a whole and the content were brought out. The contents were then subjected to centrifugation (1000 rpm for 10 min) and then analyzed for volume and *p*H (Esico-1013). Free acidity and total acidity were estimated by titrating 1 mL of the centrifuged sample diluted to 10 mL with 0.01*N* NaOH, using Topfer's reagent and phenolphthalein indicator respectively. For estimation of ulcer index, the stomach was cut open and the inner surface was examined for ulceration with the help of a magnifying glass. Omepazole (1 mg/kg) was used as the standard drug for comparison purpose. The differences between the groups was determined using the one-way analysis of variance (ANOVA) followed by Dunnett's test with 5% significance level (P <0.05) **(Table II)**.

Experimental Section

TLC was used to assess the reactions and purity of the compounds. The melting points were determined in open capillary tubes (Sisco) and are uncorrected. IR spectra were recorded on a Shimadzu-FTIR-8400S spectrophotometer using KBr powder. ^{1}H NMR ($CDCl_3$) spectra of title compounds were recorded on a Bruker DRX-300 NMR spectrometer (300 MHz) using TMS as internal standard. Mass spectra were recorded on an API-4000 (MDS-SCIEX) mass spectrometer. All the compounds gave satisfactory elemental analysis (Perkin Elmer-2400). Molecular weights of the compounds determined by Rast's method were close to the theoretical values.

Synthesis of 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-(3′-methoxy-4′-hydroxy phenyl)-pyridine 1b. A solution of vanilin (0.2 mole), ethyl acetoacetate (0.2 mole) and concentrated ammonium

Table II — Antiulcer screening results of compounds **1a-e** and **2a-e**

Group	Dose mg/kg	Vol. (mL)	*p*H	Free acid (mEq/L)	Total acid (mEq/L)	Ulcer index
Contr	-	5.61±0.12	2.46±0.17	65±1.229	162±0.120	5.25±0.17
1a	10	4.85±0.12*	2.21±0.12*	59±1.29**	159±1.02*	4.25±0.12***
1b	10	4.22±0.28*	3.14±0.16*	56.4±1.56***	141±1.76***	0.83±0.17***
1c	10	4.41±0.12*	2.51±0.16*	61±2.06*	151±1.47***	2.25±0.33***
1d	10	3.3±0.11***	3.66±0.10*	43±1***	121±1.68***	0.33±0.12***
1e	10	4.08±0.24**	2.1±0.13*	59.2±0.09**	158±2.78*	4.08±0.24***
2a	10	4.6±0.06*	2.3±.0.07*	59.2±1.536**	158.8±2.23*	4.2±0.214***
2b	10	2.5±0.05***	4.52±0.07**	50.4±0.89***	115.8±2.14***	0.35±0.02***
2c	10	3.7±0.16***	4.36.±0.80*	52.05±1.7***	104.8±2.3***	0.18±0.01***
2d	10	2.6±0.12***	4.8±0.09***	40±1.67***	115.8±2.14***	0.34±0.11***
2e	10	3.2±0.07***	3.8±0.133*	55.3±1.02***	147±0.99***	2.5±0.26***
Ompr.	1	2.01±0.05	5.01±0.1	34±0.80	98±1.12	0.08±0.08
ANOVA	F	6.873	4.412	33.170	132.35	108.55

Values are expressed as mean ± SEM.n = 6 in each group. df =10, 55. *** $P < 0.01$, ** $P < 0.05$,* $P > 0.05$. Ompr.= Omeprazole

hydroxide (8 mL) in ethanol (60 mL) was heated under reflux for 3 hr. To the resulting mixture, warm water (40 mL) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallized from alcohol to give **1b**.

Similarly, compounds **1a-e** were prepared by condensation of ethyl acetoacetate and ammonium hydroxide with other aromatic aldehydes.

The IR, ^{1}H NMR and selective mass and elemental analysis of some compounds are reported below.

1a: IR (KBr, cm^{-1}): 3329.25 (N-H-str), 3099.71 (O-H str broadened), 1693.56 (C=O-str), 1211.34 [C (=O)-O-str], 2976.26 [C-H-str (aliphatic)], 3053.42 [C-H-str (aromatic)], 786.98 [C-H-out of plane bend (substituted benzene)]; ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H, $-CH_3$), 1.4 (m, 6H, $-CH_3$), 3.7 (m, 4H,$-CH_2$), 5.1 (s, 1H,-CH-), 5.7 (s, 1H, NH), 7.2-7.7 (m, 4H, Ar-H), 6.4 (s, 1H, OH); Mass spectra : *(m/e)* 345 (M^+, 100); Mol. wt: 345.39. Found: 346.2; Found: C, 66.07; H, 6.7; N, 4.12. $C_{19}H_{23}NO_5$ required: C, 66.03; H, 6.71; N, 4.05% .

1b: IR (KBr, cm^{-1}): 3344.68 (N-H-str), 3000 (O-H str broadened), 1689.70 (C=O-str), 1213.27 [C (=O)-O-str], 2972.40 [C-H-str (aliphatic)], 1301.99 (C-O-C -asymmetric str). 1031.95 (C-O-C -symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H,$-CH_3$), 1.3 (m, 6H, $-CH_3$), 4.1 (m, 4H, $-CH_2$), 3.8 (s, 3H, $-OCH_3$), 4.9 (s, 1H,-CH), 5.7 (s, 1H,-NH), 6.6-6.9 (m, 3H, Ar-H), 5.9 (s, 1H, OH); Mass spectra : *(m/e)* 374.1(M-1, 100), 375 (M^+); Mol. wt: 375.46. Found: 375.4; Found: C, 64.1; H, 6.6; N, 3.71. $C_{20}H_{25}NO_6$ required: C, 63.92; H, 6.71; N, 3.7% .

1c: IR (KBr, cm^{-1}): 3346.61 (N-H-str), 1697.41 (C=O-str), 1211.34 [C (=O)-O-str], 2983.83 [C-H-str (aliphatic)]; ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.7 (s, 6H, $-CH_3$), 1.25 (m, 6H, $-CH_3$), 5.1 (s, 1H,-CH), 3.8 (m, 4H, $-CH_2$), 5.4 (s, 1H, NH), 6.2-6.5 (m, 3H, Ar-H); Mol. wt: 319.36. Found: 320; Found: C, 64.1; H, 6.34; N, 4.5. $C_{17}H_{21}NO_5$ required: C, 63.93; H, 6.63; N, 4.38% .

1d: IR (KBr, cm^{-1}): 3344.68 (N-H-str), 1689.7 (C=O-str), 1213.27 [C(=O)-O-str], 2972.40 [C-H-str(aliphatic)], 3091.99 (C-H-aromatic), 835.21 [C-H-out of plane bending (substituted benzene)], 1253.77 (C-O-C-asymmetric str), 1031.95 (C-O-C-symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H $-CH_3$), 1.3 (m, 6H $-CH_3$), 3.7 (m, 4H, $-CH_2$), 6.2 (s, 1H, NH), 7.2-7.7 (m, 4H, Ar-H), 4.9 (s, 1H, -CH), 3.9 (s, 3H,$-OCH_3$); Mol. wt: 359.42. Found: 360.15.

1e: IR (KBr, cm^{-1}): 3329.25 (N-H-str), 1701.4 (C=O-str), 1213.72[C (=O)-O-str], 2953.45 [C-H-str (aliphatic)], 3053.46 (C-H-aromatic); ^{1}H NMR (300 MHz, δ, ppm, $CDCl_3$): 2.4 (s, 6H, $-CH_3$), 1.3 (m, 6H $-CH_3$), 4.9 (s, 1H, -CH), 5.3 (s, 1H, NH), 3.7 (m, 4H, $-CH_2$), 6.7-6.9 (m, 5H, Ar-H); Mass spectra : *(m/e)* 329.3 (M^+), 328.2 (M-1,100); Mol. wt: 329.37. Found: 330.07; Found: C, 69.1; H, 6.84; N, 4.5. $C_{19}H_{23}NO_4$ required: C, 69.28; H, 7.03; N, 4.25%.

Synthesis of 3,5-diethoxycarbonyl-1-[(4′-sulfamoyl-1′-amino methyl) phenyl]-1,4-dihydro-2,6-dimethyl-4-(3′-methoxy-4′-hydroxy phenyl)-pyridine 2b. A mixture of 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-(3′-methoxy-4′-hydroxy phenyl)-pyridine (0.01 mole), sulfanilamide (0.01 mole) and paraformaldehyde (0.02 mole) was taken in 15 mL of rectified spirit and heated under reflux for 4 hr. The reaction-mixture was poured onto crushed ice. The product was filtered and recrystallised from aqueous ethanol to give **2b**.

Similarly, compounds **2a-e** were prepared by condensation of sulfanilamide and paraformaldehyde with 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-(substituted)-pyridine **1a-e**.

The IR, ^{1}H NMR and selective mass and elemental analysis of some compounds.

2a: IR (KBr, cm^{-1}): 3321.5 (N-H-str), 3028.25(O-H str broadened), 1689.35 (C=O-str), 1211.34 [C (=O)-O-str], 665.35 (C-S-str), 1369.50 (S=O asymmetric str), 1215.25 (S=O symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H, $-CH_3$), 4.9 (s, 1H,-CH-), 3.5 (s, 2H, $-CH_2$), 1.3 (m, 6H, $-CH_3$), 3.8 (m, 4H, $-CH_2$), 6.2 (s, 1H, OH), 5.4 (s, 1H, NH), 7.2-7.7 (4H, m, Ar-H); Mol. wt: 529.61. Found: 528.8; Found: C, 58.4; H, 6.2; N, 7.3. $C_{26}H_{31}N_3O_7S$ required: C, 58.96; H, 5.89; N, 7.93% .

2b: IR (KBr, cm^{-1}): 3352.39 (N-H-str), 3099(O-H str broadened), 1681.98 (C=O-str), 1222.91 [C(=O)-O-str], 2983.98 [C-H-str (aliphatic)], 3064.99 [C-H-str (aromatic str)],1033.68 (C-O-C -symmetric str), 1369.50 (S=O asymmetric str), 1217.12 (S=O symmetric str), 696.33 (C-S-str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H, $-CH_3$), 3.4 (s, 2H, $-CH_2$), 1.29 (m, 6H, $-CH_3$), 3.8 (s, 3H, $-OCH_3$), 3.7 (m, 4H, $-CH_2$), 5.2 (s, 1H,-CH-), 6.2 (s, 1H, OH), 5.6 (s, 1H, NH), 7.2-7.7 (4H, m, Ar-H); Mass spectra : *(m/e)* 559.1 (M^+), 558 (M-1), 374.1 (100); Mol. wt: 559.6. Found: 558.76; Found: C, 58.4; H, 5.1; N, 6.9. $C_{27}H_{33}N_3O_8S$ required: C, 57.95; H, 5.9; N, 7.51% .

2c: IR (KBr, cm^{-1}): 3346.61 (N-H-str), 1697.41 (C=O-str), 1211.34 [C (=O)-O-str], 2983.83 [C-H-str (aliphatic)]. 696.33 (C-S-str), 1374.12 (S=O asymmetric str), 1217.24 (S=O symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.5 (s, 6H, $-CH_3$), 3.6 (s, 2H, $-CH_2$), 1.3 (m, 6H, $-CH_3$), 3.9 (m, 4H, $-CH_2$), 4.8 (s, 1H,-CH-), 5.7 (s, 1H, NH) 6.2-6.8 (m, 3H, Ar-H); Mol. wt: 503.57. Found: 504; Found: C, 56.94; H, 5.3; N, 8.1. $C_{24}H_{29}N_3O_7S$ required: C, 57.19; H, 5.8; N, 8.34% .

2d: IR (KBr, cm^{-1}): 3342.75 (N-H-str), 1689.70 (C=O-str), 1240.27 [C(=O)-O-str], 2983.98 [C-H-str(aliphatic)], 3063.06 [C-H-str (aromatic)], 1031.95 (C-O-C symmetric str), 664.78 (C-S-str), 1369.22 (S=O asymmetric str.), 1211.68 (S=O symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.4 (s, 6H $-CH_3$), 3.7 (m, 4H, $-CH_2$), 3.4 (s, 2H,$-CH_2$), 1.3 (m, 6H, $-CH_3$), 3.9 (s, 3H, $-OCH_3$), 5.1 (s, 1H, -CH), 5.7 (s, 1H, NH), 6.7-7.2 (m, 4H, Ar-H); Mol. wt: 543.6. Found: 543; Found: C, 59.6; H, 6.07; N, 7.72 $C_{27}H_{33}N_3O_7S$ required: C, 59.19; H, 5.8; N, 8.2% .

2e: IR (KBr, cm^{-1}): 3329.25 (N-H-str), 1698.654 (C=O-str), 1213.72[C (=O)-O-str], 3053.46 (C-H-str, aromatic), 1373.65 (S=O asymmetric str), 1217.23 (S=O symmetric str); ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.3 (s, 6H $-CH_3$), 1.19 (m, 6H, $-CH_3$), 3.2 (s, 2H, $-CH_2$), 3.7 (m, 4H, $-CH_2$), 5.1 (s, 1H,-CH-), 5.8 (s, 1H, NH) 7.4-7.7 (m, 5H, ArH); Mol. wt: 513.6. Found: 514.2; Found: C, 60.14; H, 6.23; N, 7.78. $C_{26}H_{31}N_3O_6S$ required: C, 59.6; H, 6.03; N, 8. 17%.

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