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Synthesis and Characterization of Pyrazine-2-Carbohydrazide Derivatives as Antimicrobial Agents

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ABSTRACT

A series of pyrazine-2-carbohydrazide derivatives has been synthesized and assessed for their *in vitro* antimicrobial activity against Gram-positive and Gram-negative strains of bacteria. In the present study, pyrazinamide was hydrolyzed and esterified to obtain ethyl-pyrazinoate and was reacted with hydrazine hydrate to yield the pyrazinoic acid hydrazide. This pyrazinoic acid hydrazide was then condensed with various substituted aromatic aldehydes to obtain different derivatives of pyrazine-2-carbohydrazide. The newly synthesized compounds were characterized by IR, 1HNMR and MS spectral data. The antimicrobial activity of the synthesized compounds was found to be potent against the selected strains of Gram-positive bacteria as compared with Gram-negative bacteria.

Key words: Antimicrobial, carbohydrazide, pyrazine

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INTRODUCTION

The incidence of microbial infections is increasing worldwide despite active research devoted to the discovery and development of novel antimicrobial agents. Emergence of multiresistant strains of bacteria has increased the problem of drug resistance dramatically. Furthermore, treatment of infectious diseases is more difficult in immunocompromised patients such as those infected with human immunodeficiency virus (HIV). Various novel approaches were carried out to the current existing drugs for reducing the microbial resistance. These mainly involve structural modification of existing antimicrobial agents so as to increase microbial intracellular concentration of drug, and thereby to increase antimicrobial activity. The thorough literature survey reveals that the parent molecules having hydrazide and hydrazones groups in their structure is shown potent antimicrobial activity.[1,2] The literature survey reveals that the molecules having hydrazide skeleton possess antimicrobial activity, viz., anti-TB and antibacterial activity.[3] This endless requirement resulted in further work on pyrazine-2-carbohydrazide. In the present study, pyrazinamide was hydrolyzed and esterified to get obtain ethyl-pyrazinoate and was reacted with hydrazine hydrate to yield pyrazinoic acid hydrazide. This pyrazinoic acid hydrazide was then condensed with various substituted aromatic aldehydes to yield different pyrazine carbohydrazide derivatives so as to increase log P value, and thereby to increase the intracellular concentration in order to decrease the resistance developed due to decreased intracellular concentration of the drug. These synthesized compounds were subjected to preliminary biological evaluation (Scheme 1).

Experimental

Progress of the reaction and purity of the compounds was confirmed by Emerck precoated 60 F254 TLC plates, and

spots were rendered visible by exposing to UV light and iodine fumes. Column chromatographic separations were carried out by gradient elution with methanol/ethanol-ethyl acetate mixture and silica gel (60-120/100- 200 mesh). The compounds were purified by recrystallization using aqueous ethanol as a solvent. HPLC was performed on JASCO 2000 SERIES instrument using C-18 reverse-phase column and diodearray UV detector.

Melting points were determined in open capillaries using Veego VMP-1 electrothermal melting point apparatus and were uncorrected. The absorbance (λ_{max}) was taken on JASCO 630V spectrophotometer using methanol as solvent. The infrared (IR) spectra were recorded on JASCO FTIR 4100 Series spectrometer in KBr pellets and the frequencies recorded in wave numbers. ^{1}H NMR spectra were recorded on "VARIAN MERCURY YH-300" spectrophotometer at 300 MHz in DMSO–d6/ $CDCl_3$ solutions and their chemical shifts are recorded in δ (parts per million) units with respect to tetramethyl silane (TMS) as an internal standard. The mass spectra were recorded on Waters Q-ToF micro on electron spray ionization (ESI) source. Elemental analyses observed for all the newly synthesized compounds were found to be within the limits of accuracy.

Procedure for synthesis of pyrazinoic acid hydrazide [I]

Pyrazinamide (0.1 mol) was hydrolyzed to obtain pyrazinoic acid by alkaline hydrolysis. The acid obtained was dissolved in ethanol and few drops of conc. H_2SO_4 was added and refluxed for 24 h; hydrazine hydrate 100% (3.0 mol) was added to it and further refluxed for a period of 8 h. The excess of solvent was distilled off to obtain the resulting product. The product was crystallized from aqueous ethanol.[4,5]

Procedure for synthesis of pyrazine-2-carbohydrazide [PH 1-14] (General Procedure)

A solution of aromatic/substituted aldehyde (0.05 mol) in ethanol was added to a solution of so obtained pyrazinoic acid hydrazide (0.05 mol) in 10 ml ethanol. The mixture was refluxed for 4 h. After cooling the mixture, the precipitate was filtered, dried and recrystallized from aqueous ethanol.[6,7]

PH 1: N'-[phenyl methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH_3OH):304; IR (KBr): 3298 (NH), 3034 (Ar-CH), 1682 (C=O), 1539 (C=N); ^{1}H NMR (300 MHz, DMSO): 8.56-8.77 (m, 3H, pyrazine), 7.38-8.41 (m, 5H, aromatic), 7.20 (s, 1H, =CH) 9.39 (s, 1H, N-H); MS (*m/z*): 227.1782 [M+1]

PH 2: N-[3-phenylprop-2-en-1-ylidene] pyrazine-2-carbohydrazide

UV λ_{max} (CH_3OH):329; IR (KBr): 3296 (NH), 3026 (Ar-CH), 1682 (C=O), 1454 (C=N); ^{1}H NMR (300 MHz, DMSO): 8.73-8.87 (m, 3H, pyrazine), 7.03-7.61 (m, 5H, aromatic), 7.50 (s, 1H,= CH) 4.26-6.97 (s, 32H, =CH-CH), 10.08 (s, 1H, N-H); MS (*m/z*): 253.1835 [M+1]

PH 3: N'-[3-furyl methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):320; IR (KBr): 3294 (NH), 3034 (Ar-CH), 1682 (C=O), 1458 (C=N); ^{1}H NMR (300 MHz, DMSO): 8.72-8.87 (s, 3H, pyrazine), 6.92-7.83 (m, 3H, furyl), 6.62 (s, 1H, =CH) 12.25 (s, 1H, N-H); MS (*m/z*):217.1340 [M+1]

PH 4: N'-[(4-methoxyphenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH): 323; IR (KBr): 3296 (NH), 3032 (Ar-CH), 1682 (C=O), 1471 (C=N)**;** ^{1}H NMR (300 MHz, DMSO): 8.72-8.86 (m, 3H, pyrazine), 6.97-7.64 (m, 4H, aromatic), 7.00 (s, 1H, =CH), 3.36 (s, 3H, -OCH_3), 12.08 (s, 1H, N-H); MS (*m/z*): 257.1180 [M+1], 279.1151 [M+Na]

PH 5: N'-[(2-chlorophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH): 264; IR (KBr): 3306 (NH), 3024 (Ar-CH), 1684 (C=O), 1473 (C=N), 780 (C-Cl); ^{1}H NMR (300 MHz, DMSO): 8.74-8.87 (m, 3H, pyrazine), 7.37-7.98 (m, 4H, aromatic), 7.69 (s, 1H, =CH), 12.54 (s, 1H, N-H); MS (*m/z*): 261.0184 [M+1]

PH 6: N'-[(3-chlorophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH): 263; IR (KBr): 3304 (NH), 3078 (Ar-CH), 1695 (C=O), 1651 (C=N), 631 (C-Cl); ^{1}H NMR (300 MHz, DMSO): 8.54-8.78 (m, 3H, pyrazine), 7.47-7.88 (m, 4H, aromatic), 7.59 (s, 1H, =CH), 12.44 (s, 1H, N-H); MS (*m/z*): 260.0084 [M^+]

PH 7: N'-[(4-chlorophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH): 221; IR (KBr): 3298 (NH), 3051 (Ar-

CH), 1684 (C=O), 1529 (C=N), 797 (C-Cl); ^{1}H NMR (300 MHz, DMSO): 8.45-8.87 (m, 3H, pyrazine), 7.34-7.78 (m, 4H, aromatic), 7.29 (s, 1H, =CH), 12.42 (s, 1H, N-H); MS (*m/z*): 261.1084 [M^++ 1]

PH 8: N'-[(2-nitrophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):265; IR (KBr): 3288 (NH), 3101 (Ar-CH), 1626 (C=O), 1537 (C=N), 1564 (C-NO_2); ^{1}H NMR (300 MHz, DMSO): 8.74-8.87 (m, 3H, pyrazine), 7.61-7.80 (m, 4H, aromatic), 7.43 (s, 1H, =CH), 12.63 (s, 1H, N-H); MS (*m/z*): 272.1102 [M+1]

PH 9: N'-[(3-nitrophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):269; IR (KBr): 3285 (NH), 3010 (Ar-CH), 1684 (C=O), 1699 (C=N), 1521 (C-NO_2); ^{1}H NMR (300 MHz, DMSO): 8.47-8.78 (m, 3H, pyrazine), 7.65-7.83 (m, 4H, aromatic), 7.24 (s, 1H, =CH), 12.36 (s, 1H, N-H); MS (*m/z*): 295.1012 [M+Na]

PH 10: N'-[(4-nitrophenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):263; IR (KBr): 3292 (NH), 3103 (Ar-CH), 1701 (C=O), 1527 (C=N), 1577 (C-NO_2); ^{1}H NMR (300 MHz, DMSO): 8.64-8.87 (m, 3H, pyrazine), 7.65-7.88 (m, 4H, aromatic), 7.54 (s, 1H, =CH), 12.60 (s, 1H, N-H); MS (*m/z*): 272.1112 [M+1]

PH 11: N'-[(2-hydroxyphenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):292; IR (KBr): 3211 (NH), 3024 (Ar-CH), 1676 (C=O), 1541 (C=N), 3450 (C-OH); ^{1}H NMR (300 MHz, DMSO): 8.84-8.98 (m, 3H, pyrazine), 6.96-7.58 (m, 4H, aromatic), 7.50 (s, 1H, =CH), 12.65 (s, 1H, N-H), 5.23 (s, 1H, OH of phenyl); MS (*m/z*): 243.1610 [M+1]

PH 12: N'-[(3-hydroxyphenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):263; IR (KBr): 3231 (NH), 3042 (Ar-CH), 1660 (C=O), 1575 (C=N), 3452 (C-OH); ^{1}H NMR (300 MHz, DMSO): 8.48-8.68 (m, 3H, pyrazine), 6.69-7.35 (m, 4H, aromatic), 7.52 (s, 1H, =CH), 12.56 (s, 1H, N-H), 5.33 (s, 1H, OH of phenyl); MS (*m/z*): 243.1660 [M+1]

PH 13: N'-[(4-hydroxyphenyl) methylene] pyrazine-2-carbohydrazide

UV λ_{max} (CH3OH):266; IR (KBr): 3273 (NH), 2984 (Ar-CH), 1684 (C=O), 1575 (C=N), 3462 (C-OH); ^{1}H NMR (300 MHz, DMSO): 8.44-8.88 (m, 3H, pyrazine), 6.66-7.38 (m, 4H, aromatic), 7.50 (s, 1H, =CH), 12.42 (s, 1H, N-H), 5.43 (s, 1H, OH of phenyl); MS (*m/z*): 243.2760 [M+1]

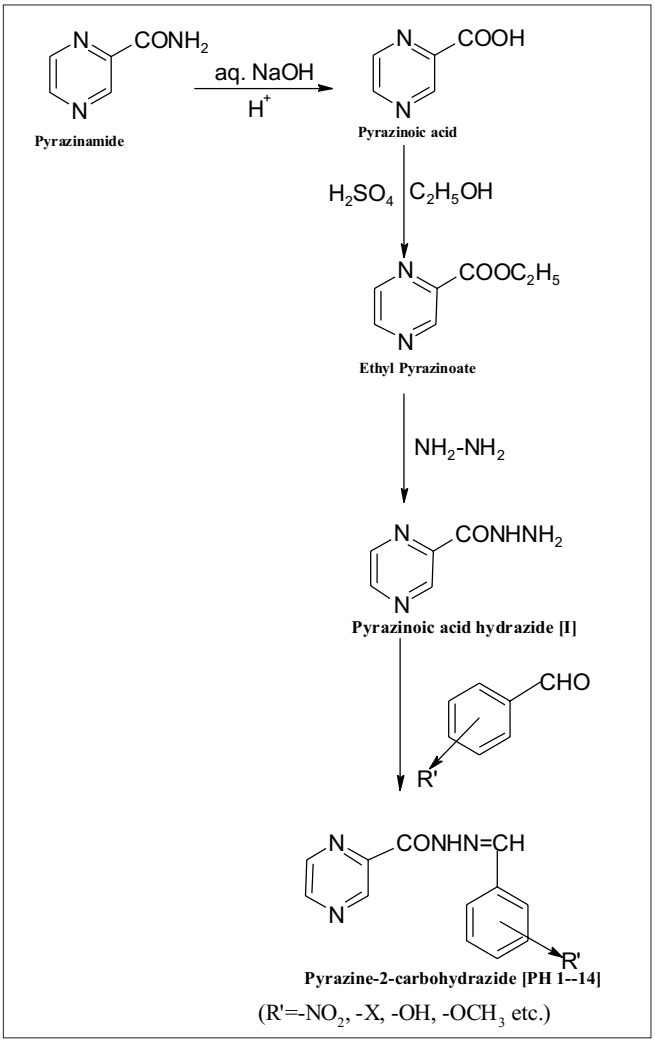
PH 14: N'-{[4-(dimethylamino) phenyl] methylene} pyrazine-2-carbohydrazide

UV λmax (CH3OH):386; IR (KBr): 3302 (NH), 2994 (Ar-CH), 1682 (C=O), 1471 (C=N); 1H NMR (300 MHz, DMSO): 8.42-8.85 (m, 3H, pyrazine), 6.71-7.54 (m, 4H, aromatic), 7.48 (s, 1H, =CH), 11.91 (s, 1H, N-H), 2.50 (s, 3H, CH3), 2.97 (s, 3H, CH3); MS (*m/z*): 270.1776 [M+1]

Evaluation of biological activity

Antibacterial activity

Different bacterial strains such as *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2711), *Salmonella typhi* (NCIM 2501) and *Escherichia coli* (NCIM 2685) were obtained from



Scheme 1: Synthetic route of the compounds

the National Collection of Industrial Microorganism (NCIM)-NCL, Pune. The *in vitro* antibacterial activity of the compounds PH 1-14 were carried out by the agar cup plate method.[8,9] The concentration of compound (250 µg/ml) were prepared in dimethyl sulfoxide (DMSO). Ofloxacin was used as standard.

The antibacterial activity was evaluated by using 24-h cultures of *S. aureus*, *B. subtilis*, *Salmonella typhi* and *E. coli* using Muller Hinton agar medium. The medium was sterilized by autoclaving at 120°C (15 lb/in^2) for 30 min. About 30 ml of molten nutrient agar medium inoculated with the respective strains of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred aseptically into each sterilized petridishes (10 cm diameter). The plates were left at room temperature to allow solidification of the media. In each plate, 3 wells of 6 mm diameter were made using a sterile borer. Accurately 0.1 ml of the test and standard solution were transferred to the wells aseptically by micropipette and labeled accordingly. The plates were then maintained at room temperature for 2 h to allow the diffusion of the solution in the medium. The petridishes used for antibacterial screening were incubated at 37±1°C for 24 h. The diameter of zone of inhibition surrounding each well was recorded.

The compounds exhibited antibacterial activity against *S. aureus*, *B. subtilis*, *Salmonella typhi* and *E. coli*, which were assayed on a quantitative basis determining the Minimal Inhibitory Concentration (MIC) for that microorganisms.

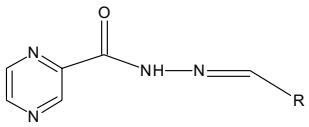
RESULTS AND DISCUSSION

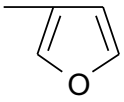
In series 1, seven compounds showed antibacterial activity against *S. aureus* and *B. subtilis*. The derivatives PH01, PH02, PH03, PH04, PH08, PH09 and PH10 have been found to be active among the compounds tested, whereas in the same series only three and two compounds showed antibacterial activity against *E. coli* and *Salmonella typhi*, respectively [Table 1]. The compounds PH05, PH06 and PH 07 were found to be active against *E. coli* and compound PH12 and PH 14 were found to be active against *Salmonella typhi* as compared with the standard ofloxacin [Table 2].

CONCLUSION

From the IR, ^{1}HNMR and MS spectral data, the assigned structure were established. The synthesized compounds showed potent antimicrobial activity against the selected strains of Gram-positive bacteria as compared to Gram-negative bacteria.

Table 1: Physical data of synthesized compounds (Series 1)



Comp. code	Substituents (-R)	M.F.	M.W.	m.p. (°C)	(%) Yield	Rf value
PH 1	-C_6H_5	$C_{12}H_{10}N_4O$	226.234	238-239	72	0.673
PH 2	-CH=CH-C_6H_5	$C_{14}H_{12}N_4O$	252.271	249-250	54	0.808
PH 3		$C_{10}H_8N_4O_2$	216.196	202-204	78	0.693
PH 4	-C_6H_4-*p*-OCH_3	$C_{13}H_{12}N_4O_2$	256.259	207-209	45	0.591
PH 5	-C_6H_4-*o*–Cl	$C_{12}H_9ClN_4O$	260.679	202-204	53	0.638
PH 6	-C_6H_4-*m*–Cl	$C_{12}H_9ClN_4O$	260.679	197-199	50	0.617
PH 7	-C_6H_4-*p*–Cl	$C_{12}H_9ClN_4O$	260.679	249-250	63	0.510
PH 8	-C_6H_4-*o*–NO_2	$C_{12}H_9N_5O_3$	271.231	208-211	72	0.617
PH 9	-C_6H_4-*m* -NO_2	$C_{12}H_9N_5O_3$	271.231	215-217	42	0.638
PH 10	-C_6H_4-*p*–NO_2	$C_{12}H_9N_5O_3$	271.231	308-310	56	0.523
PH 11	-C_6H_4-*o*–OH	$C_{12}H_{10}N_4O_2$	242.233	202-203	47	0.632
PH 12	-C_6H_4-*m*–OH	$C_{12}H_{10}N_4O_2$	242.233	252-254	70	0.510
PH 13	-C_6H_4-*p*–OH	$C_{12}H_{10}N_4O_2$	242.233	296-297	76	0.456
PH14	-C_6H_4-N $(CH_3)_2$	$C_{14}H_{15}N_5O$	269.301	255-256	81	0.617

All the compounds in Table 1 were crystallized from aq. ethanol. Purity of the compounds in Table 1 was determined using Chloroform: Methanol (4:1)

Table 2: Antibacterial activity of Series 1

Compound code	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
PH 1	9.8 ± 0.05	6.8 ± 0.05	–	–
PH 2	9.5 ± 0.07	8.2 ± 0.07	–	–
PH 3	9.5 ± 0.07	8.2 ± 0.07	–	–
PH 4	10.8 ± 0.05	6.3 ± 0.05	–	–
PH 5	–	–	8.2 ± 0.1	–
PH 6	–	–	7.9 ± 0.09	–
PH 7	–	–	8.2 ± 0.10	–
PH 8	9.8 ± 0.1	9.5 ± 0.1	–	–
PH 9	8.0 ± 0.09	8.4 ± 0.09	–	–
PH 10	8.5 ± 0.10	8.8 ± 0.10	–	–
PH 11	–	–	–	–
PH 12	–	–	–	7.2 ± 0.1
PH 13	–	–	–	–
PH 14	–	–	–	8.2 ± 0.10
Control (DMSO)	–	–	–	–
Standard (Ofloxacin)	18.5 ± 0.08	14.3 ± 0.08	13.4 ± 0.08	10.5 ± 0.08

Zone of Inhibition in mm at 250 µg/ml concentration (n = 6; ± standard error)

Table 3: MIC of Series 1

Compound code	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
PH 1	180	180	–	–
PH 2	200	180	–	–
PH 3	190	190	–	–
PH 4	210	200	–	–
PH 5	–	–	190	–
PH 6	–	–	200	–
PH 7	–	–	210	–
PH 8	180	180	–	–
PH 9	190	200	–	–
PH 10	190	190	–	–
PH 11	–	–	–	–
PH 12	–	–	–	230
PH 13	–	–	–	–
PH 14	–	–	–	200

Results are expressed in microgram per milliliter concentration

Table 3 shows the MIC values of active antibacterial compounds in the range 180-230 µg/ml concentrations against common bacterial infection causing species. This indicates that the synthesized compounds were active against routinely infection causing bacteria. Further molecular manipulations of the lead molecule may improve the spectrum of antimicrobial activity. Hence, further studies are going in this direction in our laboratory by using modern sophisticated tools of drug design, *viz.*, QSAR and molecular modeling to obtain new broad-spectrum antimicrobial agents.

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