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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 'CONH' BRIDGED INDOLE AND PYRAZINE DERIVATIVES

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ABSTRACT

A new series of N'-[(E)-phenyl methylidene] pyrazine-2-carbohydrazide and N'-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) pyrazine-2-carbohydrazide derivatives (RBK1–RBK12) were synthesized and evaluated for their antituberculosis, antibacterial and antifungal activities. All the synthesized compounds were in good agreement with spectral analysis. Three synthesized compounds RBK3, RBK4 and RBK9 have shown significant anti-tubercular activity as compared to the reference drug. Compounds RBK7, RBK8 and RBK9 shown good antibacterial activity against gram positive bacteria and compounds RBK6, RBK9, RBK10 and RBK12 shown significant activity against gram negative bacteria. Compounds RBK3, RBK7, RBK10, RBK11 and RBK12 shown significant antifungal activity when compared with standard pyrizinamide, streptomycin, ciprofloxacin, fluconazole and were used as reference standard drug for the biological activity respectively. The purity and structure confirmation of the synthesized compounds were done by TLC, IR and ^{1}H-NMR spectral study.

KEYWORDS: Indole, Pyrazine, Antibacterial, Antifungal, Anti Tubercular Activity.

INTRODUCTION

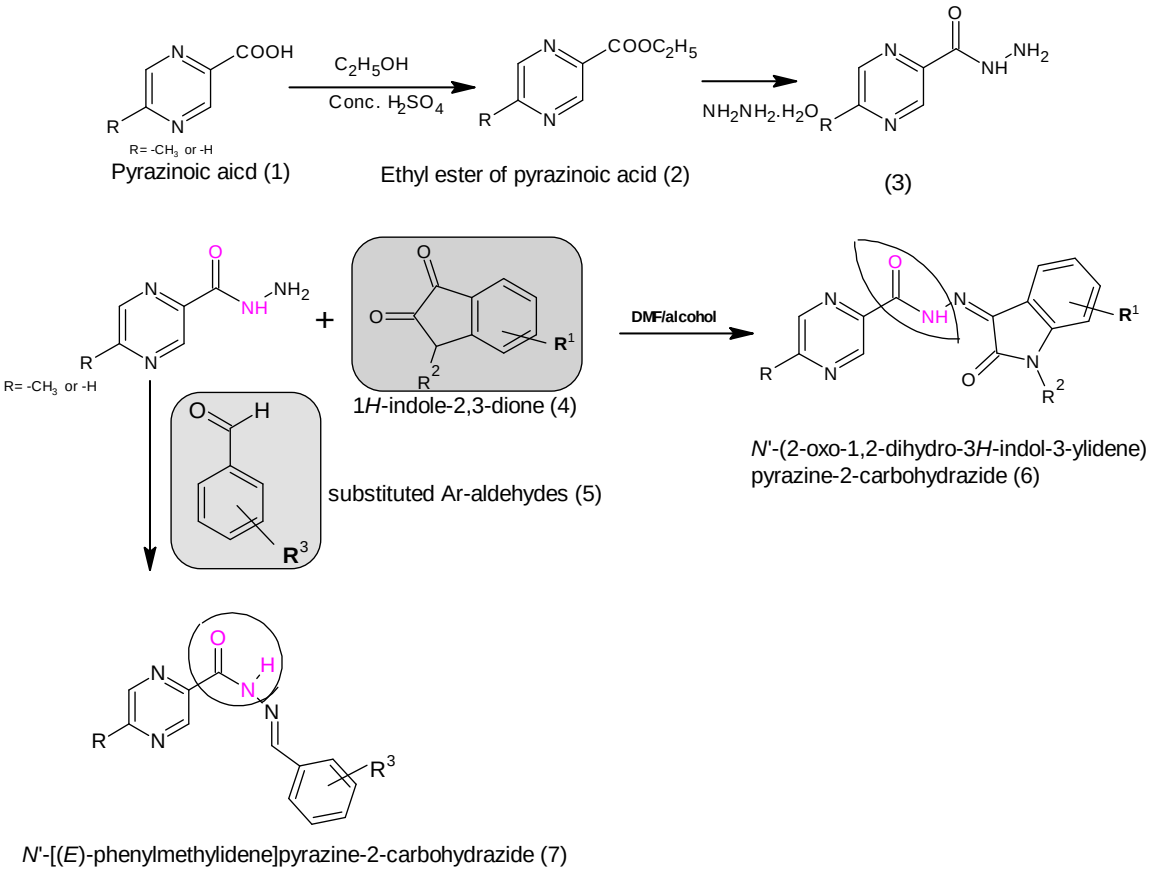
Despite the presence of large number of antibiotics, drug resistant species have become a major concern. The quest for better antimicrobial compounds against drug resistant species exhibiting minimum toxicity and higher therapeutic index is very relevant in present scenario. Indole and Pyrazine have been explored earlier for their biological activity in particular for their antimicrobial activity and antitubercular activity[1-4]. In the present work, synthesis and

characterization of a series of novel derivatives containing pyrazine and indole is reported along with results of *in vitro* studies for the determination of their activity against M Tuberculosis, Escherichia coli, Staphylococcus aureus and Candida albicans. Indole is widely distributed in the natural environment and can be produced by a variety of bacteria like *Bacillus alvei*, *Escherichia coli*, *Flavobacterium sp.* and *Haemophilus influenza*. It has been reported as an important signaling molecule in bacteria and it regulates various aspects of bacterial physiology, including resistance to drugs, spore formation, biofilm formation and plasmid stability. The amino acid tryptophan is an indole derivative and the precursor of the neurotransmitter serotonin. The indole-derived phytochemicals and bacterial metabolites are a result of biosynthesis via coupling of tryptophan with other amino acids. Chemically this heterocyclic ring is a fusion of benzene and pyrrol ring system. Indole is an important nitrogen heterocycle found in countless natural products, part of an essential amino acid (tryptophan), and a key structural component of many value added chemicals including pharmaceuticals.[5-8]

MATERIALS AND METHODS

The starting 5-methylpyrazine-2-carboxylic (1) acid was reacted with ethanol in the presence of catalytic amounts of concentrated sulfuric acid to yield pyrazinoic acid ester (2). The obtained ester was then reacted with 99% hydrazine hydrate (0.03 mol) in presence of alcohol and the mixture was refluxed for 4hrs on water bath. After cooling, the precipitate was filtered off, washed with cold water and dried under vacuum (60°C) to obtain title compound (3). The crude product was recrystalized from 50% aqueous ethanol.

SCHEME



RESULTS AND DISCUSSION

Preparation of *N*'-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) pyrazine-2-carbohydrazide (6) RBK 1-10: The obtained carbohydrazide (3) was then reacted with substituted and un-substituted indoles (4) in presence of dimethyl formamide (DMF) or in presence of alcohol gives N'-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) pyrazine-2-carbohydrazide derivatives(6) to obtain targeted molecules RBK1-RBK10 (table-3) and are in good yields and complying with spectral data.

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK1

IR Spectra (cm^{-1}): 3161, N-H stretch of 2^o amine, 2910 aromatic C-H stretch, 1693 C = O stretch, 1616 C = N stretch, 1398 $-CH_3$ bend. ^{1}H NMR spectrum (δppm): 2.642, 3H $-CH_3$ (s), 7.33-7.77, 4H, Ar-H (m), 8.1-9.3 1H, -NH (s) hydrazide, 10.37, 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK2

IR Spectra (cm^{-1}): 3161 N-H stretch of 2^o amine, 2910 aromatic C-H stretch, 1693 C = O stretch, 1616 C = N stretch. ^{1}H NMR spectrum (δppm): 7.34-7.64 4H, Ar-H (m), 8.6-9.5 1H, -NH (s) hydrazide, 10.71 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK3

IR Spectra (cm^{-1}): 3392 N-H stretch of 2^o amine, 3136 aromatic C-H stretch, 1703 C = O stretch, 1618 C = N stretch, 1398–CH_3 bend, 1276 C - F stretch. ^{1}H NMR spectrum (δppm): 2.667 3H -CH_3 (s), 7.10-7.61 4H Ar-H (m), 8.4-9.4 1H -NH (s) hydrazide.

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK4

IR Spectra (cm^{-1}): 3157 N-H stretch of 2^o amine, 1687 C = O stretch, 1616 C = N stretch, 1261 cm^{-1} C - F stretch. ^{1}H NMR spectrum (δppm): 7.10-7.61 4H, Ar-H (m), 8.6-9.5 1H, -NH (s) hydrazide, 10.87 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK5:

IR Spectra (cm^{-1}): 3599 N-H stretch of 2^o amine, 3352 aromatic C-H stretch, 1759 C = O stretch, 1608 C = N stretch. 1402–CH_3 bends. ^{1}H NMR spectrum (δppm): 2.720 3H, -CH_3 (s), 7.413-7.442 4H, Ar-H (m), 9.419 1H, -NH (s) hydrazide, 10.1 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK6

IR Spectra (cm^{-1}): 3184 N-H stretch of 2^o amine, 1703 C = O stretch, 1624 C = N stretch, 1400–CH_3 bend, 1213 C - F stretch. ^{1}H NMR spectrum (δppm): 3.333 3H, -CH_3 (s), 7.02-7.44 4H, Ar-H (m), 8.7-9.5 1H, -NH (s) hydrazide, 10.4 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK7

IR Spectra (cm^{-1}): 3180 N-H stretch of 2^o amine, 1701 C = O stretch, 1612 C = N stretch, 1332 N = O stretch. ^{1}H NMR spectrum (δppm): 7.8-8.0 4H, Ar-H (m), 8.3-8.8 1H, -NH (s) hydrazide, 10.59 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK8

IR Spectra (cm^{-1}): 3126 N-H stretch of 2^o amine, 2924 aromatic C-H stretch, 1720 cm^{-1} C = O stretch, 1608 C = N stretch, 1400–CH_3 bend , 1325 N = O stretch ^{1}H NMR spectrum (δppm): 2.624 3H, -CH_3 (s), 7.03-7.76 4H, Ar-H (m), 8.1-9.2 1H, -NH (s) hydrazide, 11.41 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK9

IR Spectra (cm^{-1}): 3068 N-H stretch of 2o amine, 2677 aromatic C-H stretch, 1656 C = O stretch, 1512 C = N stretch, 680 Br- stretch. ^{1}H NMR spectrum (δppm): 7.3-8.6 4H, Ar-H (m), 8.7-9.2 1H, -NH (s) hydrazide, 10.19 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK10

IR Spectra (cm^{-1}): 3392 N-H stretch of 2o amine, 3136 aromatic C-H stretch, 1703 C = O stretch, 1618 C = N stretch, 1400–CH_3 bend, 680 Br- stretch. ^{1}H NMR spectrum (δppm): 2.638 3H, -CH_3 (s), 7.26-7.76 4H, Ar-H (m), 7.8-9.3 1H, -NH (s) hydrazide, 10.66 3H, Pyrazine (m).

Preparation of N'-[(E)-phenyl methylidene] pyrazine-2-carbohydrazide (7)

RBK 11-12: The obtained carbohydrazide then treated with substituted and un-substituted aromatic aldehyde and few drops of glacial acetic acid in ethanol refluxed for 5 hours the residue was stirred with ice cold water and filtered off, and dried under vacuum to gives N'-[(E)-phenyl methylidene] pyrazine-2-carbohydrazide (7). The crude product was recrystalized from aqueous ethanol, Physical data of compounds RBK1-RBK12 presented in table 1.

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK11

IR Spectra (cm^{-1}): 3196 N-H stretch of 2o amine, 2610 aromatic C-H stretch, 1656 C = O stretch, 1614 C = N stretch, 1404–CH_3 bend, 1356 N = O stretch. ^{1}H NMR spectrum (δppm): 2.62 3H, -CH_3 (s), 7.50-7.98 4H, Ar-H (m), 8.23-9.21 1H, -NH (s) hydrazide, 12.05 3H, Pyrazine (m).

IR Spectra and ^{1}H NMR spectrum data of synthesized compound RBK12

IR Spectra (cm^{-1}): 3749 -OH stretch, 3165 N-H stretch of 2o amine, 2411 aromatic C-H stretch, 1691 C = O stretch, 1606 C = N stretch, 1377–CH_3 bend. ^{1}H NMR spectrum (δppm): 3.3-2.4 3H, -CH_3 (s), 8.1-7.0 4H, Ar-H (m), 9.3 -1H,(s)-OH, 8.6 1H, -NH (s) hydrazide 10.8 3H, Pyrazine (m).

BIOLOGICAL ACTIVITY[9-13]

Anti-Tubercular

Test compounds were evaluated for *in vitro* anti-tubercular activity. Minimum inhibition concentration (MICs) were determined and interpreted for *Mycobacterium tuberculosis* H_{37}Rv. Compounds were taken at concentrations of 100µg mL^{-1}, 50µg mL^{-1} and 25µg mL^{-1} in DMF. *M. tuberculosis* H_{37}Rv strain was used in Middle brook 7H-9 broths which was inoculated with standard as well test compounds and incubated at 37°C for 4 weeks, Table 2.

Antitubercular activity

Test compounds were evaluated for *in vitro* antitubercular activity. MICs were determined and interpreted for *Mycobacterium tuberculosis* H37Rv according to the procedure of the approved macro dilution reference method of antimicrobial susceptibility testing. Compounds were taken at concentrations of 100 µg/mL, 50 µg/mL and 25 µg/mL in DMF. *M.tuberculosis* H37Rv strain was used in Middle brook 7H-9 broth which was inoculated with standard as well test compounds and incubated at 37°C for 4 weeks. The bottles were inspected for growth twice a week for a period of three weeks. Readings were taken at the end of 4 weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain.

Antibacterial activity

Compounds were subjected to *in vitro* screening against Gram-negative and Gram-positive. The minimum inhibitory concentration (MIC) was determined using tube dilution method. Muller Hilton broth was used as culture medium. Sterilized medium was dispensed in each borosilicate glass test tubes. The drug solution was added in order to attain final concentration of 500, 250, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1 µgmL^{-1} in DMF. Inoculums' of standard suspension (0.1mL of the test organism strain which contains 5x10^{2} colony forming unit's mL^{-1}) was added to tubes. The tubes were incubated at 37°C for 24 hr. and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point.

Antifungal activity

The test compounds were evaluated at 500, 250, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1 µgmL^{-1} concentrations for their *in-vitro* antifungal activity using Sabouraud's Dextrose agar medium. Dimethyl formamide (DMF) was used as solvent for sample preparation. The Minimum Inhibitory Concentration (MIC) was determined. Sabouraud's Dextrose agar medium containing flucanozole as well as control Sabouraud's dextrose agar medium was inoculated with the microorganisms and incubated at 28° for 48 hr.

Table No 1: Data showing physical constants of compounds (RBK1-RBK12).

Sl. No	Product code	Mol formula	Mol weight	Melting point (°C)	% yield	Rf value
1	RBK 1	$C_{14}H_{11}N_5O_2$	281	292-94	77.7	0.63
2	RBK2	$C_{13}H_9N_5O_2$	267	286-88	65.5	0.66
3	RBK 3	$C_{14}H_{10}N_5O_2F$	299	280-82	53.1	0.70
4	RBK 4	$C_{13}H_8N_5O_2F$	285	300-02	84.4	0.59
5	RBK 5	$C_{15}H_{13}N_5O_2$	295	272-74	74.74	0.77
6	RBK 6	$C_{14}H_{11}N_5O_2$	281	298-300	91.62	0.63
7	RBK 7	$C_{13}H_8N_6O_4$	312	288-90	97.19	0.69
8	RBK 8	$C_{14}H_{10}N_6O_4$	326	278-80	88.95	0.72
9	RBK 9	$C_{13}H_8N_5O_2Br$	346	268-70	92	0.77
10	RBK 10	$C_{14}H_{10}N_5O_2Br$	356	308-10	91.1	0.73
11	RBK 11	$C_{13}H_{11}N_5O_3$	285	300-02	97.39	0.67
12	RBK 12	$C_{13}H_{12}N_4O_2$	256	286-88	54.7	0.69

Table 2: Biological activity of synthesized compounds RBK1-RBK12.

Sl. No.	Compound	*M.tuber-culosis* $H_{37}Rv$	Gram –ve bacteria		Gram –ve bacteria		Fungi	
			E. Coli	*K. Pneumoniae*	*S. Aureus*	*B. sps*	*C. albicans*	*A. Niger*
	RBK-1	6.25	0.8	0.4	50	12.5	0.4	50
	RBK-2	12.25	0.4	3.12	50	50	0.4	25
	RBK-3	100	0.4	6.25	50	25	1.6	25
	RBK-4	100	6.25	12.5	25	25	0.4	25
	RBK-5	12.5	0.4	25	25	50	0.4	25
	RBK-6	50	1.6	50	100	25	0.2	50
	RBK-7	12.5	25	50	50	50	3.12	50
	RBK-8	12.5	50	100	50	50	0.8	6.25
	RBK-9	100	50	25	100	50	0.8	12.5
	RBK-10	3.12	6.25	50	100	25	1.6	50
	RBK-11	12.5	12.5	50	50	100	0.6	100
	RBK-12	12.5	6.5	50	100	50	0.4	100

Table 3: Showing substitution on CONH bridged Pyrazines and Indoles.

Sl. No	Product code	Mol formula	R	R^1	R^2	R^3
1	RBK 1	$C_{14}H_{11}N_5O_2$	5-CH_3	-H	-H	---
2	RBK2	$C_{13}H_9N_5O_2$	-H	-H	-H	---
3	RBK 3	$C_{14}H_{10}N_5O_2F$	5-CH_3	-F	-H	---
4	RBK 4	$C_{13}H_8N_5O_2F$	-H	-F	-H	---
5	RBK 5	$C_{15}H_{13}N_5O_2$	5-CH_3	-H,	-CH_3	---
6	RBK 6	$C_{14}H_{11}N_5O_2$	-H	7-F,	-CH_3	---
7	RBK 7	$C_{13}H_8N_6O_4$	-H	5-NO_2	-H	---
8	RBK 8	$C_{14}H_{10}N_6O_4$	5-CH_3	5-NO_2	-H	---
9	RBK 9	$C_{13}H_8N_5O_2Br$	-H	6-Br	-H	---
10	RBK 10	$C_{14}H_{10}N_5O_2Br$	5-CH_3	6-Br	-H	---
11	RBK 11	$C_{13}H_{11}N_5O_3$	5-CH_3	-	-H	*o*-NO_2
12	RBK 12	$C_{13}H_{12}N_4O_2$	5-CH_3	-	-H	*o*-OH

CONCLUSION

In this work, I report the synthesis of N'-[(E)-phenyl methylidene] pyrazine-2-carbohydrazide and N'-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) pyrazine-2-carbohydrazide derivatives (RBK1–RBK12) and evaluated for their antituberculosis, antibacterial and antifungal activities. All the synthesized compounds were in good agreement with spectral analysis. Three synthesized compounds RBK3, RBK4 and RBK9 have shown significant anti-tubercular activity as compared to the reference drug. Compound RBK7, RBK8 and RBK9 shown good antibacterial activity against gram positive bacteria and compound RBK6, RBK9, RBK10 and RBK12 shown significant activity against gram negative bacteria. Compound RBK3, RBK7, RBK10, RBK11 and RBK12 shown significant antifungal activity when compared with standard drugs. Pyrizinamide, streptomycin, ciprofloxacin, fluconazole were used as the standard drug for the biological activity respectively. The purity and structure confirmation of the synthesized compounds were done by TLC, IR and ^{1}H-NMR spectral study. This study extends the scope exploration of novel CONH bridged pyrazines and indoles as promising antitubercular and antimicrobial agents.

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