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Design, synthesis of certain triazole derivatives bearing pyrazine moiety

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ABSTRACT

Several 4-pyrazinoyl 3 aryl/aryloxy methyl substituted 1,2,4-triazolo (3,4-b) (1,3,4) thiadiazolidine derivatives incorporating pyrazinoyl group have been synthesized. The compounds were screened for *in vitro* antibacterial,antifungal and antitubercular activities. Some compounds exhibited significant antitubercular and antifungal activity.

Key words: Triazole derivatives, pyrazine moiety, N-bridged heterocycles, pathogenic organisms, antitubercular activity.

INTRODUCTION

Certain 1,2,4-triazoles and thiadiazoles have displayed diverse biological activities. Further it was also reported that substituted 1,2,4-triazoles and their N-bridged heterocycles have received considerable attention during past two decades as they are endowed with variety of biological activities and have a wide range of therapeutic properties[1-4] . The wide spectrum of biological activities exhibited by various triazole derivatives derived from 4-amino/ substituted amino-5-mercapto-1,2,4-triazoles have made them an important class of chemotherapeutic agents. 1,2,4-Triazole nucleus has been incorporated into a good number of interesting drug candidates including CNS stimulants, antianxiety agents, sedatives, H_1/H_2 histamine receptor blockers, cholinesterase active agents[5-9] etc.,

Promted by these observations and in continuation of our work on nitrogen heterocycles of medicinal interest , we report here in the design, synthesis and biological evaluation of certain triazole derivatives bearing pyrazine moiety. The investigation appeared interesting because of compactness and planarity of triazole ring system. Over the past several years the emergence of organisms resistance to nearly all the classes of antimicrobial agents has become a serious public health concern .The emergence of bacterial resistance to the variety of antimicrobial agents containing different biological active moieties is quite serious. The triazole derivatives are expected to have chemotherapeutic intervention against bacteria, virus and other pathogens. This lead us to synthesize title compounds and evaluate them for different biological activities with expectation to have chemotherapeutic intervention against different pathogenic organisms.

The studies were extended to the synthesis of 4-pyrazinoyl-3-aryl/aryloxy methyl substituted-1,2,4-triazolo(3,4-b) (1,3,4)-thiadiazolidine derivatives incorporating pyrazinoyl group that is present in pyrazinamide a well known antitubercular drug.

The required potassium dithiocarbazinates of aryl/aryloxy acid hydrazides were prepared by heating the hydrazides with carbon disulphide in

presence of alcoholic potassium hydroxide. The potassium dithiocarbazinates thus prepared were heated with pyrazinic acid hydrazide in an oil bath for 6-8 hrs until profuse evolution of hydrogen sulphide was observed to obtain the required 3 -aryl /ayloxy methyl -4-(N-pyrazin-2^1-yl-carboxamido)- 5-mercapto 1,2,4-triazoles. A mixture of 5-mercapto triazoles and excess of formaldehyde was refluxed for 40-48 hrs and the reaction mixture on cooling was poured on crushed ice when title compound i.e. 4-pyrazinoyl-3 aryl/aryloxy methyl substituted -1,2,4-triazolo (3,4-b) (1,3,4) thiadiazolidine derivatives were obtained in good yields. The structures of all the newly synthesized compounds were established on the basis of their elemental and spectral data.

MATERIAL AND METHODS

All the chemicals used were of analytical grade. The drugs used for screening biological activity were procured from different pharmaceutical industries as gift samples.

The melting points were determined in open capillaries and are uncorrected. TLC was performed on silica gel G coated plates using chloroform and ethyl acetate as irrigant and iodine vapour as visualizing agent. IR spectra in KBR (cm^{-1}) were recorded on simadzu FTIR 8000 series spectrophotometer and ^{1}H NMR spectra on EM-390 MHZ spectrometer in DMSO-d_6 using TMS as internal standard (chemical shifts are expressed in δ ppm) . All the compounds gave satisfactory elemental analysis for C, H and N.

Synthesis of 3-aryl/aryloxy methyl-4(N-pyrazin-2^1–yl caboxamido)-5-mercapto-1,2,4-triazoles

These are obtained by heating a mixture of potassium dithiocarbazinates of aryl/aryloxy acids as per the reported procedure.

Synthesis of 4-pyrazinoyl-3-aryl/aryloxy methyl substituted -1,2,4-triazolo (3,4-b) (1,3,4)-thiadiazolidine derivatives

A mixture of mercapto triazole ($3a_1$–a_{15}, 0.1 mol) and excess of formaldehyde was refluxed for 40-48 hrs and the reaction mixture on cooling was poured on crushed ice, when the required thiadiazolidine derivatives ($3a_1$ –a_{15}) were separated out. The product is filtered, washed with water, dried and recrystallized from aqueous ethanol. The purity of the compounds was established by single spot on TLC silica gel-G plate using the solvent system chloroform : ethyl acetate (1:3). All the compounds of this series are prepared following the same procedure. Their characterization data is given in table 1. $3a_1$ IR (cm^{-1}) 3065 (aromatic C-H stretching 2840 (C-H stretching to CH_2)absence of thiol group at 2625, 1690 (C=0), 1610 (C=N), 1600 (C=C), 1348 (C-N), 740 (mono substituted benzene), 690 (C-S-C), ^{1}HNMR δ 4.4 (2H,s,S-CH_2) 6.8 – 8.0 (5H,m,Ar-H), 8.8,9.4 (3H,m,pyrazinyl)MS m/z 309 (M^+) [Found C,54.14; H,3.12; N,27.06; $C_{14}H_{10}ON_6S$ requires C,54.19; H,3.20; and N 27.10%]

$3a_4$ IR (cm^{-1}) 3070 (aromatic C-H stretching). 2820C-H stretching (-CH_2-) absence of thiol group at 2625, 1690 (C=O), 1620 (C=N),1605 (C=C), 1530, 1352 (NO_2), 1380 (C-N), 695 (C-S-C), 660 (C-Cl). ^{1} HNMR δ 4.4 (2H,s,S-CH_2), 6.8-7.9 (3H,m,Ar-H),8.8-9.4 (3H,m,pyrazinyl),MS m/z 389 (M^+), 391 (M^++2), 282, 222,156, 122,107, 79,77,69,57,43. [Found C,43.12; H,1.99;N,25.02; $C_{14}H_8O_3N_7SCl$ requires C,43.19; H,2.05 and N,25.19%]

$3a_6$ IR (cm^{-1})3064 (aromatic C-H stretching), absence of thiol group at 2625, 2380(OCH_2), 1618(C=O), 1618(C=N),1608(C=C), 1390(C-N),1162 (C-0-C),728 (mono substituted benzene), 692(C-S), ^{1}HNMR δ 4.5(2H,s,S-CH_2), 5.4 (2H,s,OCH_2) 6.9-8.1 (54,m,Ar-H),8.8-9.4 (34,m,pyrazinyl)

$3a_9$ IR (CM^{-1}),3070 (aromatic C-H stretching, absence of bond at 2625 for –SH of mercapto triazole, 2382 (OCH_2),1690 (C=O), 1615 (C=N), 1602(C=C),1387 (C-N),1165 (C-O-C),840 (1,4–disubstituted benzene), 696 (C-S), ^{1}HNMR d 2.4(3H,s,CH_3),4.5 (2H,s,S-CH_2), 6.9-8.1 (4H,m,Ar-H), 8.8-9.4 (3H,m,pyrazinyl)MS m/z 354,247,121,107,79.77,69,57,43 [Found C,54.17; H,3.86;N,23.65;$C_{16}H_{14}O_2N_6S$ requires C,54.24; H,3.95; N,23.73%]

Biological activity

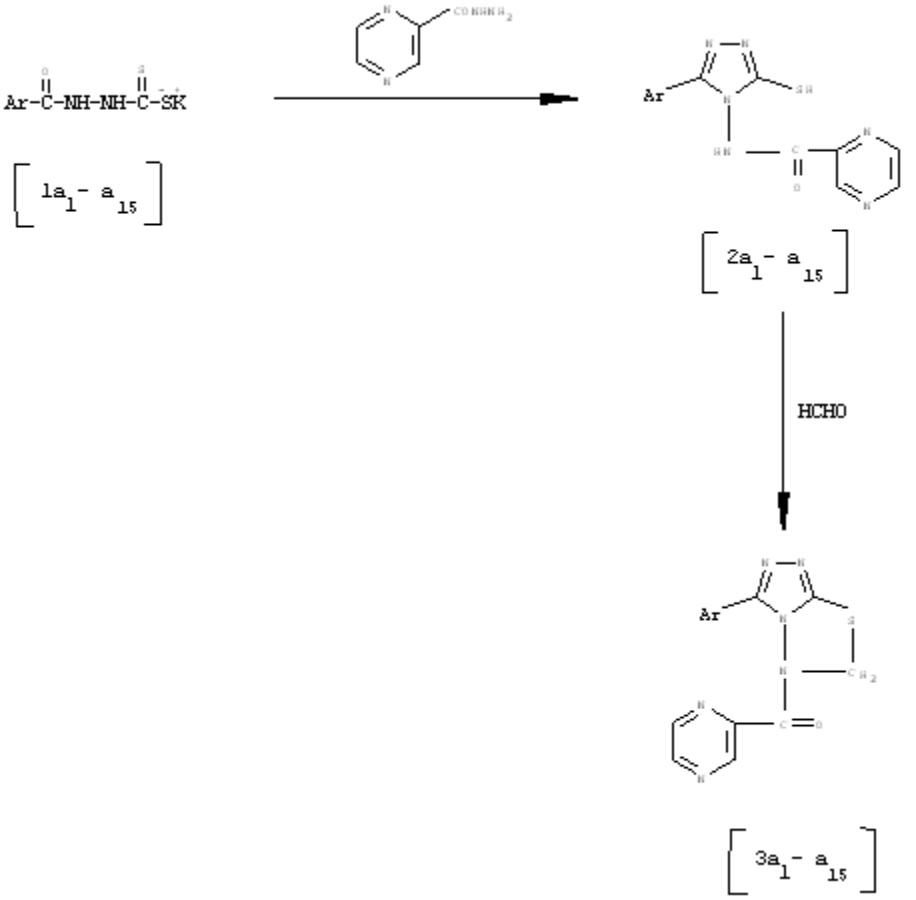
The compounds were screened for invitro antibacterial, antifungal, antitubercular activities using the reported standard procedures[10]. The

invitro antitubercular activity of the compounds were studied at the concentrations 100mg, 10mg and 1mg/ml against the highly virulent $H_{37}RV$ strain *Mycobacterium tuberculosis* LJ medium was used for inoculation, LJ tubes without the compounds were used as control. LJ tubes with the solvent DMSO and standard drug Streptomycin of above concentrations were used for the study. The tubes after 28 days of inoculation were taken for observation to note the presence or absence of pellicular growth. Absence of any growth was considered as inhibition. All the newly synthesized compounds were screened for their invitro antibacterial activity[10] against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeuroginosa* in DMF at the concentrations 50µg & 100µg/ml by cup plate method. Ampicillin was used as standard. The compounds were also screened for their invitro antifungal activity[11] against *Aspergillus niger & E.albicans* in DMF at concentrations 50µg & 100g/µl. Griseofulvin was used as standard.

Compounds A_1, A_3, A_5, A_6, A_{15} exhibited almost equipotent antitubercular activity incomparision with the standard while majority of the compounds except A_7, A_8, A_9 and A_{14} showed significant antifungal activity. However all the compounds showed weak antibacterial activity.

RESULTS AND DISCUSSION

All the synthesized compounds screened for their invitro antibacterial activity showed only weak activity much against our expectation. Generally, the compounds which have exhibited significant antitubercular activity do possess potent antibacterial activity also. But during the present study even though the compounds exhibit antibacterial activity it is the weak activity and we



Scheme 1

Table 1: Characterization data of triazole-thiadiazolidines ($3a_1$-a_{15})

S. No.	Compound	Ar	MP °C	Yield %	Rf value	Antifuntal activity (µg/ml) *A. niger* 50	*A. niger* 100	*C. albicans* 50	*C. albicans* 100	Antitubercula activity 1µg	10µg	100µg
1	$3a_1$	phenyl	184	64	0.71	12	16	12	16	+	-	-
2	$3a_2$	2-chloro phenyl	196	60	0.65	13	16	13	16	+	+	+
3	$3a_3$	4-chloro phenyl	173	68	0.74	14	17	13	16	-	-	-
4	$3a_4$	4-chloro-3-nitro phenyl	235	62	0.66	13	17	12	16	+	++	++
5	$3a_5$	5-chloro-2-hydroxy phenyl	277	60	0.64	14	18	13	17	+	-	-
6	$3a_6$	phenoxy methyl	230	63	0.66	13	18	13	17	+	-	-
7	$3a_7$	2-methy phenoxy methyl	228	62	0.60	9	12	8	11	+	++	++
8	$3a_8$	3- methyl phenoxy methyl	219	65	0.66	8	11	7	10	+	+	+
9	$3a_9$	4- methyl phenoxy methyl	206	68	0.65	9	12	7	11	+	+	+
10	$3a_{10}$	2-chloro phenoxy methyl	233	65	0.62	12	16	12	16	+	++	++
11	$3a_{11}$	4-chloro phenoxy methyl	212	68	0.68	14	17	13	18	+	+	+
12	$3a_{12}$	4-bromo phenoxy methyl	246	58	0.61	13	16	12	17	+	++	++
13	$3a_{13}$	4-nitro phenoxy methyl	259	70	0.63	13	17	12	16	+	+	+
14	$3a_{14}$	1-naphthoxy methyl	197	64	0.65	8	10	7	11	+	+	+
15	$3a_{15}$	2-naphthoxy methyl	209	68	0.68	12	16	13	17	+	-	-
	Griseofulvin					13	17	12	16			
	Streptomycin									+	-	-

(-) = no growth (indicates activity)

(+) = less than 20 colonies

(++) = more than 20 colonies.

felt its not worth mentioning. However the design of title triazolo - thiadiazoline molecules showed quite significant antifungal activity may be due to the fact that the fused system of the rings and compactness of the molecules may provide the required structural necessities for the enhancement of antifungal activity. C-S-C linkage of thiadiazolidine moiety helps to improve the antifungal activity. The compounds with 2-chloro phenyl, 4-chloro phenyl, 4-chloro-3-nitrophenyl, 5-chloro-2-hydroxy phenyl, 2-chloro, 4-chloro phenoxy methyl, 4-nitro phenoxy methyl substitutents which are the electronegative groups on the phenyl ring system at 3rd position of the triazole may contribute for much improved antifungal activity. This is due to the reason that none of the compounds having electron donating group on the phenyl ring showed significant antifungal activity. Thus, overall the phenyl ring at 3rd position of triazole, C-S-C system in thiadiazolidine ring, the fused ring system and compactness of the molecule play vital role in the improved antifungal activity.

Among the compounds screened for antitubercular activity against the highly virulent human strain *mycobacterium tuberculosis* H_{37}RV the aryl groups containing chlorine atoms at different positions of aryl ring and phenoxy ring having methyl substituent have exhibited equipotent activity with a standard Streptomycin at the tested concentrations particularly at 10 µg/ml. Rest of the compounds did not show activity at the above concentrations. The cell wall of the tubercle bacilli is known to be rich in lipids. Therefore, lipid soluble compounds may be expected to penetrate the bacterial cell wall better than those which are water soluble. Perhaps the active one may enter the lipoidal layer of the bacterial cell and may produce the same type of mechanism as INH, the first line of drug used for the therapy.

The screening of the biological activity in general revealed that the compounds possess the potent antifungal and antitubercular activity. Though the general nature of the substituents in the moiety is described, it is not possible to draw conclusive structure activity relationship of the compounds with respect to each activity. Hence the further modification of the moiety or detailed study on biological activity is necessary. Hence detailed toxicity studies of the compounds is necessary and it may be quite rewarding.

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