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Antibacterial and antitubercular screening of amine-amide linked disubstituted 1,2,3-triazoles

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Abstract: Antibacterial evaluation of twenty five synthesized amine-amide linked 1,4-disubstituted 1,2,3-triazoles has been carried out against – *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Staphylococcus aureus*, while, antitubercular activity was examined against – *Mycobacterium tuberculosis* H_{37}Rv. Biological screening of synthesized 1,2,3-triazoles revealed moderate to good antibacterial and antitubercular activity against tested strains.

Keywords: Antibacterial activity, Antitubercular activity, 1,4-Disubstituted 1,2,3-triazoles

Introduction

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, is creating a havoc in the society and responsible for millions of death worldwide every year [1]. Present drugs used for treatment of tuberculosis are also loosing the grip slowly on this infectious disease. So it is need of time to come out with new broad spectrum antitubercular drug to curb this disease effectively. On the other hand, commonly available antibacterial agents are also facing a challenge for ever increasing to resistant bacterial infections. Owing to this researchers must focus their theme of work for developing broad spectrum and effective antibacterials.

Different kinds of heterocyclic compounds are widespread in nature, among them; five-member heterocyclic compounds are of key important compounds because of their enormous biological activity. Triazole derivatives are five member heterocycles containing nitrogen atoms which, readily able to bind with active sites in biological system *via* diverse non-covalent interactions, and thus display versatile biological spectrum. The triazole nucleus possess various pharmacological activities like antimicrobial [2-4], antimalarial [5-7], anti-inflammatory [8], antitubercular [9-11], antidiabetic [12], anticonvulsant [13], antitrypanosomal [14], ,

anti-HIV [15], anticancer [16-18], antiallergic [19], antioxidant [20,21, 22] etc.

Encouraged from above fact, we have screened earlier synthesized [23, **Figure 1**] amine-amide linked 1,4-disubstituted 1,2,3-triazoles against – *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Staphylococcus aureus* bacterial strains by the serial dilution method and antitubercular activity against – *Mycobacterium tuberculosis* $H_{37}Rv$ by Lowenstein – Jensen method.

Experimental section

Biological activity

The antimicrobial evaluation was carried out with the help of Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar. The *in vitro* antitubercular was carried out from Microcare laboratory & TRC, Surat, Gujarat.

Antibacterial activity

The antibacterial testing of newly synthesized compounds was assessed *in vitro* against Gram-negative bacteria – *Escherichia coli* (MTCC 443), *Enterobacter aerogenes* (NCDC 106), *Klebsiella pneumoniae* (NCDC 138); Gram-positive bacteria – *Staphylococcus aureus* (MTCC 3160) as per serial dilution method [24] using a stock solution of 200 µg/mL concentration. Sabouraud dextrose broth was used as nutrient media while dimethylsulfoxide as a solvent control. Ciprofloxacin was used as a standard drug for bacterial strains. A stock solution of testing compound and control drug was serially diluted to get concentration of 100, 50, 25, 12.5, 6.25, 3.12 µg/mL. All these dilutions were inoculated with respective bacteria in saline solution and incubated at 37 ºC for 24 h. Results were recorded in terms of minimum inhibitory concentration (MIC) expressed in µmol/mL in **Table 1.**

Antitubercular activity

All the synthesized compounds were screened for anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv [MTCC 200] by Lowenstein Jensen method [25]. Isoniazid was used as standard drug for comparison. Lowenstein Jensen was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum size for test strain was adjusted to 1 mg/mL. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Each synthesized drug was diluted obtaining 2000 µg/mL concentration, as a stock solution. In primary screening 500 µg/mL, 250 µg/mL and 125 µg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

The compounds found active in primary screening were similarly diluted to obtain 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL and 1.5625 µg/mL concentrations. The highest dilution showing at least 99 % inhibition is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/mL. The recommended drug concentrations were 0.2 mg/L for Isoniazid. **Table 2**

Results and discussion

The amine-amide linked 1,4-disubsituted 1,2,3-triazoles **(1-25)** were synthesized from reaction of *N*-substituted(prop-2-yn-1yl)amines, 2-bromo-*N*-arylacetamides and sodium azide in the presence of copper sulphate pentahydrate and sodium ascorbate in dimethylformamide [23].

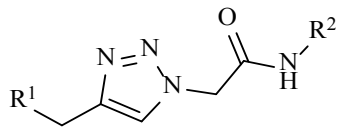
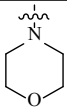
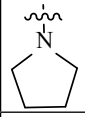
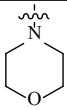
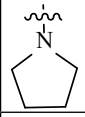
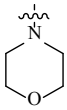
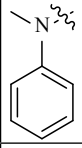
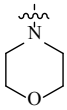
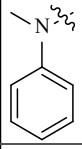
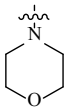
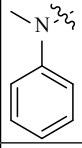
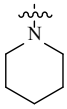
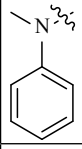
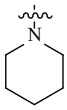
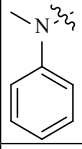
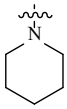
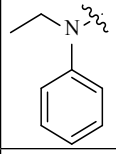
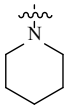
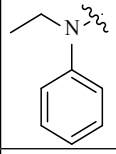
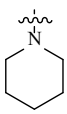
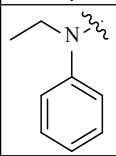
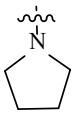
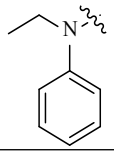
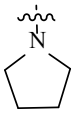
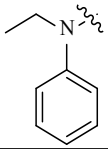
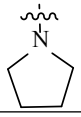


Figure 1

Compound	R^1	R^2		Compound	R^1	R^2
1		C_6H_5		**14**		4-$NO_2C_6H_4$
2		4-$CH_3OC_6H_4$		**15**		α-$C_{10}H_7$
3		4-FC_6H_4		**16**		C_6H_5
4		4-$NO_2C_6H_4$		**17**		4-$CH_3OC_6H_4$
5		α-$C_{10}H_7$		**18**		4-FC_6H_4
6		C_6H_5		**19**		4-$NO_2C_6H_4$
7		4-$CH_3OC_6H_4$		**20**		α-$C_{10}H_7$
8		4-FC_6H_4		**21**		C_6H_5
9		4-$NO_2C_6H_4$		**22**		4-$CH_3OC_6H_4$
10		α-$C_{10}H_7$		**23**		4-FC_6H_4

11		C_6H_5		**24**		4-$NO_2C_6H_4$
12		4-$CH_3OC_6H_4$		**25**		α-$C_{10}H_7$
13		4-FC_6H_4				

Amine-amide linked 1,4-disubstituted 1,2,3-triazoles

Biological activity

Antibacterial Activity

All the compounds **(1-25)** were tested for *in vitro* antibacterial activity against three Gram-negative bacteria – *Escherichia coli* (MTCC 443), *Enterobacter aerogenes* (NCDC 106), *Klebsiella pneumonia* (NCDC 138) and one Gram-positive bacteria – *Staphylococcus aureus* (MTCC 3160) by using serial dilution method [24]. Ciprofloxacin was used as standard drug and the minimum inhibitory concentrations (MIC) were presented in μmol/mL, listed in **Table 1**.

From the antibacterial screening, it was observed that the compounds exhibited moderate to good activity. Based on biological data in Table **1,** compound **5** (MIC, 0.0178 μmol/mL) displayed promising activity against *E. coli* and *K. pneumoniae*. Compounds **24** (MIC 0.0328 μmol/mL) and **25** (MIC 0.0324 μmol/mL) displayed moderate activity against the tested bacterial strains.

Table 1. *In vitro* antibacterial activity of 1,4-disubstituted 1,2,3-triazoles (1-25)

	Minimum Inhibitory Concentration (MIC, μmol/mL)			
Compound	***Escherichia Coli***	***Enterobacter aerogenes***	***Klebsilla pneumoniae***	***Staphylococcus aureus***
1	0.0830	0.0830	0.0415	0.0415
2	0.0377	0.0754	0.0377	0.0377
3	0.0391	0.0391	0.0391	0.0391
4	0.0361	0.0361	0.0361	0.0361
5	**0.0178**	**0.0356**	**0.0178**	**0.0356**
6	0.0418	0.0418	0.0836	0.0836
7	0.0759	0.0759	0.0379	0.0379
8	0.0394	0.0788	0.0394	0.0394
9	0.0726	0.0726	0.0726	0.0363
10	0.0358	0.0716	0.0358	0.0358
11	0.0876	0.0876	0.0438	0.0438
12	0.0396	0.0793	0.0396	0.0396
13	0.0412	0.0824	0.0412	0.0412
14	0.0378	0.0378	0.0757	0.0378
15	0.0373	0.0373	0.0373	0.0373
16	0.0778	0.0389	0.0389	0.0389
17	0.0711	0.0356	0.0356	0.0356
18	0.0368	0.0368	0.0368	0.0368
19	0.0682	0.0341	0.0341	0.0341
20	0.0336	0.0336	0.0336	0.0336
21	0.0373	0.0373	0.0745	0.0745
22	0.0342	0.0342	0.0342	0.0342
23	0.0354	0.0354	0.0354	0.0354
24	**0.0328**	0.0657	**0.0328**	**0.0328**
25	**0.0324**	**0.0324**	**0.0324**	**0.0324**
Ciprofloxacin	0.0189	0.0189	0.0189	0.0189

***Bold MIC values reflects comparatively better antibacterial activity in respective series**

Antitubercular activity

All the 1,4-disubstituted 1,2,3-triazoles were also tested for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTCC 200) by Lowenstein – Jensen method [25]. Isoniazid was used as standard drug. The results were tabulated in term of MIC in µmol/mL as given in **Table 2.**

All triazole exhibited moderate *in vitro* antimycobacterial activity with MIC ranging from 1.5854 to 0.1314 µmol/mL. Compound **4** (MIC, 0.1805 µmol/mL), **10** (MIC, 0.1790 µmol/mL), **15** (MIC, 0.1491 µmol/mL), **22** (MIC, 0.1710 µmol/mL), **24** (MIC, 0.1314 µmol/mL) displayed good antitubercular activity.

The preliminary structure-activity relationship analysis suggested that compounds with naphthyl ring displayed better activity than the compounds containing phenyl ring. The results also supported the fact that the presence of morpholine contributed to better efficacy as compared to piperdine against the tested stains. N-ethyl aniline linked triazoles showed better results than the N-methyl aniline linked triazoles.

Table 2. *In vitro* antitubercular activity of 1,4-disubstituted 1,2,3-triazoles (1-25)

	Minimum Inhibitory Concentration (MIC, µmol/mL)
Compound	***M. tuberculosis* ($H_{37}Rv$)**
1	0.2075
2	0.3018
3	0.7829
4	0.1805
5	0.2046
6	0.2089
7	0.3038
8	1.5764
9	1.4528
10	0.1790
11	0.2190
12	1.5854
13	0.3297
14	0.7568
15	0.1491
16	0.3112
17	0.1779
18	0.7367
19	0.6824
20	1.3461
21	0.2982
22	0.1710
23	0.2890
24	0.1314
25	0.2594
Isoniazid	0.0015

Conclusion

All the triazoles were evaluated for *in vitro* antibacterial against four bacterial strains, while antitubercular activity was carried out against *Mycobacterium tuberculosis* $H_{37}Rv$. The preliminary structure-activity relationship analysis suggested that the presence of naphthyl ring enhanced activities in comparison to phenyl ring. Triazole derivatives having morpholine moiety showed better activity as compared to derivatives having piperdine moiety.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgment

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