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Original article

Synthesis and evaluation of *in vitro* anti-tuberculosis activity of *N*-substituted glycolamides

Fereidoon Daryaee [a], Farzad Kobarfard [b,*], Ali Khalaj [c], Parisa Farnia [d]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy and Neuroscience Research Center, Kerman University of Medical Science, Kerman, Iran*
[b] *Department of Medicinal Chemistry, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, 10 Shams Alley, Vali-e-Asr Avenue, P.O. Box 14155-6153, Tehran, Iran*
[c] *Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran*
[d] *National Research Institute of Tuberculosis and Lung Diseases, Shaheed Beheshti University of Medical Science, Tehran, Iran*

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Abstract

On the basis of the structural similarity of *N*-substituted glycolamides with *N*-glycolyl muramic acid residues of the cell wall of *Mycobacterium tuberculosis*, a series of these compounds were designed and synthesized by the reaction of glycolic acid acetonide **1** (2,2-dimethyl-5-oxo-1,3-dioxolane) with the proper amines. The minimum inhibitory concentration (MIC) was determined against *M. tuberculosis* $H_{37}Rv$ in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA). Among the synthesized compounds, all those with disubstituted amide structure accompanied by one or two heteroatom(s) with loan pair(s) of electrons atom(s) β to the amide nitrogen demonstrated moderate anti-tuberculosis activity and all the monosubstituted amides showed no activity at all.

Keywords: Anti-tuberculosis; Glycolamide; Alamar blue assay; *N*-Glycolyl muramic acid; Cell wall

1. Introduction

Tuberculosis is one of the most common infectious diseases known to man. About 32% of the World's population – or 1.86 billion people – are infected with TB. Every year, approximately 8 million of these develop active tuberculosis (TB), and almost 2 million of them will die from the disease [1]. In India alone, one person dies of TB every minute [2]. With the global spread of HIV, similar increases in TB incidence rates and mortality are to be feared [3]. Moreover there has been a recent and disturbing increase in the number of TB cases that are caused by organisms which are resistant to the first-line drugs: isoniazid (INH), rifampicin (RIF), ethambutol (ETH), streptomycin (STR), and pyrazinamide (PYR) [4]. Therefore, the necessity for new drugs against *Mycobacterium tuberculosis* is well documented [5].

An attractive target for new TB agents is the mycobacterial cell wall [6], which is necessary for viability. Several known drugs such as isoniazid [7] and ethambutol [8] inhibit cell wall synthesis. Since *N*-glycolyl muramic acid (Fig. 1) is an essential and rather specific component of mycobacterium cell wall, this compound was considered as an attractive target for drug development [9]. Therefore, we chose to design new potential anti-tuberculosis compounds based on the structure of *N*-glycolyl muramic acid. Being one of the main components of the framework of the mycobacterium cell wall, *N*-glycolyl muramic acid is synthesized in the early stages of cell wall biosynthesis. This compound is then subjected to a series of further enzymatic transformations in which it plays the role of substrate [9]. It seems that there is a great possibility that compounds with similar structures to *N*-glycolyl muramic acid could interfere with the process of cell wall biosynthesis by inhibiting one of these enzymatic reactions and thus inhibit mycobacterial growth.

Based on this logic, we decided to keep the glycolamide moiety of *N*-glycolyl muramic acid and synthesize compounds

* Corresponding author. Tel.: +98 21 8877 3521; fax: +98 21 8879 5008.
E-mail address: farzadkf@yahoo.com (F. Kobarfard).

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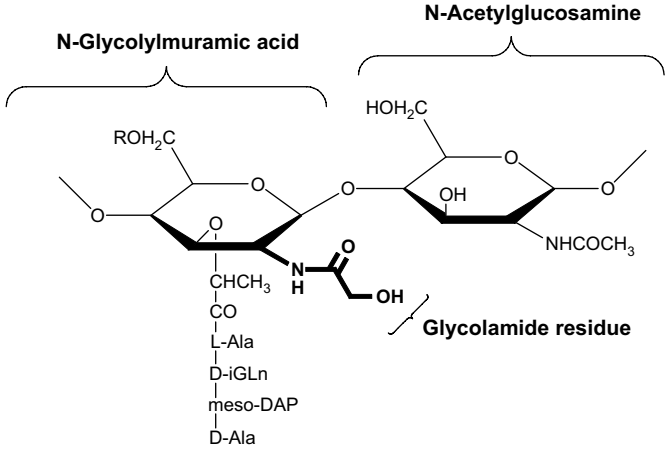


Fig. 1. Structure of the basic peptidoglycan unit of mycobacterial cell wall.

in which the muramic acid moiety is replaced by a diverse group of structures such as aliphatic, alicyclic and aromatic compounds possessing different types of functional groups.

2. Chemistry

The target compounds **3a**–**m** (Table 1) were synthesized by the synthetic route reported in Scheme 1. Glycolic acid acetonide (2,2-dimethyl-5-oxo-1,3-dioxolane) **1** was prepared by the reaction of glycolic acid and acetone in the presence of concentrated sulfuric acid at −5 °C. In the next step, through the reaction of the appropriate amines **2a**–**m** with the obtained acetonide in dichloromethane, as a solvent, at room temperature (RT) our desired glycolamides **3a**–**m** were prepared.

3. Biology

3.1. Anti-tuberculosis activity

Anti-mycobacterial activities of compounds **3a**–**m** against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) were evaluated by Alamar Blue Assay [10]. The MIC (minimum inhibitory concentration) values were determined and compared with ethambutol **4** as a reference drug (Table 1).

4. Results and discussion

Anti-mycobacterial activities of compounds **3a**–**m** are presented in Table 1.

From the results it is apparent that none of the *N*-monosubstituted amides showed the MIC less than 57 μg/mL. The exception is compound **3b**, which in spite of being a *N*-monosubstituted amide, demonstrated good minimum inhibitory activity (29.5 μg/mL) compared to the others. Comparing the structure of compound **3b** with the structure of ethambutol (**4**) reveals an interesting similarity between the two compounds in having 2-amino-1-butanol moiety. This common structural feature might be responsible for higher activity of compound **3b**. Among the *N*,*N*-disubstituted amides tested, the most interesting results were from those containing heteroatom(s) (nitrogen or oxygen), β to the amide nitrogen in their structures. For example, compound **3f** with an oxygen atom β to the amide nitrogen, showed higher inhibitory activity compared to compounds **3d** and **3e** which do not have any electron pair donating atom at that position of their structures. Based on this suggestion, higher inhibitory activity of compound **3f** relative to compound **3k** (in spite of having hydroxyl group at position 4 of its piperidine ring) can be explained by the existence of oxygen atom β to the amide nitrogen of morpholine ring in the structure of compound **3f** and lack of this atom in the same position in the structure of compound **3k**. The most interesting interpretation can be suggested when the inhibitory activities of compounds **3g** and **3j** are compared with each other. As the β nitrogen atom in compound **3g** is connected to a carbonyl group, its electron pair donating effect is lower than the β nitrogen atom in compound **3j** which is not connected to a carbonyl group. Therefore the lower inhibitory activity of compound **3g** compared to that of compound **3j** can be possibly attributed to the higher electron donating properties of oxygen at β position relative to the amide nitrogen in compound **3j**. Furthermore, the highest activity observed for compound **3c** can be because of the existence of two oxygen atoms β to the amide nitrogen in its structure.

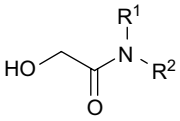
Interestingly, the distance between the amide nitrogen and hydroxyl oxygen atom in compounds **3c** and **3f** is the same distance observed between the nitrogen and the oxygen atom of pyranose ring in *N*-glycolyl muramic acid which is the part of Mycobacterium cell wall which these compounds were designed and synthesized based on its structure (Fig. 2).

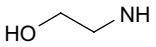
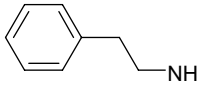
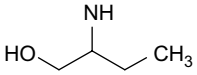
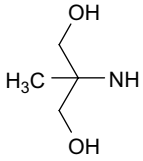
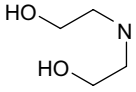
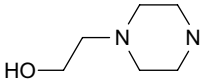
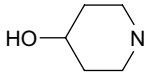
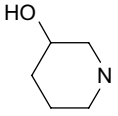
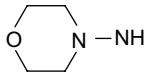
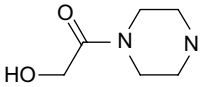
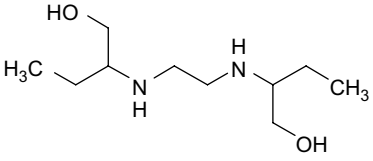
For the synthesis of **3b**, a racemic mixture of *S* and *R* enantiomers of 2-amino-1-butanol was used and hence a racemic mixture of *S* and *R* enantiomers of this compound was obtained. In ^{1}H NMR spectrum of this compound the multiplet at 3.65 ppm is indicative of the diastereotopic hydrogens of CH_2 attached to the hydroxyl group. The other two diastereotopic hydrogens (CH_2 next to CH_3) have appeared separately at 1.59 and 1.36 ppm each of them as multiplets as a result of both geminal and vicinal couplings.

In ^{1}H NMR spectrum of compound **3c** the two methylene groups which are attached to hydroxyl groups have appeared at 3.53 ppm as a multiplet with an integration equivalent to four hydrogens. On the other hand the other two methylene groups (Fig. 3) which are attached to amide nitrogen have appeared as two separate and clear triplets at 3.36 and 3.26 ppm. These observations which may seem unusual at the first sight, are due to the well known double bond character of amide bond which makes the two identical substituents on the amide nitrogen to appear with different chemical shifts.

The same pattern is observed for compounds **3d**–**3l**. For instance in **3d** which contains a pyrrolidine ring, methylene groups at positions 2 and 5 appear at 3.54 and 3.29 ppm as triplets and methylene groups at positions 3 and 4 appear at 1.99 and 1.90 ppm as quintets. The only compound which does not show the same pattern is compound **3m** in which morpholine ring is not directly attached to the carbonyl group and therefore the methylene groups at positions 2 and 6 appear with an

Table 1
Minimum inhibitory concentration of some glycolamides, **3a–m**



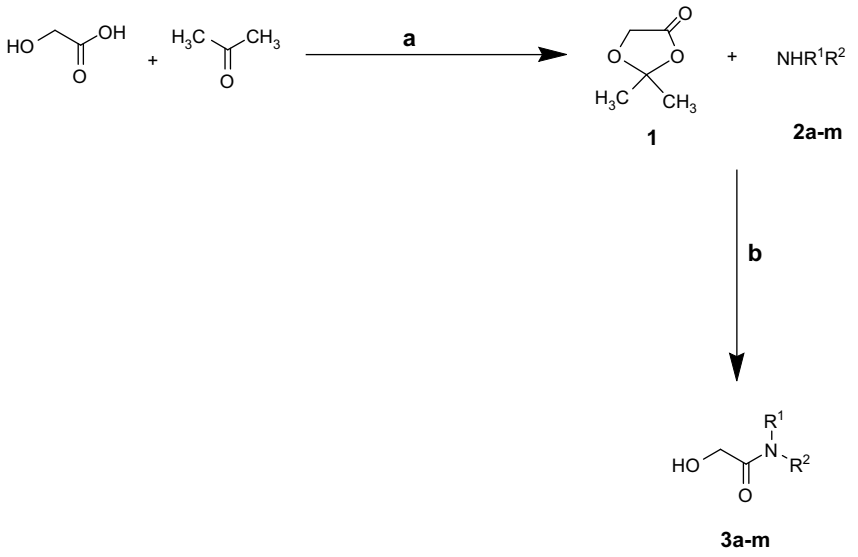
Compound	NR^1R^2	MIC ($\mu g\ mL^{-1}$)	Compound	NR^1R^2	MIC ($\mu g\ mL^{-1}$)
a		87.5	**h**		74
b		29.5	**i**		67.5
c		12.5	**j**		14.25
d		25.5	**k**		33.25
e		38.75	**l**		60
f		13.75	**m**		57
g		15.75	**4**[a]		5

[a] Ethambutol.

identical chemical shift as a single triplet at 2.74 ppm with the integration equivalent to four hydrogens. The two methylene groups at positions 3 and 5 of the morpholine ring are also equivalent and show up at 3.61 ppm as a single triplet.

5. Conclusion

According to this study, it seems that disubstituted glycolamides with more electron donating atoms β to the amide nitrogen show the highest activity. Since this is the first time that amide derivatives of glycolic acid are reported as potential anti-tuberculosis agents, compounds **3c** and **3f** could be considered as new hits in these series of studies and further optimization is underway on these compounds since the highest activities observed (compounds **3c** and **3f**) are still lower than the activity of ethambutol (MIC = 5 μg/ml). Progressing from these hits into promising leads calls for a comprehensive assessment of chemical integrity and further SAR studies on these compounds. In the process of hit-to-lead generation our main objective would be finding the hits with potencies equal or higher than the existing anti-mycobacterium substances such as ethambutol and conducting more comprehensive biological activity assessments on them.



Scheme 1. Synthesis of compounds **3a–m**. Reagents and conditions: (a) conc. H_2SO_4, −5 °C, 30 min; (b) under argon gas, CH_2Cl_2, RT, overnight.

6. Experimental protocols

6.1. General procedure

Chemicals and all solvents used in this study were purchased from Merck AG Chemical (Merck, Darmstadt, Germany). The melting points were obtained using an Electrothermal IA-9100 capillary apparatus and are uncorrected. The IR spectra were obtained using a Nicolet Magna IR 550 spectrometer. The ^{1}H NMR spectra were recorded on a Bruker DRX-500 Advance spectrometer, and the chemical shift (δ) are in ppm relative to tetramethylsilane (TMS), which was used as an internal standard. Mass spectra were obtained on a Finningan TSQ-70 instrument. Elemental analyses were carried out on a HERAEUS CHN rapid elemental analyzer (Heraeus GmbH, Germany) for C, H and N and the results are within ±0.4% of the theoretical values.

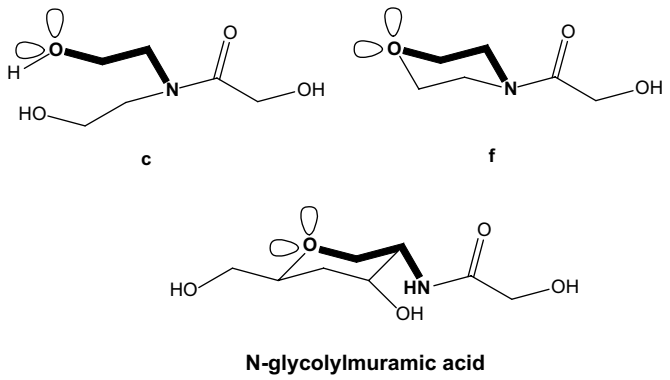


Fig. 2. Comparative illustration of *N*-glycolyl muramic acid and compounds **c** and **f**.

6.2. Synthesis of 2,2-dimethyl-5-oxo-1,3-dioxolane (glycolic acid acetonide) (*1*)

To a solution of glycolic acid (6.77 g, 89 mmol) in dry acetone (40 mL) was added concentrated sulfuric acid (1 mL) at −5 °C and the mixture was stirred at this temperature for 30 min. It was then added into crushed ice and neutralized by sodium bicarbonate powder and extracted by dichloromethane (100 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give 3.2 g of **1** as a pure oily compound in 48% yield. IR (cm^{-1}): 3000, 2960 (CH, aliphatic), 1800 (CO). ^{1}H NMR ($CDCl_3$): δ 4.35 (s, 2H, CH_2), 1.5 (s, 6H, CH_3). Anal. Calcd for $C_5H_8O_3$: C, 51.72; H, 6.94%. Found: C, 51.92; H, 6.95%.

6.3. Synthesis of N-glycolyl-ethanolamine (*3a*)

A mixture of glycolic acid acetonide **1** (116 mg, 1 mmol) and ethanolamine **2a** (61 mg, 1 mmol) in dichloromethane (5 mL) was stirred under argon gas at ambient temperature overnight. The crude oily product was purified by column chromatography (methanol) to give 12 mg of **3a** as a pure oily compound in 10% yield. IR (cm^{-1}): 3220–3480 (OH, alcohol), 1640 (CO, amide). ^{1}H NMR (DMSO): δ 7.654 (s, 1H, amide NH), 5.6 (s, 1H, OH), 4.8 (s, 1H, OH), 3.78 (s, 2H, glycolyl CH_2), 3.42 (t, 2H, CH_2, $J = 5.6$ Hz), 3.17 (q, 2H, CH_2, $J = 5.6$ Hz). MS (EI) *m/z* (%): 119.2 ($M^{\bullet+}$, 62), 88.1 (100).

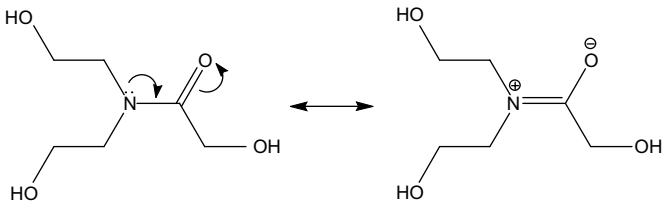


Fig. 3. Resonance structures of compound **3c**.

Anal. Calcd for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%. Found: C, 40.18; H, 7.65; N, 11.79%.

6.4. Synthesis of N-glycolyl-2-amino-1-butanol (**3b**)

The title compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 2-amino-1-butanol (**2a**, 89 mg, 1 mmol) as described for compound **3a**. The crude solid thus obtained was crystallized from ethylacetate, to give 59 mg of **3b**, in a 40% yield. Mp: 80–82 °C. IR (cm^{-1}): 3380 (NH, amide), 3280 (OH, alcohol), 1620 (CO, amide). ^1H NMR (DMSO-d_6): δ 7.28 (d, 1H, amide NH, $J = 8$ Hz), 5.50 (s, 1H, OH), 4.54 (s, 1H, OH), 3.79 (s, 2H, glycolyl CH_2), 3.65 (m, 2H, CH_2), 3.35 (m, 1H, CH), 1.59 (m, 1H, CH), 1.36 (m, 1H, CH), 0.82 (t, 3H, CH_3, $J = 7.2$ Hz). MS (EI) *m/z* (%): 147.2 ($M^{\bullet+}$, 30), 116.1 (100). Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52%. Found: C, 49.07; H, 8.93; N, 9.55%.

6.5. Synthesis of N-glycolyl-diethanolamine (**3c**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and diethanolamine (**2c**, 105 mg, 1 mmol) as described for compound **3a**. The crude oily product was purified by column chromatography (methanol) to give 24.5 mg of **3c** as a pure oily compound in a 15% yield. IR (cm^{-1}): 3200–3520 (OH, alcohol), 1640 (CO, amide). ^1H NMR (DMSO-d_6): δ 4.80 (s, 2H, OH), 4.30 (s, 1H, OH), 4.12 (s, 2H, glycolyl CH_2), 3.53 (m, 4H, CH_2), 3.36 (t, 2H, CH_2, $J = 5.6$ Hz), 3.26 (t, 2H, CH_2, $J = 5.6$ Hz). MS (EI) *m/z* (%): 163.2 (M^+, 45), 120.1 (100). Anal. Calcd for $C_6H_{13}NO_4$: C, 44.16; H, 8.03; N, 8.58%. Found: C, 44.43; H, 7.95; N, 8.25%.

6.6. Synthesis of 1-glycolyl-pyrrolidine (**3d**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and pyrrolidine (**2d**, 71 mg, 1 mmol) as described for compound **3a**. The crude product was purified by column chromatography on silica gel (hexane/chloroform, 7:3, 1:1, chloroform) to give 26 mg of **3d**, in a 20% yield. Mp: 40 °C (lit. [11]: 42–44 °C). IR (cm^{-1}): 3200–3480 (OH, alcohol), 1640 (CO, amide). ^1H NMR ($CDCl_3$): δ 4.08 (s, 2H, glycolyl CH_2), 3.59 (s, 1H, OH), 3.54 (t, 2H, CH_2, $J = 6.4$ Hz), 3.29 (t, 2H, CH_2, $J = 6.8$ Hz), 1.99 (qu, 2H, CH_2, $J = 6.4$ Hz), 1.90 (qu, 2H, CH_2, $J = 6.8$ Hz). MS (EI) *m/z* (%): 129.2 ($M^{\bullet+}$, 37), 98.2 (100). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84%. Found: C, 55.45; H, 8.83; N, 10.64%.

6.7. Synthesis of 1-glycolyl-piperidine (**3e**)

The title compound was prepared by the reaction between **1** (116 mg, 1 mmol) and piperidine (**2e**, 85 mg, 1 mmol) as described for compound **3a**. The final solution was washed with hydrochloric acid (3 M, 5 mL), the organic layer was separated and the solvent was evaporated under reduced pressure to give 43 mg of **3e**, in a 30% yield. Mp: 39–40 °C (lit. [11]: 39–41 °C). IR (cm^{-1}): 3400–3440 (OH, alcohol), 1650 (CO, amide). ^1H NMR ($CDCl_3$): δ 4.14 (s, 2H, glycolyl CH_2), 3.76 (s, 1H, OH), 3.61 (t, 2H, CH_2, $J = 5.4$ Hz), 3.20 (t, 2H, CH_2, $J = 5.4$ Hz), 1.68 (m, 2H, CH_2), 1.59 (m, 4H, CH_2). MS (EI) *m/z* (%): 143.3 ($M^{\bullet+}$, 59), 112.2 (100). Anal. Calcd for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78%. Found: C, 58.50; H, 8.95; N, 10.13%.

6.8. Synthesis of 4-glycolyl-morpholine (**3f**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and morpholine (**2f**, 87 mg, 1 mmol) as described for compound **3a**. After evaporation of the solvent under reduced pressure, the residue was crystallized from ethylacetate to give 44 mg of **3f**, in a 30% yield. Mp: 80–83 °C (lit. [11]: 80–82 °C). IR (cm^{-1}): 3420 (OH, alcohol), 1650 (CO, amide). ^1H NMR ($CDCl_3$): δ 4.17 (s, 2H, glycolyl CH_2), 3.70 (m, 6H, CH_2), 3.62 (s, 1H, OH), 3.29 (t, 2H, CH_2, $J = 4.8$ Hz). MS (EI) *m/z* (%): 145.2 ($M^{\bullet+}$, 34), 114.2 (100). Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65%. Found: C, 49.78; H, 7.84; N, 9.34%.

6.9. Synthesis of 1,4-diglycolyl-piperazine (**3g**)

The title compound was prepared by the reaction between **1** (232 mg, 2 mmol) and piperazine (**2g**, 86 mg, 1 mmol) as described for compound **3a**. After evaporation of organic solvent under reduced pressure, the residue was crystallized from methanol to give 40 mg of **3g**, in a 20% yield. Mp: 194–197 °C (lit. [12]: 187–190 °C). IR (cm^{-1}): 3240–3400 (OH, alcohol), 1640 (CO, amide). ^1H NMR (DMSO-d_6): δ 4.67 (t, 2H, OH, $J = 5.6$ Hz), 4.10 (d, 4H, glycolyl CH_2, $J = 5.6$ Hz), 3.48 (m, 8H, CH_2). MS (EI) *m/z* (%): 202.3 (M^+, 5), 113.2 (100). Anal. Calcd for $C_8H_{14}N_2O_4$: C, 47.52; H, 6.98; N, 13.85. Found: C, 47.12; H, 7.01; N, 13.98.

6.10. Synthesis of N-glycolyl-2-phenyl-ethylamine (**3h**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 2-pheny-ethylamine (**2h**, 121 mg, 1 mmol) as described for compound **3a**. The crude product was purified by column chromatography on silica gel (chloroform) to give 18 mg of **3h**, in a 10% yield. Mp: 71–73 °C (lit. [13]: 74–75 °C). IR (cm^{-1}): 3360 (NH, amide), 3160 (OH, alcohol), 1640 (CO, amide), 1550, 1470 (C–C, aromatic). ^1H NMR ($CDCl_3$): δ 7.26 (m, 5H, Ar–H), 6.56 (s, 1H, OH), 4.06 (s, 2H, CH_2), 3.57 (q, 2H, CH_2, $J = 6$ Hz), 2.84 (t, 2H, CH_2, $J = 6$ Hz). MS (EI) *m/z* (%): 179.3 ($M^{\bullet+}$, 21), 104.2 (100). Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82%. Found: C, 67.27; H, 7.41; N, 7.65%.

6.11. Synthesis of 2-glycolylamino-2-methyl-1, 3-propanediol (**3i**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 2-amino-2-methyl-1,3-propanediol (**2i**, 133 mg, 1 mmol) as described for compound **3a**. The solvent was removed under vacuum to give 182 mg of **3i**, in

a 95% yield as pure oily compound. IR (cm^{-1}): 3200–3480 (OH, alcohol), 1650 (CO, amide). ^1H NMR (DMSO-d_6): δ 7.17 (s, 1H, amide NH), 5.72 (s, 1H, OH), 4.98 (s, 2H, OH), 3.72 (s, 2H, glycolyl CH_2), 3.50 (d, 2H, CH_2, $J = 10.4$ Hz), 3.38 (d, 2H, CH_2, $J = 10.8$ Hz), 1.18 (s, 3H, CH_3). MS (EI) *m/z* (%): 163.2 ($M^{\bullet+}$, 52), 132.2 (100). Anal. Calcd for $C_6H_{13}NO_4$: C, 44.16; H, 8.03; N, 8.58%. Found: C, 44.29; H, 8.08; N, 8.49%.

6.12. Synthesis of 1-(2-hydroxyethyl)-4-glycolyl-piperazine (**3j**)

The title compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 1-(2-hydroxyethyl)-piperazine (**2j**, 130 mg, 1 mmol) as described for compound **3a**. After evaporation of the organic solvent under reduced pressure, the residue was crystallized from ethylacetate to give 38 mg of **3j**, in a 20% yield. Mp: 72–74 °C. IR (cm^{-1}): 3200–3480 (OH, alcohol), 1640 (CO, amide). ^1H NMR (DMSO-d_6): δ 4.52 (s, 1H, OH), 4.44 (s, 1H, OH), 4.05 (s, 2H, glycolyl CH_2), 3.49 (t, 2H, CH_2, $J = 6$ Hz), 3.44 (m, 2H, CH_2), 3.29 (m, 2H, CH_2), 2.38 (m, 6H, CH_2). MS (EI) *m/z* (%): 188.2 ($M^{\bullet+}$, 38), 157.1 (100). Anal. Calcd for $C_8H_{16}N_2O_3$: C, 51.05; H, 8.57; N, 14.88%. Found: C, 50.87; H, 8.61; N, 14.76%.

6.13. Synthesis of 4-hydroxy-1-glycolyl-piperidine (**3k**)

The title compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 4-hydroxy-piperazine (**2k**, 101 mg, 1 mmol) as described for compound **3a**. After evaporation of the organic solvent under reduced pressure, the residue was crystallized from ethylacetate to give 32 mg of **3k**, in a 20% yield. Mp: 116–118 °C. IR (cm^{-1}): 3200–3480 (OH, alcohol), 1640 (CO, amide). ^1H NMR ($CDCl_3$): δ 4.18 (d, 2H, glycolyl CH_2, $J = 4.8$ Hz), 4.02 (m, 1H, CH); 3.673 (t, 1H, OH, $J = 4.8$ Hz), 3.50 (m, 1H, CH), 3.38 (m, 1H, CH), 3.09 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 1.63 (s, 1H, OH), 1.56 (m, 2H, CH_2). MS (EI) *m/z* (%): 159.3 ($M^{\bullet+}$, 52), 128.2 (100). Anal. Calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80%. Found: C, 53.08; H, 8.21; N, 8.73%.

6.14. Synthesis of 3-hydroxy-1-glycolyl-piperidine (**3l**)

The title compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 3-hydroxy-piperazine (**2l**, 101 mg, 1 mmol) as described for compound **3a**. After evaporation of the organic solvent under reduced pressure, the residue was crystallized from ethylacetate to give 16 mg of **3l**, in a 10% yield. Mp: 66–68 °C. IR (cm^{-1}): 3200–3480 (OH, alcohol), 1640 (CO, amide). ^1H NMR ($CDCl_3$): δ 4.18 (d, 2H, glycolyl CH_2, $J = 4.8$ Hz), 3.70 (br s, 1H, OH), 3.59 (dd, 1H, CH, $J = 6.4$, 10.4 Hz), 3.45 (dd, 1H, CH, $J = 8$, 14 Hz); 3.37 (dd, 1H, CH, $J = 2.8$, 13.2 Hz), 3.19 (m, 2H, CH_2), 2.34 (s, 1H, OH), 1.90 (m, 2H, CH_2), 1.52 (m, 2H, CH_2). MS (EI) *m/z* (%): 159.2 ($M^{\bullet+}$, 18), 84.2 (100). Anal. Calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80%. Found: C, 52.70; H, 8.27; N, 8.99%.

6.15. Synthesis of 4-glycolylamino-morpholine (**3m**)

This compound was prepared by the reaction between **1** (116 mg, 1 mmol) and 4-amino-morpholine (**2m**, 102 mg, 1 mmol) as described for compound **3a**. After evaporation of the organic solvent under reduced pressure, the residue was crystallized from ethylacetate to give 32 mg of **3m**, in a 20% yield. Mp: 139–141 °C. IR (cm^{-1}): 3360 (NH, amide), 3200 (OH, alcohol), 1660 (CO, amide). ^1H NMR (DMSO-d_6): δ 4.24 (t, 1H, OH, $J = 6$ Hz), 4.12 (d, 2H, glycolyl CH_2, $J = 6$ Hz), 3.61 (t, 4H, CH_2, $J = 4.8$ Hz), 2.74 (t, 4H, CH_2, $J = 4.8$ Hz). MS (EI) *m/z* (%): 160.2 ($M^{\bullet+}$, 10), 101.1 (100). Anal. Calcd for $C_6H_{12}N_2O_3$: C, 44.99; H, 7.55; N, 17.49%. Found: C, 44.73; H, 7.62; N, 17.52%.

6.16. Biology

6.16.1. Anti-tuberculosis activity

All the compounds were evaluated for *in vitro* anti-tuberculosis activity against *M. tuberculosis*, in National Research Institute of Tuberculosis and Lung Disease of Shahid Beheshti University of Medical Sciences of Iran. Ethambutol (**4**) was used as a reference drug.

The synthesized compounds were tested by serial dilution against *M. tuberculosis* $H_{37}Rv$ (ATTCC 27294), in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA) [10] to determine the actual minimum inhibitory concentration (MIC). The MIC was defined as the lowest concentration effecting a reduction in the fluorescence of 90% relative to the control [14].

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