SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF 2-HYDROXYACETAMIDES

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ABSTRACT

On the basis of the structural similarity of 2-hydroxyacetamides (glycolamides) with N-glycolylmuramic acid residues of the cell wall of *Mycobacterium tuberculosis* several of these compounds were prepared mainly by the reaction of 5-oxo-2,2-dimethyl-1,3-dioxolane **1** (glycolic acid acetonide) with corresponding amines and their antimycobacterial activities were determined by Alamar blue Assay. Of the synthesized compounds disubstituted amides bearing hydrophilic moieties showed moderate activity.

Keywords: 2-Hydroxyacetamides, Glycolic acid acetonide, Alamar blue Assay, *Mycobacterium tuberculosis*, Hydrophilicity.

INTRODUCTION

In recent years due to the emergence of monodrug or multidrug resistant strains of *Mycobacterium tuberculosis* and the AIDS epidemic, the incidence of this infection and rates of mortality from tuberculosis have increased considerably (1), and search for new drug leads of new structural classes and with novel mechanism of action have been intensified in this field. An attractive target for such new agents is the mycobacterial cell wall of which many structural components are not present in mammalian system or other bacteria (2) and several known antituberculosis drugs such as ethambutol is believed to act against mycobacterial cell wall biosynthesis.

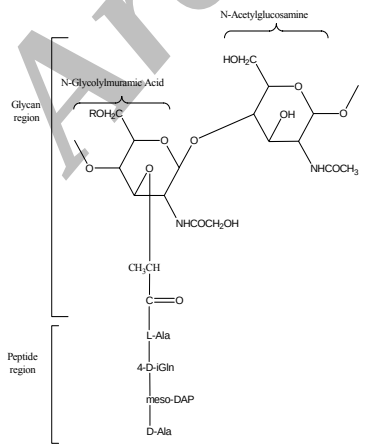

Figure 1. Structure of the basic peptidoglycan unit of mycobacterial cell walls

One of the differentiating features of the cell envelope of *Mycobacterium tuberculosis* is that the N-acetyl group on the muramic acid residues of the peptidoglycan structures which is common in other bacteria is oxidized to N-glycolyl function (1). This essential and rather specific component of the mycobacterium cell wall which is formed in the early stages of the cell wall biosynthesis acts as a substrate for a number of enzymatic transformations (Fig. 1)

In the search for drugs targeting *M.tuberculosis* cell wall biosynthesis it seemed that N-substituted glycolamides $\mathbf{3}_{\mathbf{a-n}}$ could serve as inhibitors due to their structural similarity with N-glycolylmuramic acid residues of the cell wall structure. (Fig. 2)

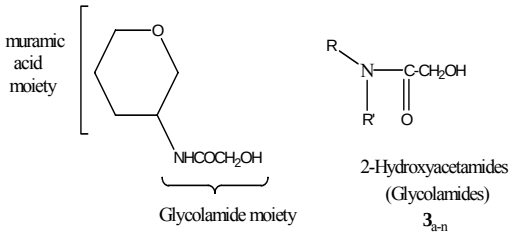

Figure 2

This article describes the synthesis and physicochemical properties of several known ($\mathbf{3}_{\mathbf{e-i}}$) and novel 2-hydroxyacetamides ($\mathbf{3}_{\mathbf{a-d,\ j-n}}$) and preliminary evaluation of antimycobacterial activity of these compounds by Alamar blue Assay (4) in comparison with ethambutol as the reference drug.

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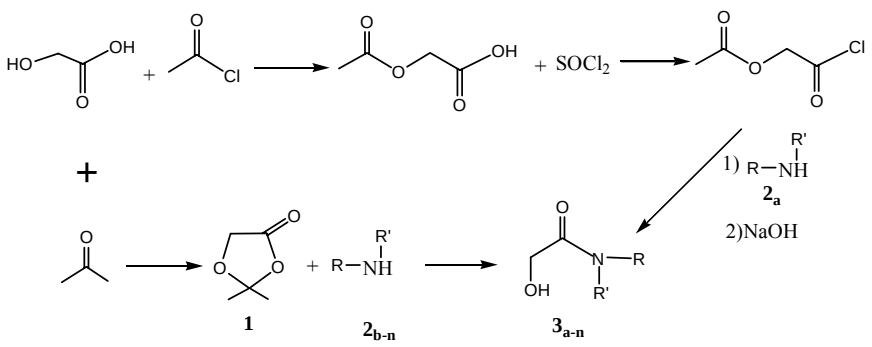
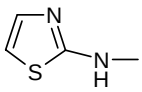
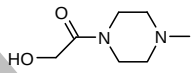
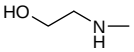
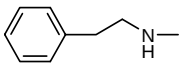
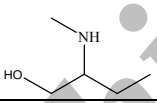
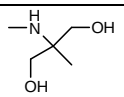
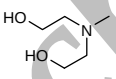
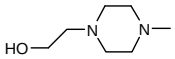
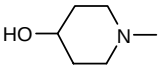
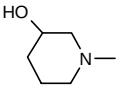
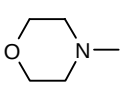
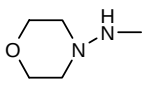
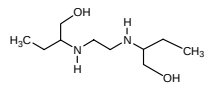


Figure 3. synthesis of 2-hydroxyacetamides $\mathbf{3_{a-n}}$

Table 1. structures and antimycobacterial activities of 2-hydroxyacetamides $\mathbf{3_{a-n}}$

	R'—N(R')—	MIC *μg/mL*		R'—N(R')—	MIC *μg/mL*
a		>100	h		15.75
b		87.5	i		74
c		29.5	j		67.5
d		12.5	k		14.25
e		25.5	l		33.25
f		38.75	m		60
g		13.75	n		57
	ethambutol			5	

MATERIALS AND METHODS

Chemistry

The 2-hydroxyacetamides $\mathbf{3_{b-n}}$ were prepared through the reaction of the corresponding amines $\mathbf{2_{b-n}}$ with 2,2-dimethyl-5-oxo-1,3-dioxalane **1** (glycolic acid acetonide) (5) which in turn was prepared (6) by the reaction of glycolic acid with acetone (Fig 3). Attempted synthesis of the amide $\mathbf{3_a}$ by a similar method failed and this compound could be prepared through the reaction of 2-aminothiazole with acetoxyacetylchloride (7) followed by hydrolysis of the acetyl group (Fig 3).
Of compounds which were synthesized in this investigation, preparation of $\mathbf{3_{e-g}}$ (8) and $\mathbf{3_h}$ (9), through the reaction of the corresponding amines with glycolic acid and preparation of $\mathbf{3_i}$ through the reaction of amine $\mathbf{2_i}$ with glycolic acid methyl ester (10) have previously been reported. All the new compounds were characterized by ^{1}HNMR, IR, and Mass spectral data and the physical and spectral data of the known compounds were consistent with the literature values.
Melting points were determined on a Reichert hot plate and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity Plus 400 MHz spectrometer using *DMSO-d_6* and *$CDCl_3$* as solvent. Chemical shifts (δ) are reported in ppm relative to *TMS* as internal standard. Mass spectra were obtained on a Finningan TSQ-70 instrument. Infrared spectra were recorded on a Nicolet Magna IR 550 spectrometer. Glycolic acid, amines $\mathbf{2_{a-n}}$, solvents and silica gel 60 for column chromatography were purchased from Merck (Germany).

N-(hydroxyacetyl)-2-aminothiazole 3_a

To a mixture of 2-aminothiazole (2 gr, 20 mmol) and 0.2 N sodium hydroxide (100 mL) under continuous stirring at 0°C was added dropwise acetoxyacetylchloride (2.734 gr, 6.02 mL) and the mixture was then stirred at room temperature for 20 min (7). The precipitate (0.68 gr) was collected by filtration and without further purification, refluxed in 1N sodium hydroxide (10 mL) for 1 hrs. The reaction mixture was then cooled to room temperature, the precipitate was filtered and crystallized from ethyl acetate to afford 0.5 gr (11%) of compound $\mathbf{3_a}$ which was characterized by the physicochemical data listed below.
m.p.: 186-188 °C.
^{1}HNMR(δ) (DMSO) : 11.73 (1H; s; amide NH); 7.47 (1H; d, *J*=3.2 HZ; H-4); 7.23 (1H; d, *J*=3.2 HZ; H-5); 5.51 (1H; t, *J*=6 HZ ; OH); 4.13 (2H; d, *J*=6 HZ; CH_2).
IR (cm^{-1}): 3280-3400 (OH, alcohol); 1700 (CO, amide); 1580 (C-C, aromatic).
Mass m/z (%): 158.2 (30); 100.1 (100); 58.1 (22).

2,2- dimethyl- 5-oxo- 1,3- dioxolane (glycolic acid acetonide) 1

This compound was prepared by some modifications in the reported method (6). To a solution of glycolic acid (6.77 gr 0.089 mole) in dry acetone (40 mL) was added conc. sulfuric acid (1 mL) at –5 °C and the mixture was stirred at –5 °C for 30min. The mixture was then added into crushed ice , neutralized by the gradual addition of 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 gr (48%) of an oil which was pure by TLC examination (EtOAc).
^{1}HNMR(δ) ($CDCl_3$): 4.35 (2H; s; H-4); 1.50 (6H; s; CH_3).
IR (cm^{-1}): 3000, 2960 (CH, aliphatic); 1800 (CO, acetonide).

General Procedure for the synthesis of Glycolamides 3_{b-n}

For the preparation of glycolamides $\mathbf{3_{b-g}}$ and $\mathbf{3_{i-n}}$, 1 mmole (0.11 6gr) of 5-oxo-2,2-dimethyl-1,3-dioxolane **1** (glycolic acid acetonide) (6) and for the preparation of glycolamide $\mathbf{3_h}$, 2 mmole (0.232 gr) of glycolic acid acetonide **1** with 1 mmole of appropriate amines (5) were stirred at room temperature under argon for 12 hrs. Except for $\mathbf{3_j}$ which was pure and needed no further purification, the resulting mixture was dissolved in chloroform (5 mL), the obtained solution was washed successively with 3N HCl (5 mL), water (5 mL) and dried over anhydrous $MgSO_4$. The residues after evaporation of the solvent were subjected to column chromatography on silica gel or crystallized from organic solvents to obtain pure compounds. In the case of amides $\mathbf{3_{f,n}}$ residues after evaporation of the solvent were pure and used for antimycobacterial evaluation without further purification.

N-(hydroxyacetyl)-Ethanolamine 3_b

Column chromatography of the residue (MeOH) gave pure product as an oil.
Yield: 0.1 gr (10%).
^{1}HNMR(δ) (DMSO) : 7.65 (1H; s; amide NH); 5.60 (1H; s; OH); 4.80 (1H; s; OH); 3.78 (2H; s; glycolyl CH_2); 3.42 (2H; t, *J*=5.6 HZ; CH_2); 3.17 (2H; q, *J*=5.6 HZ; CH_2).
IR (cm^{-1}): 3220-3480 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 120.2 (62); 88.1 (100); 76.2 (100); 70.2 (60).

N-(hydroxyacetyl)-2-amino-1-butanol 3_c

The residue was crystallized from EtOAc to obtain pure product.
Yield: 0.51 gr (40%); m.p.: 80-82 °C.
^{1}HNMR(δ) (DMSO) : 7.28 (1H ; d, *J*=8 HZ ; amide NH) ; 5.50 (1H ; s ; OH) ; 4.54 (1H ; s ; OH); 3.79 (2H; s; glycolyl CH_2); 3.65 (2H; m; H-

1); 3.35 (1H; m; H-2); 1.59 (1H; m; H-3); 1.36 (1H; m; H-3); 0.82 (3H; t, *J*=7.2 HZ; CH_3).
IR (cm^{-1}): 3380 (NH, amide); 3280 (OH, alcohol); 1620 (CO, amide).
Mass m/z (%): 148.2 (30); 116.1 (100); 58.1 (100).

N-(hydroxyacetyl)-Diethanolamine 3_d
Column chromatography of the residue (MeOH) gave pure product as an oil.
Yield: 0.2 gr (15%).
^{1}HNMR(δ) (DMSO) : 4.80 (2H; s; OH); 4.30 (1H; s; OH); 4.12 (2H; s; glycolyl CH_2); 3.53 (4H; m; CH_2); 3.36 (2H; t, *J*=5.6 HZ; CH_2); 3.26 (2H; t, *J*=5.6 HZ; CH_2).
IR (cm^{-1}): 3200-3520 (OH, alcohol); 1640 (CO, amoide).
Mass m/z (%): 164.2 (45); 132.2 (85); 120.1 (100); 74.1 (100).

1-(hydroxyacetyl)-Pyrrolidine 3_e
Column chromatography of the residue (hexane-chloroform, 7:3 → 5:5 →chloroform only) gave pure product.
Yield: 0.48 gr (20%); m.p.: 40 °C (Reference 8 m.p. 42-44°C).
^{1}HNMR(δ) ($CDCl_3$) : 4.08 (2H ; s ; glycolyl CH_2); 3.59 (1H; s; OH); 3.54 (2H; t, *J*=6.4 HZ; CH_2); 3.29 (2H ; t, *J*=6.8 HZ ; CH_2) ; 1.99 (2H ; qu, *J*=6.4 HZ ; CH_2) ; 1.90 (2H ; qu, *J*=6.8 HZ ; CH_2).
IR (cm^{-1}): 3200-3480 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 129.2 (37); 98.2 (100); 55.1 (57).

1-(hydroxyacetyl)-Piperidine 3_f
TLC of the residue (EtOAc) confined purity of the product.
Yield: 0.37 gr (30%); m.p.: 39-40 °C (Reference 8 m.p. 39-41°C).
^{1}HNMR(δ) ($CDCl_3$) : 4.14 (2H; s; glycolyl CH_2); 3.76 (1H; s; OH); 3.61 (2H; t, *J*=5.4 HZ; CH_2); 3.20 (2H ; t, *J*=5.4 HZ ; CH_2) ; 1.68 (2H ; m ; CH_2) ; 1.59 (4H ; m ; CH_2).
IR (cm^{-1}): 3400-3440 (OH, alcohol); 1650 (CO, amide).
Mass m/z (%): 143.3 (59); 112.2 (100); 69.2 (95).

4-(hydroxyacetyl)-Morpholine 3_g
The residue was crystallized from EtOAc to obtain pure product.
Yield: 2.18 gr (30%); m.p.:80-83 °C. (Reference 8 m.p. 80-82°C).
^{1}HNMR(δ) ($CDCl_3$) : 4.17 (2H; s; glycolyl CH_2); 3.70 (6H; m; CH_2); 3.62 (1H; s; OH); 3.29 (2H; t, *J*=4.8 HZ; CH_2).
IR (cm^{-1}): 3420 (OH , alcohol); 1650 (CO, amide)
Mass m/z (%): 145.2 (34); 114.2 (100); 70.1 (60).

1,4-di(hydroxyacetyl)-Piperazine 3_h
The residue was crystallized from MeOH to obtain pure product.
Yield: 0.53 gr (20%); m.p.: 194-197 °C. (Reference 9 m.p. 187-190°C).
^{1}HNMR(δ) (DMSO) : 4.67 (2H ; t, *J*=5.6 HZ ; OH) ; 4.10 (4H ; d, *J*=5.6 HZ ; glycolyl CH_2) ; 3.48 (8H ; m ; CH_2).
IR (cm^{-1}): 3240-3400 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 202.3 (5); 171.3 (95); 113.2 (100); 85.2 (50).

N-(hydroxyacetyl)-2-phenyl-ethylamine 3_i
Column chromatography of the residue (chloroform) gave pure product.
Yield: 0.15 gr (10%); m.p.: 71-73 °C (Reference 10 m.p. 74-75°C).
^{1}HNMR(δ) ($CDCl_3$) : 7.26 (5H ; m ; Ar-H) ; 6.56 (1H ; s ; OH) ; 4.06 (2H ; s ; CH_2); 3.57 (2H; q, *J*=6 HZ; CH_2); 2.84 (2H; t, *J*=6 HZ; CH_2).
IR (cm^{-1}): 3360 (NH, amide); 3160 (OH, alcohol); 1640 (CO, amide); 1550, 1470 (C-C, aromatic).
Mass m/z (%): 179.3 (21); 104.2 (100); 91.2 (28).

2-(hydroxyacetylamino)-2-methyl-1,3-propanediol 3_j
TLC of the residue (EtOAc) confined purity of the product.
Yield: 1.41 gr (100%); oil.
^{1}HNMR(δ) (DMSO) : 7.17 (1H; s; amide NH); 5.72 (1H; s; OH); 4.98 (2H; s; OH); 3.72 (2H; s; glycolyl CH_2); 3.50 (2H; d, *J*=10.4 HZ; CH_2); 3.38 (2H; d, *J*=10.8 HZ; CH_2); 1.18 (3H; s; CH_3).
IR (cm^{-1}): 3200-3480 (OH, alcohol); 1650 (CO, amide).
Mass m/z (%): 164.2 (52); 132.2 (100); 115.1 (59); 74.1 (100); 56.1 (63).

4-(hydroxyacetyl)-1-(2-hydroxyethyl)-Piperazine 3_k
The residue was crystallized from EtOAc to obtain pure product.
Yield: 033 gr (20%); m.p.: 72-74 °C.
^{1}HNMR(δ) (DMSO) : 4.52 (1H; s; OH); 4.44 (1H; s; OH); 4.05 (2H; s; glycolyl CH_2); 3.49 (2H; t, *J*=6 HZ; CH_2) ; 3.44 (2H ; m ; CH_2) ; 3.29 (2H ; m ; CH_2) ; 2.38 (6H; m; CH_2).
IR (cm^{-1}): 3200-3480 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 189.2 (38); 158.3 (47); 157.1 (100); 99.1 (87); 70.1 (53).

4-hydroxy-1-(hydroxyacetyl)-Piperidine 3_l
The residue was crystallized from EtOAc to obtain pure product.
Yield: 0.27 gr (20%); m.p.: 116-118 °C.
^{1}HNMR(δ) ($CDCl_3$) : 4.18 (2H; d, *J*=4.8HZ; glycolyl CH_2); 4.02 (1H; m; CH); 3.673 (1H; t, *J*=4.8 HZ; OH); 3.50 (1H ; m ; CH) ; 3.38 (1H ; m ; CH) ; 3.09 (2H; m; CH_2); 1.90 (2H; m; CH_2);

1.63 (1H; s; OH); 1.56 (2H; m; CH_2).
IR (cm^{-1}): 3200-3480 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 159.3 (52); 128.2 (100); 84.2 (52); 57.2 (45).

3-hydroxy-1-(hydroxyacetyl)-Piperidine 3_m
The residue was crystallized from EtOAc to obtain pure product.
Yield: 0.14 gr (10%); m.p.: 66-68 °C.
^{1}HNMR(δ) ($CDCl_3$): 4.18 (2H; d, *J*=4.8; glycolyl CH_2); 3.70 (1H; bs; OH); 3.59 (1H; dd, *J*=6.4,10.4 HZ; CH); 3.45 (1H; dd, *J*=8,14 HZ; CH); 3.37 (1H; dd, *J*=2.8,13.2 HZ; CH); 3.19 (2H; m; CH_2); 2.34 (1H; s; OH); 1.90 (2H; m; CH_2); 1.52 (2H; m; CH_2).
IR (cm^{-1}): 3200-3480 (OH, alcohol); 1640 (CO, amide).
Mass m/z (%): 159.2 (18); 128.2 (45); 84.2 (100); 43.1 (88).

4-(hydroxyacetylamino)-morpholine 3_n
The residue was crystallized from EtOAc to obtain pure product.
Yield: 0.28 gr (20%); m.p.: 139-141 °C.
^{1}HNMR(δ) (DMSO) : 4.24 (1H; t, *J*=6 HZ; OH); 4.12 (2H; d, *J*=6 HZ; glycolyl CH_2); 3.61 (4H; t, *J*=4.8 HZ; CH_2) ; 2.74 (4H ; t, *J*=4.8 HZ ; CH_2).
IR (cm^{-1}): 3360 (NH, amide); 3200 (OH, alcohol); 1660 (CO, amide).
Mass m/z (%): 161.2 (10); 101.1 (100); 55.1 (45).

Antituberculosis activity
Antimycobacterial activities of compounds $\mathbf{3_{a-n}}$ against Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) were evaluated by Alamar blue Assay (4) in comparison with ethambutol as the reference drug, in National Research Institute of Tuberculosis and Long Disease of Shahid beheshti University of Medical Sciences of Iran and results are listed in Table 1.
To prepare inoculum, Microorganisms were grown in middle brook 7H9 broth medium until a bacterial density corresponding to a 0.5 Mc Farland turbidity standard. Stock solution of each compound was prepared in distillated water and subsequence two fold dilutions were performed in 0.1 mL of 7H9 broth media to obtain dilution of the tested compounds in the range of 0.25-100 µg/mL. After addition of diluted suspension of 0.5 McFarland to the medium containing different dilution of the tested compounds and incubation at 37 °C for 5 days, 20 µL of Alamar blue indicator and 20µL of 10% tween were added to each sample and then they were reincubated at 37°C. Samples were observed at 12 and 24 hours for a color change from blue to pink. MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls which consisted of bacteria and medium only (4).

RESULTS AND DISCUSSION

From the MIC values of the tested compounds listed in table 1 it appears that in comparison with ethambutol as the reference drug (MIC = 5) with the exception of $\mathbf{3_c}$ (MIC = 29.5) other monosubstituted amides displayed weak activity (MIC > 50) and most of disubstituted amides showed moderate activity (MIC < 15). While higher activities of disubstituted amides $\mathbf{3_d}$ (MIC =12.5), $\mathbf{3_g}$ (MIC = 13.75), $\mathbf{3_k}$ (MIC = 14.25) and $\mathbf{3_h}$ (MIC = 15.75) in comparison with those of $\mathbf{3_e}$ (MIC = 25.5) and $\mathbf{3_f}$ (MIC = 38.75) may be attributed to higher hydrophilicity of these compounds, amide of 3-hydroxy piperidine $\mathbf{3_m}$ (MIC = 60) was less active than the corresponding analogue without hydroxyl substituent ($\mathbf{3_f}$, MIC = 38.75).
Electron withdrawing effect and/or specific interaction of the hydroxyl group may be responsible for this decreased in activity and it may be speculated that parameters other than hydrophilicty such as electronic and steric parameters might have influences on the activity. Although the MIC value of the most active compound ($\mathbf{3_d}$, MIC = 12.5) is not comparable to that of ethambutol as the reference drug, (MIC = 5), the ease of their synthesis enable facile development of this group of compounds as effective agents for tuberculosis chemotherapy.

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