

SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF 2-HYDROXYACETAMIDES

¹FEREIDOON DARYAEE, ²FARZAD KOBARFARD, ¹ALI KHALAJ, ³PARISSA FARNIA

¹Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, ²Department of Medicinal Chemistry, Faculty of Pharmacy, ³National Research Institute of Tuberculosis and Lung Diseases, Shaheed Beheshti University of Medical Sciences

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.

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 **3_{a-n}** could serve as inhibitors due to their structural similarity with N-glycolylmuramic acid residues of the cell wall structure. (Fig. 2)

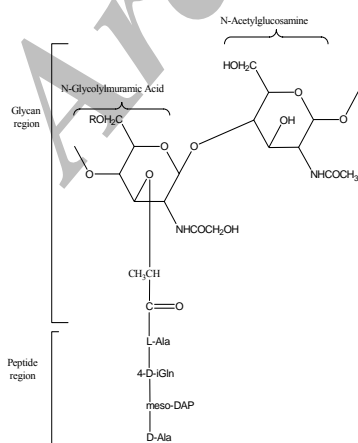


Figure 1. Structure of the basic peptidoglycan unit of mycobacterial cell walls

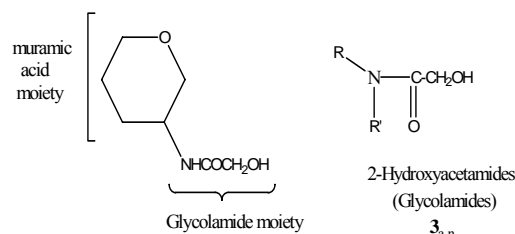


Figure 2

This article describes the synthesis and physicochemical properties of several known (**3_{e-i}**) and novel 2-hydroxyacetamides (**3_{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.

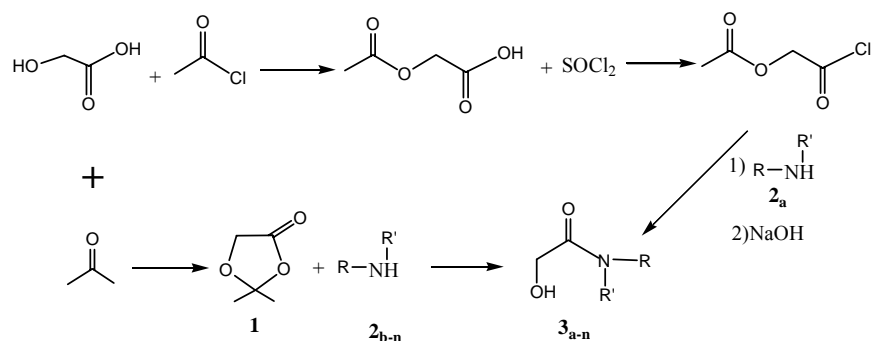


Figure 3. synthesis of 2-hydroxyacetamides 3_{a-n}

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

	$\text{R}'-\text{N}-\text{R}'$	MIC $\mu\text{g/mL}$		$\text{R}'-\text{N}-\text{R}'$	MIC $\mu\text{g/mL}$
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 **3_{b-n}** were prepared through the reaction of the corresponding amines **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 **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 **3_{e-g}** (**8**) and **3_h** (**9**), through the reaction of the corresponding amines with glycolic acid and preparation of **3_i** through the reaction of amine **2_i** with glycolic acid methyl ester (**10**) have previously been reported. All the new compounds were characterized by ¹HNMR, 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. ¹H NMR spectra were recorded on a Varian Unity Plus 400 MHz spectrometer using *DMSO-d₆* and *CDCl₃* 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 **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 **3_a** which was characterized by the physicochemical data listed below.
m.p.: 186-188 °C.

¹HNMR(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; CH₂).

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).

¹HNMR(δ) (CDCl₃): 4.35 (2H; s; H-4); 1.50 (6H; s; CH₃).

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 **3_{b-g}** and **3_{i-n}**, 1 mmole (0.11 gr) of 5-oxo-2,2-dimethyl-1,3-dioxolane **1** (glycolic acid acetonide) (**6**) and for the preparation of glycolamide **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 **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 MgSO₄. 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 **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%).

¹HNMR(δ) (DMSO) : 7.65 (1H; s; amide NH); 5.60 (1H; s; OH); 4.80 (1H; s; OH); 3.78 (2H; s; glycolyl CH₂); 3.42 (2H; t, *J*=5.6 Hz; CH₂); 3.17 (2H; q, *J*=5.6 Hz; CH₂).

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.

¹HNMR(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 CH₂); 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; CH₃).
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%).

¹HNMR(δ) (DMSO) : 4.80 (2H; s; OH); 4.30 (1H; s; OH); 4.12 (2H; s; glycolyl CH₂); 3.53 (4H; m; CH₂); 3.36 (2H; t, $J=5.6$ HZ; CH₂); 3.26 (2H; t, $J=5.6$ HZ; CH₂).

IR (cm⁻¹): 3200-3520 (OH, alcohol); 1640 (CO, amide).

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).

¹HNMR(δ) (CDCl₃) : 4.08 (2H; s; glycolyl CH₂); 3.59 (1H; s; OH); 3.54 (2H; t, $J=6.4$ HZ; CH₂); 3.29 (2H; t, $J=6.8$ HZ; CH₂); 1.99 (2H; qu, $J=6.4$ HZ; CH₂); 1.90 (2H; qu, $J=6.8$ HZ; CH₂).

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).

¹HNMR(δ) (CDCl₃) : 4.14 (2H; s; glycolyl CH₂); 3.76 (1H; s; OH); 3.61 (2H; t, $J=5.4$ HZ; CH₂); 3.20 (2H; t, $J=5.4$ HZ; CH₂); 1.68 (2H; m; CH₂); 1.59 (4H; m; CH₂).

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).

¹HNMR(δ) (CDCl₃) : 4.17 (2H; s; glycolyl CH₂); 3.70 (6H; m; CH₂); 3.62 (1H; s; OH); 3.29 (2H; t, $J=4.8$ HZ; CH₂).

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).

¹HNMR(δ) (DMSO) : 4.67 (2H; t, $J=5.6$ HZ; OH); 4.10 (4H; d, $J=5.6$ HZ; glycolyl CH₂); 3.48 (8H; m; CH₂).

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).

¹HNMR(δ) (CDCl₃) : 7.26 (5H; m; Ar-H); 6.56 (1H; s; OH); 4.06 (2H; s; CH₂); 3.57 (2H; q, $J=6$ HZ; CH₂); 2.84 (2H; t, $J=6$ HZ; CH₂).

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-(hydroxyacetyl-amino)-2-methyl-1,3-propanediol 3_j

TLC of the residue (EtOAc) confined purity of the product.

Yield: 1.41 gr (100%); oil.

¹HNMR(δ) (DMSO) : 7.17 (1H; s; amide NH); 5.72 (1H; s; OH); 4.98 (2H; s; OH); 3.72 (2H; s; glycolyl CH₂); 3.50 (2H; d, $J=10.4$ HZ; CH₂); 3.38 (2H; d, $J=10.8$ HZ; CH₂); 1.18 (3H; s; CH₃).

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: 0.33 gr (20%); m.p.: 72-74 °C.

¹HNMR(δ) (DMSO) : 4.52 (1H; s; OH); 4.44 (1H; s; OH); 4.05 (2H; s; glycolyl CH₂); 3.49 (2H; t, $J=6$ HZ; CH₂); 3.44 (2H; m; CH₂); 3.29 (2H; m; CH₂); 2.38 (6H; m; CH₂).

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.

¹HNMR(δ) (CDCl₃) : 4.18 (2H; d, $J=4.8$ HZ; glycolyl CH₂); 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; CH₂); 1.90 (2H; m; CH₂);

1.63 (1H; s; OH); 1.56 (2H; m; CH₂).
 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.

¹HNMR(δ) (CDCl₃): 4.18 (2H; d, *J*=4.8; glycolyl CH₂); 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; CH₂); 2.34 (1H; s; OH); 1.90 (2H; m; CH₂); 1.52 (2H; m; CH₂).

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-(hydroxyacetyl-amino)-morpholine 3_n

The residue was crystallized from EtOAc to obtain pure product.

Yield: 0.28 gr (20%); m.p.: 139-141 °C.

¹HNMR(δ) (DMSO) : 4.24 (1H; t, *J*=6 Hz; OH); 4.12 (2H; d, *J*=6 Hz; glycolyl CH₂); 3.61 (4H; t, *J*=4.8 Hz; CH₂); 2.74 (4H; t, *J*=4.8 Hz; CH₂).

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 3_{a-n} against Mycobacterium tuberculosis H₃₇Rv (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 McFarland turbidity standard. Stock solution of each compound was prepared in distilled water and subsequent 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 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 3_d (MIC = 12.5), 3_g (MIC = 13.75), 3_k (MIC = 14.25) and 3_n (MIC = 15.75) in comparison with those of 3_e (MIC = 25.5) and 3_f (MIC = 38.75) may be attributed to higher hydrophilicity of these compounds, amide of 3-hydroxy piperidine 3_m (MIC = 60) was less active than the corresponding analogue without hydroxyl substituent (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 hydrophilicity such as electronic and steric parameters might have influences on the activity. Although the MIC value of the most active compound (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.

ACKNOWLEDGEMENT

Author would like to thank Deputy of the Research of Tehran Medical Science University for the financial support of this investigation

REFERENCES

1. Bleed, D, watt C, Dye C. World Health Organization, Global Tuberculosis Control, WHO, Geneva, Switzerland, WHO/ CDS/ TB/ 2000. 275: 1-75.
2. Yufang MA, Stern RJ, Scherman MS, Vissa VD, Yan W, Johnes VC. Zhang F, Franzbau SG, Lewis HL, Mcneil MR. Drug targeting Mycobacterium tuberculosis cell wall synthesis: Genetics of dTDP-Rhamnose Synthetic Enzymes and Development of a Microtiter Plate-Based Screen for Inhibition of Conversion of dTDP-Glucose to dTDP-Rhamnose.

3. Deng I, Mikusova KG, Robuck M, Scherma P, Brennan J, McNeil M. Recognition of multiple effects of ethambutol on the metabolism of the mycobacteria cell envelope. *Antimicrob. Agents. Chemother.* 1995; 39: 649-701.
4. Collins LA, Franzblau SG, Microplate Blue Assay Versus BACTEC 460 System for High-Throughput Screening of Compounds Against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents. Chemother* 1997; 41:1004-1009
5. Khalaj A, Nahid E. Synthesis of α -hydroxyamides from acetonides of α -hydroxycarboxylic acids and primary amines. *Synthesis.* 1985; 1153-1155.
6. Willstatter R, Konigsberger F, Uber Aceton-Verbindungen von α -oxy-sauren. *Chem.Ber.*1923; 56: 2107.
7. Walter W, Steffen M, Heyns K, Oxidations reaction an der saureamid gru ppII. *Chem.Ber.*1966; 99: 3204-3214.
8. Fisher A, Rohr W, Heribicidal (Sulfamoyloxy) acetamides. *Ger. Off.* 1972; 2219923, 1979; *Chem. Abst* 80: 27127k
9. Barrows TH. Synthetic absorbale surgical devices of Polyester amides. *Eur. Pat. Appl* 30882, 1982; *Chem. Abst*, 95: 207606r
10. Seymour L, Shapiro.IRA MR, Freedman L. α -hydroxyacetamides and related Compounds *J. AM. Chem. Soc* 1959: 6322.

Archive of SID