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Synthesis, characterization and evaluation of antituberculosis activity of some hydrazones

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Abstract

Several new hydrazone derivatives were prepared by the reaction of some active hydrogen compounds with the diazonium salts of 4-amino-3,5-di/1,3,5-trimethylpyrazoles at 0–5 °C. Structures of the new substances were confirmed using UV, IR, ^{1}H NMR, ^{13}C NMR and EI-mass spectral data. In vitro antituberculosis activity of these compounds were tested on *Mycobacterium tuberculosis* H37Rv at 6.25 μg/ml. Both hydrazone products, ethyl 2-[(3,5-dimethylpyrazole-4-yl)hydrazono]-3-oxobutyrate (**3d**) and methyl 2-[(3,5-dimethylpyrazole-4-yl)hydrazono]-4-methoxy-3-oxobutyrate (**3e**) showed 29 and 28% inhibition against *M. tuberculosis*, respectively.

Keywords: 4-Amino-3,5-di/1,3,5-trimethylpyrazoles; Hydrazones; Antituberculosis activity

1. Introduction

Tuberculosis is presently regarded as the most dangerous infective disease world-wide and one of the major AIDS-associated infections. The simultaneous presence of HIV infection, spreading of drug resistant strains of *Mycobacterium tuberculosis* and the scarce compliance with the lengthy complex therapies often complicate the treatment of tuberculosis. Therefore, the search for new antituberculosis agent is required, in spite of the availability of effective drugs such as isoniazid and rifampin. In literature, it is reported that hydrazone derivatives have antitubercular activity [1,2]. With the aim of contributing to the literature, we decided to synthesize some hydrazones beginning with 4-amino-3,5-di/1,3,5-trimethylpyrazole and observe them for their antituberculosis activity.

Aryldiazonium salts of the compounds **1** and **2** coupled with acetylacetone, 1-benzoylacetone, ethyl acetoacetate, 6-methyl heptane-2,4-dione and methyl 4-methoxyacetoacetate in the presence of sodium acetate to give **3a**–**e** and **4a**–**b** (Fig. 1). The structures of the new substances were confirmed by UV, Infrared (IR), ^{1}H NMR, ^{13}C NMR, EI-mass spectral data and elementary analysis. These compounds were tested for their antituberculosis activity against *M. tuberculosis* at 6.25 μg/ml concentration.

2. Chemistry

4-Amino-3,5-dimethylpyrazole (**1**) was prepared as described previously [3]. 4-Amino-1,3,5-trimethylpyrazole (**2**) was synthesized same methods as novel compound in this study. Aryldiazonium salts of the compounds **1** and **2** coupled with acetylacetone, 1-benzoylacetone, ethyl acetoacetate, 6-methyl heptane-2,4-dione and methyl 4-methoxyacetoacetate in the presence of sodium acetate gave **3a**–**e** and **4a**–**b**.

The structure of synthesized compounds was proved by UV, IR, ^{1}H NMR, ^{13}C NMR and EI-mass spectra and elemental analysis. Some of physiochemical properties of these compounds are listed in Tables 1 and 2.

^{1}H NMR spectra of the coupling products (**3a**–**e**, **4a**–**b**) in DMSO-d_6 exhibited a hydrazone NH group at 11.00–15.00 ppm [4]. Observing hydrazone protons of compounds **3a**, **3d** and **3e** in three different fields proves the existence of isomers of **3a**, **3d** and **3e** [2,5]. In addition, in the ^{1}H NMR spectra of compounds **3a**–**e**, **4a**–**b** signals arising from >CH–N=N– structure at

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3.00–4.00 ppm were not observed [6,7]. This finding also supported that the structure of these compounds might be given in hydrazone form. The others protons are shown in Table 2.

^{13}C NMR spectral data of **3a** and **4b** were in accordance with literature values [1,7,8].

EI-mass spectra of **3a**–**e** and **4a** showed the correct molecular ion [M^+] peaks of different intensity which confirmed their molecular weights. The major fragmentation pathway appeared by the cleavage of –NH–N=C< bonds of hydrazone moiety [5,8,9]. Molecular ion was not detected in the mass spectrum of compound **4b** although characteristic fragment ions were observed. EI-mass spectra of the synthesized compounds exhibited the expected fragmentation pattern of pyrazole structures (Fig. 2).

3. Experimental

3.1. Chemistry

Aniline, benzocaine, EtOH, Et_2O, glacial AcOH, HCl, acetylacetone, AcONa and $NaNO_2$ were supplied from Merck. Methyl 4-methoxyacetoacetate, hydrazine hydrate, 1-benzoylacetone, methylhydrazine, 6-methylheptane-2,4-dione were purchased from Fluka, Sigma, BDH Chemicals.

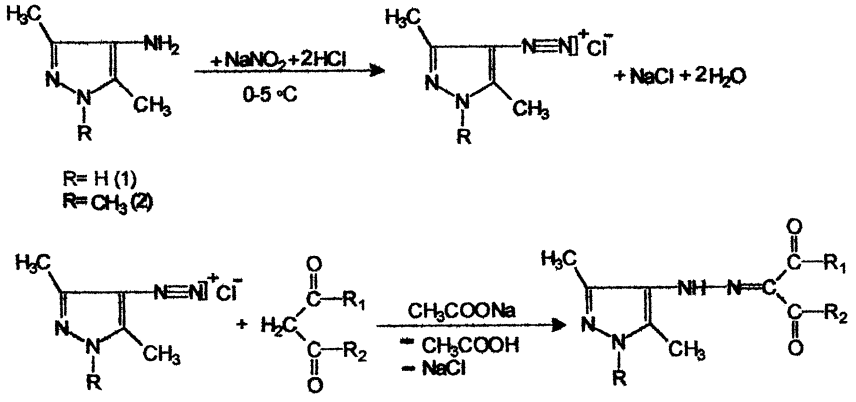

Fig. 1. Synthesis of the hydrazone derivatives.

Table 1
The physiochemical properties of the compounds

Comp.	R	R_1	R_2	M.p. (°C)	Yield (%)	UV λ_{max}, ε (EtOH) (1 mg/100 ml)	IR ν (cm^{-1}) (KBr) N–H, C–H and C=O stretching
3b	H	CH_3	$CH_2CH(CH_3)CH_3$	123–125	54	394 (13 000), 232 (6400)	3217, 2971, 1626
3c	H	CH_3	CH_3	182–183	64	393 (15 000), 232 (68 000)	3209, 1630
3d	H	CH_3	OC_2H_5	166–168	46	376 (5825), 231 (3760)	3244, 2983, 1673, 1618
3e	H	H_3COCH_2	OCH_3	165–166	28	379 (11 820), 230 (7044)	3273, 2935, 1696, 1614
4a	CH_3	CH_3	CH_3	95–97	43	385 (16 700), 239 (8300)	2929, 1662

Table 2
^1H NMR and mass data of the compounds

Comp.	^1H NMR δ (ppm)	EI MS (m/z, %)
3b	0.89 (d, 6H, –CH(CH_3)$_2$); 2.10 (m, 1H, –CH–); 2.28 (s, 6H, methyl protons to pyrazole); 2.47 (s, 3H, –$COCH_3$); 2.60 (d, 1H, –CH_2–); 2.80 (d, 1H, –CH_2–); 12.05 (s, 1H, pyrazole-NH); 14.58 (s, 1H, hydrazone-NH)	264 [M^+], 42 (100%)
3c	2.30 (s, 9H, methyl protons to pyrazole and CH_3–CO–); 2.48 (s, 3H, –CO–CH_3); 12.40 (s, 1H, pyrazole-NH); 14.63 (s, 1H, hydrazone-NH)	222 [M^+], 42 (100%)
3d	1.35 (t, 3H, CH_3–CH_2–); 2.30 (s, 9H, methyl protons to pyrazole and CH_3–CO–); 4.30 (q, 2H, –O–CH_2–); 12.40 (s, 1H, pyrazole-NH); 11.90, 12.30, 15.00 (3s, 1H, hydrazone-NH)	252 [M^+], 110 (100%)
3e	2.22 (s, 6H, methyls to pyrazole); 3.30–3.40 (s, 3H, O–CH_3); 3.65, 3.80 (2s, 3H, –COOCH_3); 4.40, 4.60 (2s, 2H, –CH_2–OCH_3); 12.30 (s, 1H, pyrazole-NH); 12.40, 12.60–14.90 (3s, 1H, hydrazone-NH)	268 [M^+], 42 (100%)
4a	2.10 (s, 3H, C_5 methyl protons to pyrazole); 2.20 (s, 3H, C_3 methyl protons to pyrazole); 2.32 (s, 3H, –COCH_3); 2.49 (s, 3H, –COCH_3); 2.50, 3.68 (s, 3H, C_1 methyl protons to pyrazole); 14.26 (s, 1H, hydrazone-NH)	236 [M^+], 56 (100%)

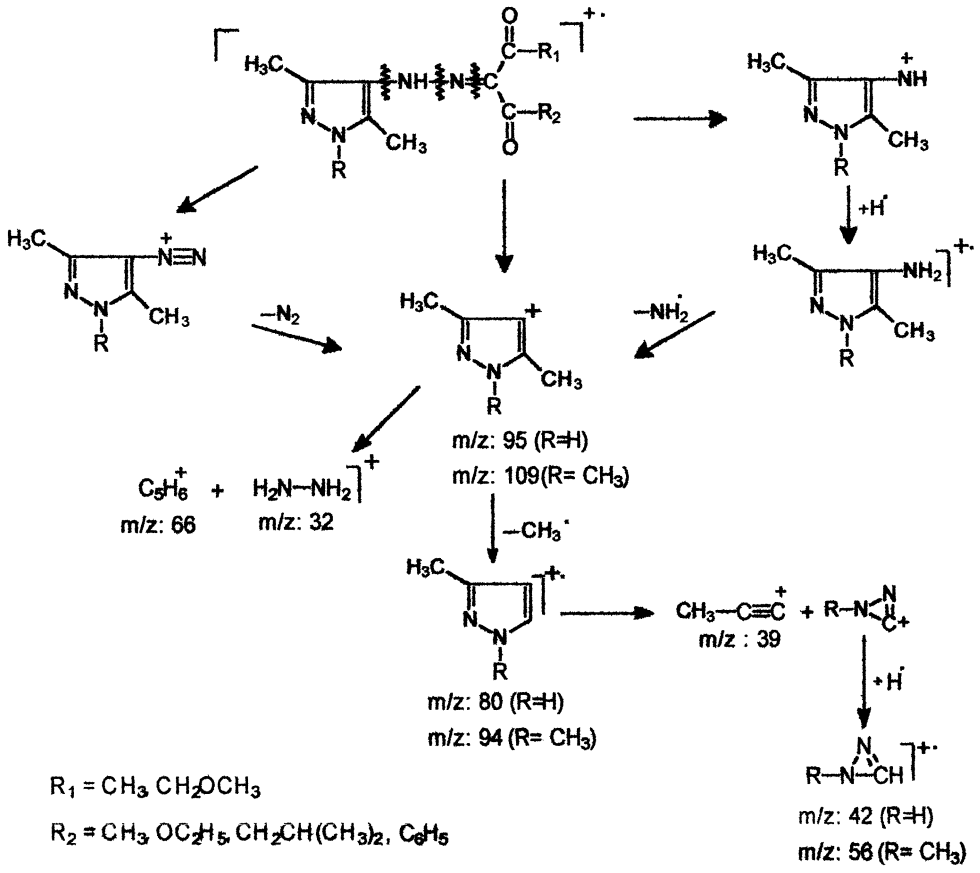

Fig. 2. Mass spectral fragmentation pattern of compounds.

Melting points were determined on a Buchi 530 melting point apparatus and uncorrected. UV spectra were obtained on a Shimadzu UV 2100 S spectrophotometer (1 mg/100 ml in EtOH). IR spectra were run as KBr disk on Perkin–Elmer 1600 FTIR. Elemental analyses were performed on a Leco CHNS-932 instruments. NMR spectra (^{1}H and ^{13}C) were taken on a Bruker AVANC–DPX 400 spectrometer in DMSO. Chemical shifts were reported in ppm related to the internal standard, TMS. Mass spectra were measured on a Fisons Instruments UG Platform II LS-MS Double focusing spectrometer.

3.1.1. Preparation of 4-amino-3,5-di/1,3,5-trimethylpyrazoles

4-Amino-3,5-dimethylpyrazole (**1**) was prepared as described previously [3]. 4-Amino-1,3,5-trimethylpyrazole (**2**) was synthesized same methods as novel compound in this study. $C_6H_{11}N_3$, 66%, m.p. 98 °C. ^{1}H NMR (δ, ppm): 1.92 (s, 3H, methyl to C_5), 2.00 (s, 3H, methyl to C_3), 3.10 (s, 2H, NH_2), 3.47 (s, 3H, methyl to C_1).

*3.1.2. General procedure for the preparation of hydrazones beginning with 4-amino-3,5-di/1,3,5-trimethylpyrazoles (**3a–e**,**4a–b**)*

Aryldiazonium salts of the **1** and **2** were formed by the action of HNO_2 on compounds **1** and **2** at 0–5 °C. These were coupled with acetylacetone, 1-benzoylacetone, ethyl acetoacetate, 6-methylheptane-2,4-dione and methyl 4-methoxyacetoacetate in the presence of AcONa. The precipitate hydrazone was filtered, washed with distilled water and recrystallized from EtOH [10].

*3.1.2.1. 1-Phenyl-1,2,3-butanetrione 2-(3,5-dimethylpyrazole-4-yl)hydrazone (**3a**).* $C_{15}H_{16}N_4O_2$: Yield: 41%, m.p.: 170–175 °C, UV (EtOH): λ_{max} (ε) 399 (12 000), 229 (9900) nm. IR (KBr): 3173, 1605, 1572, 1444, 1297, 1213, 693. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 2.00 (s, 6H, methyl protons to pyrazole), 2.10 (s, 3H, –CO–CH_3), 7.40–7.70 (m, 5H, phenyl), 12.40 (s, 1H, pyrazole-NH), 11.50, 12.30–14.90 (3s, 1H, hydrazone-NH). ^{13}C NMR (100, 6 MHz, DMSO-d_6, ppm): 10.00 (pyrazole, CH_3), 12.50 (pyrazole, CH_3), 30.73 (–CO–CH_3), 121.42 (phenyl, C2 and C6), 128.53

(phenyl, C4), 129.20 (phenyl, C3 and C5), 130.06 (pyrazole, C4), 133.22 (pyrazole, C5), 133.88 (pyrazole, C3), 140.43 (hydrazone, C=N), 193.21 (–*C*O–CH_3), 197.32 (–*C*O–C_6H_5). EI MS (70 eV, *m*/*z*): 284 [M^+], 169, 162, 161, 123, 111, 110, 105, 95, 80, 79, 78, 77, 42 (100%), 41, 39, 32.

*3.1.2.2. Methyl 2-[(1,3,5-trimethylpyrazole-4-yl)hydrazono]-4-methoxy-3-oxobutyrate (**4b**).* $C_{12}H_{18}N_4O_4$: Yield: 31%, m.p.: 105 °C, UV (EtOH): λ_{max} (ε) 376 (9700), 236 (7300) nm. IR (KBr): 2949, 2825, 1701, 1621, 1585, 1543, 1490, 1328, 1210, 1173, 1144, 1080. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 2.10 (s, 3H, C_5 methyl protons to pyrazole), 2.40 (s, 3H, C_3 methyl protons to pyrazole), 3.34 (s, 3H, C_1 methyl protons to pyrazole), 3.66, 3.67 (2s, 3H, –CH_2–OCH_3), 3.71, 3.78 (2s, 3H, –COOCH_3), 4.53, 4.42 (2s, 2H, –CH_2–OCH_3), 11.81, 14.40 (2s, 1H, hydrazone-NH). ^{13}C NMR (100, 6 MHz, DMSO-d_6, ppm): 10.00 (pyrazole, CH_3), 12.25 (pyrazole, CH_3); 37.50 (–N–CH_3), 52.50 (CH_2–O–CH_3), 60.00 (–CO–OCH_3), 74.00 (–CH_2–O–CH_3), 122.20 (pyrazole, C4), 131.50 (pyrazole, C5), 138.50 (pyrazole, C3), 138.50 (hydrazone, C=N), 164.25 (–*C*O–CH_2), 195.00 (–*C*O–OCH_3). EI MS (70 eV, *m*/*z*): 138, 137, 125, 124, 109, 94, 83, 82, 59, 56, 55, 45, 43, 39.

3.2. Microbiology

3.2.1. In vitro evaluation of antimycobacterial activity against *M. tuberculosis H37Rv*

A primary screen was conducted at 6.25 μg/ml against *M. tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system [11,12]. Compounds effecting $< 90\%$ inhibition in the primary screen ((minimal inhibitory concentration) MIC > 6.25 μg/ml) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary were re-tested at lower concentration (MIC) in a broth microdilution assay with alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

3.2.2. BACTEC radiometric method of susceptibility testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 more, or suspension of organism isolated earlier on conventional medium. The culture was well mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampicin (0.25 μg/ml). A control vial was inoculated with a 1:100 microdilution of the culture. A suspension equivalent to a McFarland No. 1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret results:

$$\Delta GI \text{ control} > \Delta GI \text{ drug} = \text{suspectible}$$

$$\Delta GI \text{ control} < \Delta GI \text{ drug} = \text{resistant}$$

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control GI is 30, the vials were read for 1 or 2 additional days to estabilish a definite pattern of ΔGI differences.

Table 3
Antituberculosis activity screen results of **3a**–**e** and **4a**–**b**

Comp.	MIC values (μg/ml)	Inhibition (%)
3a	>6.25	3
3b	>6.25	4
3c	>6.25	
3d	>6.25	29
3e	>6.25	28
4a	>6.25	
4b	>6.25	

4. Results and discussion

Antituberculosis activities of the compounds have been tested against *M. tuberculosis* using the BACTEC 460 radiometric system [11,12]. Rifampicin was used as the standard in tests. Both hydrazone products, ethyl 2-[(3,5-dimethylpyrazole-4-yl)hydrazono]-3-oxobutyrate (**3d**) and methyl 2-[(3,5-dimethylpyrazole-4-yl)hydrazono]-4-methoxy-3-oxobutyrate (**3e**) showed 29 and 28% inhibition towards *M. tuberculosis*, respectively. Compounds **3c**, **4a** and **4b** were not active against *M. tuberculosis* H37Rv. The primary antituberculosis activity screening results are reported in Table 3. The compounds, which exhibited $< 90\%$ inhibition in the primary screen (MIC > 6.25 μg/ml) were not evaluated further.

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