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Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones as potential antimycobacterial agents

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Abstract

Novel coupling products **7a**–**d** and 4-arylhydrazono-2-pyrazoline-5-ones **8a**–**e** were synthesized and evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv and *Mycobacterium avium*. Compound **7b** was found to be the most potent derivatives of the **7a**–**d** series by an MIC value of 6.25 μg/ml. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

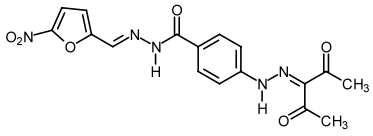
Keywords: Coupling products; 4-Arylhydrazono-2-pyrazoline-5-one; 1,2,4-Triazole; Hydrazide–hydrazones

1. Introduction

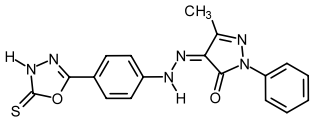
Isoniazide and hydrazones of isoniazide [1–5], some 4-aminobenzoic acid hydrazide–hydrazones [6] and their coupling products with indole [7], 1,3,4-oxadiazoline-2(3H)-thione starting from *p*-aminosalicylic acid hydrazide [8], pyrazolone derivatives [9], 1-methyl-1H-2-imidazo [4][5b] pyridine carboxylic acid hydrazide–hydrazones [10], 1,2,4-triazole derivatives [11] have been reported to possess promising antitubercular activity against *Mycobacterium tuberculosis* H37Rv. In our earlier studies, coupling products of 5-nitro-2-furylhydrazone (**A**) [12], 4-arylhydrazono-2-pyrazoline-5-ones from 1,3,4-oxadiazole-2(3H)-thione derivatives (**B**) [13], 1,2,4-triazoline-3(2H)-thione derivatives containing thiourea moiety (**C**) [11] examined by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), have been proved to possess considerable inhibition against *M. tuberculosis* H37Rv (Fig. 1). Therefore, it was planned to design more potent antimycobacterial agents, effective in lower dose accompanying with low toxicity.

2. Chemistry

During the course of this study, some novel coupling products containing hydrazide–hydrazone moiety; 2-pyrazoline-5-one derivatives obtained from either 1,3,4-oxadiazoline-2(3H)-thione or 1,2,4-triazoline-3(2H)-



A



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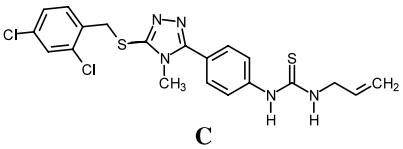

C

Fig. 1. Some compounds proved to inhibit the growth of *M. tuberculosis H37Rv*.

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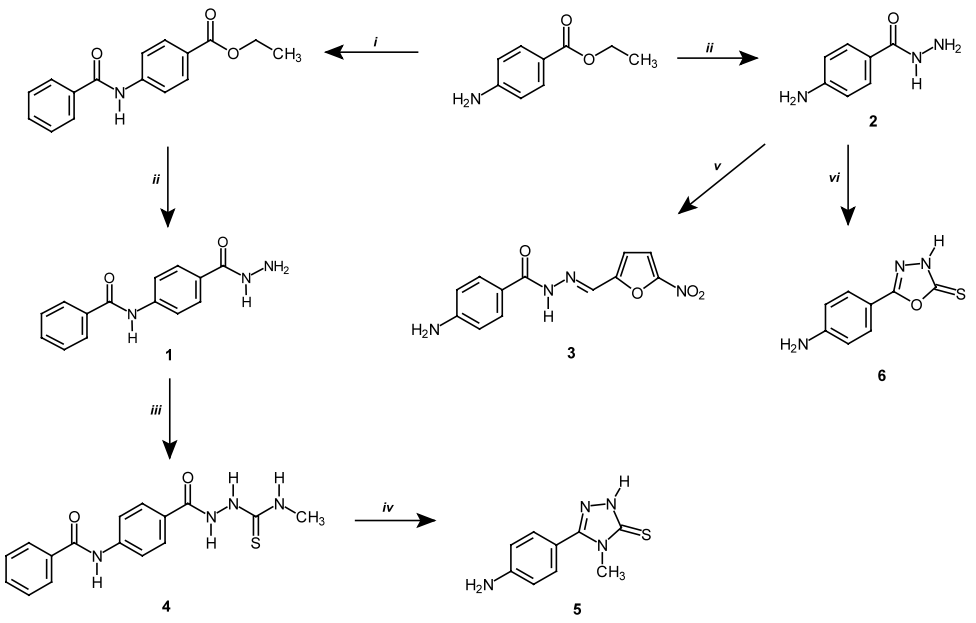


Fig. 2. Preparation of the aromatic primary amines **3**, **5** and **6**. Key to reactions: (i) C_6H_5COCl–ether; (ii) H_2N–NH_2·H_2O–EtOH-reflux; (iii) CH_3NCS–EtOH-reflux; (iv) (1) NaOH(2N)-reflux, (2) HCl; (v) 5-nitrofurfural–EtOH-reflux; (vi) (1) CS_2–KOH-reflux, (2)HCl.

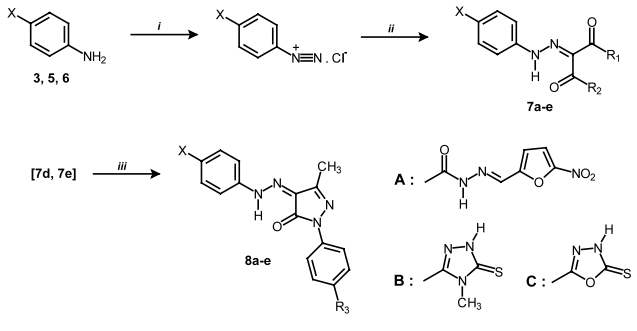
thiones have been synthesized (Figs. 2 and 3). Purity of these compounds has been confirmed by elemental analysis whilst their structures were elucidated using UV and ^{1}H NMR spectroscopy. Antituberculosis properties of the new compounds, synthesized in the present study, have been investigated in TAACF.

The UV spectra of **7a**–**d** and **8a**–**e** exhibited characteristic K bands for chromophoric C=N group at 365–385 and 395–433 nm regions, respectively, which corresponds to hydrazone groups. In the UV spectra of compounds **7a**–**d** and **8a**–**e**, absorptions arising from N=N bond at around 332–360 nm [14,15] and above 400 nm [16] were not observed. The ^{1}H NMR spectra of **7a**–**c** and **8a**–**c**, **8e** in DMSO-d_6 displayed the hydrazone N–H protons at 11.92–13.56 ppm. The hydrazone and triazoline protons of **7d** and oxadiazoline protons of **8d** were observed to exchange with deuterium in DMSO-d_6. In addition, ^{1}H NMR spectra of these compounds signals arising from possible >CH–N=N– structure at 3.00–4.00 ppm [17,18] were not observed. UV and ^{1}H NMR findings of these compounds also supported that the structures of **7a**–**d** and **8a**–**e** might be given in hydrazone form. The azomethine (CH=N) and N–H protons (CON*H*N=CH) of hydrazide–hydrazone **7a**–**c** exhibited the singlets at 8.37–8.44 ppm. The N–H protons of triazoline (**8a**–**c**) and oxadiazoline (**8e**) resonated at 13.93, 13.75, 13.93 and 14.30–15.23 ppm. ^{1}H NMR spectrum of **8d** has been taken by heating due to low solubility of this compound. For this reason, ^{1}H NMR spectrum of **8d** has been displayed shift to low energy medium. The carboxylic acid protons of compound **7a** were observed to exchange with deuterium in DMSO-d_6, which was supported by the literature [19–22]. EI-MS spectra of **7a**, **7b**, **7c** and **7d** confirmed their molecular weights displaying the parent molecular ions at m/z 389, 415, 447 and 347, respectively.

3. Experimental

3.1. Chemistry

Benzocaine, carbondisulfide and 4-nitro-phenylhydrazine were purchased from Merck. All other chemicals were purchased from Fluka. All melting points (m.p.) were recorded on a Buchi-530 m.p. apparatus



Compd.	X	R_1	R_2	Compd.	X	R_3
7a	A	OH	OH	**8a**	B	H
7b	A	CH_3	C_6H_5	**8b**	B	NO_2
7c	A	CH_3	OC_2H_5	**8c**	B	OCH_3
7d	B	CH_3	OC_2H_5	**8d**	C	NO_2
7e*	C	CH_3	OC_2H_5	**8e**	C	OCH_3

Fig. 3. Synthesis of target compounds **7a**–**e** and **8a**–**e** (*Reference [26]) Key to reactions: (i) $NaNO_2 + 2HCl/0–5$ °C; (ii) R_1–CO–CH_2–CO–R_2–AcONa; (iii) R_3–C_6H_4–NH–NH_2–AcOH-reflux.

Table 1
Physical and spectral data of **7a–d**

Comp.	M.p. (°C)	Yield (%)	Molecular weight (M.Wt.)	EI-MS (m/z, 70 eV)	UV λ_{max} log ε (1 mg per 100 ml)
7a	260–270 (decomposition)	63	$C_{15}H_{11}N_5O_8$ (389)	389 [M^+], 254, 235, 208, 207, 164, 154, 131, 121, 119, 104,93	380 (4.37) 302 (4.17)
7b	234–240 (decomposition)	71	$C_{18}H_{17}N_5O_7$ (415)	415 [M^+], 274, 273, 254, 120, 104, 92	381 (4.53) 302 (4.17)
7c	245–260 (decomposition)	68	$C_{22}H_{17}N_5O_6$ (447)	447 [M^+], 331, 309, 308, 293, 274, 273, 251, 228, 224, 223, 147, 119	206 (4.58)
7d	165–170	80	$C_{15}H_{17}N_5O_3S$ (347)	347 [M^+], 315, 302, 275, 274, 232, 207, 206, 205, 191, 190, 176, 133, 132, 118	365 (4.43) 256 (4.33)

Table 2
Physical, analytical and spectral data of **8a–e**

Compd.	M.p. (°C)	Yield (%)	Molecular weight (M.Wt.)	Elemental analysis			UV λ_{max} log ε (1 mg per 100 ml)
				C	H	S	
8a	285–287	70	$C_{19}H_{17}N_7OS\cdot 1/2H_2O$ (400.46)	56.99 57.04	4.53 4.46	8.00 7.47	401 (4.34) 245 (4.70) 210 (4.86)
8b	287–289	63	$C_{19}H_{16}N_8O_3S$ (436.46)	52.28 51.89	3.69 3.23	7.34 7.00	395 (3.71) 235 (4.54) 209 (4.82) 194 (4.10)
8c	280–282	67	$C_{20}H_{19}N_7O_2S\cdot 1/2H_2O$ (430.48)	55.80 55.88	4.68 4.53	7.45 7.39	399 (3.68) 209 (4.78)
8d	>300	56	$C_{18}H_{13}N_7O_4S\cdot 1/2H_2O$ (432.41)	50.00 50.94	3.26 3.10	7.41 7.14	433 (3.70) 301 (3.69) 234 (4.56) 209 (4.82) 192 (4.08)
8e	287–290	58	$C_{19}H_{16}N_6O_3S\cdot 1/2H_2O$ (417.44)	55.87 55.62	3.95 3.85	7.85 7.57	428 (4.47) 295 (4.23) 244 (4.69) 209 (4.86)

and uncorrected. UV spectra were recorded on a Shimadzu UV 2100S spectrophotometer (1 mg per 100 ml in ethanol). Nuclear Magnetic Resonance spectra (^{1}H NMR), were recorded on a Bruker AVANC-DPX 400 spectrometer. MS spectra were obtained on a Fisons Instruments VG Platform II LC-MS in the electron impact (EI) mode. Elemental analyses were performed on a Carlo Erba 1106 instrument.

*3.1.1. Preparation of aromatic primary amines (**3**, **5**, **6**)*

These compounds were prepared as described previously [23–25].

*3.1.2. Synthesis of coupling products (**7a–e**)*

Compounds **7a–e** were synthesized by the reactions of diazonium salts of **3**,**5**,**6** with malonic acid–ethyl acetoacetate–benzoylacetone according to literature methods [12,26]. Physical and spectral data of these compounds are given in Tables 1 and 2.

*3.1.3. Synthesis of 4-arylhydrazono-2-pyrazoline-5-ones (**8a–e**)*

The coupling products **7d** and **7e** were refluxed with phenylhydrazine–4-nitrophenylhydrazine–4-methoxyphenylhydrazine hydrochloride to yield **8a–e** using a reported procedure [13,27,28]. Physical and spectral data of these compounds are given in Tables 2 and 3.

3.2. Biological activity

Compounds were also tested for in vitro antimycobacterial activity against *M. tuberculosis* H37Rv using the BACTEC 460 radiometric system [29]. The effective compounds were also tested against *Mycobacterium avium* using the same technique. Inhibitions of the synthesized compounds against *M. tuberculosis* H37Rv and *M. avium* are given in Tables 4 and 5.

Table 3
^{1}H NMR spectral data of **7a–d** and **8a–e**

Compd.	^{1}H NMR δ (ppm)
7a	7.19–7.79 (m, 6H, Ar–H); 8.37 (s, 1H, CH=N); 11.83 (s, 1H, CON*H*N=CH); 11.92 (b, 1/2H, NH–N=C<)
7b	1.29 (t, 3H, CH_2–CH_3); 2.42 (s, 3H, CO–CH_3); 4.32 (q, 2H, CH_2–CH_3); 6.61–7.79 (m, 6H, Ar–H); 8.37 (s, 1H, CH=N); 11.93 (s, 1H, CON*H*N=CH); 12.29 (b, 1/2H, N*H*–N=C<)
7c	2.08 (s, 3H, CO–CH_3); 7.27–8.31 (m, 12H, Ar–H); 8.44 (s, 1H, CH=N); 12.21 (s, 1H, CON*H*N=CH); 12.97 (b, 1/2H, N*H*–N=C<)
7d	1.26 (t, 3H, CH_2–CH_3); 2.36 (s, 3H, CO–CH_3); 3.54 (s, 3H, N–CH_3); 4.24 (q, 2H, CH_2–CH_3); 7.50 (d, 2H, *o*-NH, $J = 8.5$ Hz); 7.65 (d, 2H, *m*-NH, $J = 8.5$ Hz)
8a	2.32 (s, 3H, pyrazoline CH_3); 3.56 (s, 3H, triazoline N–CH_3); 7.21–7.93 (m, 9H, Ar–H); 12.99–13.49 (s, 1H, hydrazone N–H); 13.93 (s, 1H, triazoline N–H)
8b	2.21 (s, 3H, pyrazoline CH_3); 3.41 (s, 3H, triazoline N–CH_3); 7.67–8.22 (m, 4H, Ar–H); 12.50–13.50 (b, 1H, hydrazone N–H); 13.75 (s, 1H, triazoline N–H)
8c	2.30 (s, 3H, pyrazoline CH_3); 3.56 (s, 3H, triazoline N–CH_3); 3.77 (s, 3H, O–CH_3); 7.01–7.82 (m, 8H, Ar–H); 12.80–13.56 (b, 1H, hydrazone N–H); 13.93 (s, 1H, triazoline N–H)
8d	4.87 (s, 3H, pyrazoline CH_3); 10.27–10.84 (m, 8H, Ar–H)
8e	2.31 (s, 3H, pyrazoline CH_3); 3.78 (s, 3H, O–CH_3); 7.02–7.94 (m, 8H, Ar–H); 12.56–13.74 (b, 1H, hydrazone N–H); 14.30–15.23 (b, 1H, oxadiazoline N–H)

4. Results and discussion

The synthesized compounds in the present study were tested for their antimycobacterial activity against *M. tuberculosis* H37Rv and *M. avium* using the BACTEC 460 radiometric system [29]. The results are shown in Tables 4 and 5. Rifampicin was used as the standard in the assays for *M. tuberculosis* H37Rv. The compounds which exhibited $< 90\%$ inhibition in the primary screen (Initial screening concentrations were 12.5 μg/ml for **7b** and **7c**, 6.25 μg/ml for the remaining compounds) were not evaluated further. Compounds **7b** and **7c** effecting $> 90\%$ inhibition in the primary screen at 12.5 μg/ml was re-tested at lower concentrations against *M. tuberculosis* H37Rv. Level II assay results of these compounds are given in the Table 4. Compound **7b** which was obtained from coupled diazonium salt of 4-aminobenzoic acid [(5-nitro-2-furyl)methylene]hydrazide with benzoylacetone was found to be active against *M. tuberculosis* H37Rv at 6.25 μg/ml. The same compounds were also tested against *M. avium* using the same technique. Clarithromycin was used as the standard in the tests. No satisfactory level of inhibition was observed with compounds **7b** and **7c** at 12.5 μg/ml, whereas clarithromycin exhibited 98% inhibition at 2 μg/ml. However, in our previous study [12], 4-aminobenzoic acid [(5-nitro-2-furyl)methylene]hydrazide **3**, which was starting compound of **7b** and **7c**, was observed to inhibit the growth of *M. tuberculosis* H37Rv by only 70%. From the given data, it can be concluded that **7b** could be a leading compound for further development.

Table 4
Antimycobacterial results of **7a–d** and **8a–e** against *M. tuberculosis* H37Rv

Compd.	MIC (μg/ml)	Inhibition (%)	Level II
7a	>6.25	0	
7b	<12.5	94	6.25
7c	<12.5	95	12.5
7d	>6.25	1	
8a	>6.25	0	
8b	>6.25	1	
8c	>6.25	1	
8d	>6.25	0	
8e	>6.25	2	
Rifampicin	0.25	98	

Table 5
Antimycobacterial results of **7b** and **7c** against *M. avium*

Compd.	MIC (μg/ml)	Inhibition (%)
7b	>12.5	45
7c	>12.5	16
Clarithromycin	2	98

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