ORIGINAL RESEARCH

Synthesis of some novel hydrazone derivatives and evaluation of their antituberculosis activity

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ABSTRACT: The heterocyclic hydrazone constitute an important class of biologically active drug molecules which have attractive attention of medicinal chemists due to their antituberculosis activities. For this purpose, new hydrazone derivatives were synthesized and evaluated for antituberculosis activity. The reaction of (5,6,7,8-tetrahydronaphthalen-1-yl) acetic acid hydrazide with various benzaldehydes gave 5,6,7,8-tetrahydronaphtalen acetic acid benzylidene hydrazide derivatives. The chemical structures of the compounds were elucidated by 1H-NMR, EI-MS spectral data and Elemental Analysis. The compounds were evaluated for antituberculosis activity against Mycobacterium tuberculosis H37Rv (ATCC 27294) using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results indicated that all of the tested compounds showed low activity against the test organism. The compound A10 showed high antituberculosis activity (IC50: 3.072 μg/mL and IC90: 3.358 μg/mL) and low cytotoxicity (CC50: >40 μg/mL).

KEY WORDS: Hydrazone, Antituberculosis activity, Mycobacterium tuberculosis

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Received:
February 01, 2010

Accepted:
March 07, 2010

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INTRODUCTION

In spite of a 5000 year history, tuberculosis (TB) remains the leading single-agent infectious disease killer in the world. Approximately one third of the world's population is infected with TB bacilli, and each year almost 8 million people develop active TB and 2 million die as a result of TB. The major challenges for tuberculosis control are the development of multidrug-resistant tuberculosis (MDRTB) strains and the increasing numbers of immunocompromised

individuals with HIV infections who are highly susceptible to the disease. As a result, there is a pressing need for new antitubercular agents acting with greater potency and efficacy than the current existing drugs (1).

To pursue this goal, our research efforts are directed to find new chemical classes of antimycobacterially active agents. The methods of investigation of structure-activity relationships (SARs) enabled us to find some new pharmacophores of the above-mentioned activity. Many studies were carried out on heterocyclic systems bearing a hydrazone structure as a pharmacophore (2-13). In this study, we planned to synthesize new molecules bearing hydrazone moieties for their potential antituberculosis activity.

Chemistry

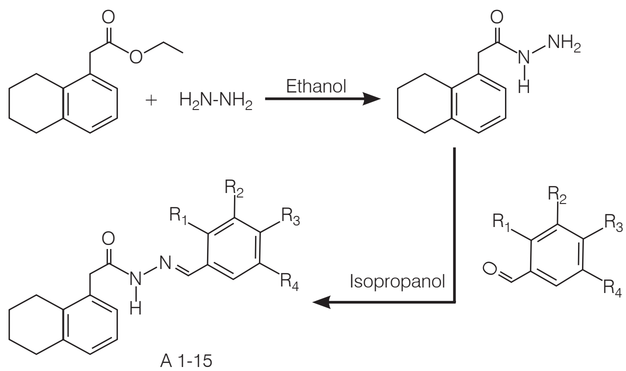
The synthetic route of the compounds is outlined in Scheme 1. For the synthesis of the title compounds, 5,6,7,8-tetrahydronaphthalene acetic acid hydrazide required as starting material was prepared by the reaction of 5,6,7,8-tetrahydronaphthalene acetic acid ethyl ester with hydrazine hydrate (14). The reaction of equimolar quantities of hydrazide with appropriate benzaldehydes in the presence of isopropyl alcohol resulted in the formation of the title compounds (**A1-15**) (Table 1).

Pharmacology

Antituberculosis activity and Cytotoxicity

The initial screen is conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) (15). One of the compounds showed significant antituberculosis activity as can be inferred from Table 2.

The VERO cell cytotoxicity assay (16) is done in parallel with the TB Dose Response assay. Viabil-



SCHEME 1. Synthetic protocol of the title compounds

ity is assessed using Promega's Cell Titer-Glo Luminescent Cell Viability Assay (17).

EXPERIMENTAL

Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded on the following instruments: ^{1}H NMR, Bruker 400 MHz NMR spectrometer in DMSO-d_6 using TMS as an internal standard; elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser; EI-MS, VG Quattro mass spectrometer.

Preparation of 5,6,7,8-tetrahydronaphthalene acetic acid hydrazide

In a flask equipped with a reflux condenser, a mixture of 5,6,7,8-tetrahydronaphthalene acetic acid ethyl ester (100 mmol) and the hydrazine hydrate (100 mmol) is reacted in ethanol (200 mL). The mixture is then refluxed for 1 h and the obtained solid is filtered and used without further purification (14).

Preparation of 5,6,7,8-tetrahydronaphtalen acetic acid benzylidene hydrazide A1-15

The reaction of equimolar quantities of hydrazide (5 mmol) with appropriate benzaldehyde (5 mmol) in the presence of isopropyl alcohol resulted in the formation of the title compounds. Some characteristics of the synthesized compounds are shown in Table 1.

A1: ^{1}H-NMR: δ 1.60-1.80 (4H, m), 2.60-2.75 (4H, m), 3.74 (2H, s), 6.90-7.10 (3H, m), 7.49 (2H, d, *J*= 8.5 Hz), 7.70 (2H, dd, *J*= 8.5, 2.1 Hz), 8.12 (1H, s), 11.51 (1H, s). EIMS (*m/z*): 326 (M$^+$, 9.5 %), 291 (1), 188 (27), 181 (8), 171 (25), 154 (17), 145 (100), 140 (58), 129 (31). For $C_{19}H_{19}ClN_2O$ calculated: 69.83 % C, 5.86 % H, 8.57 % N; found: 69.85 % C, 5.89 % H, 8.56 % N.

A2: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.33 (3H, s), 2.65-2.75 (4H, m), 3.71 (2H, s), 6.90-7.10 (3H, m), 7.24 (2H, d, *J*= 8.0 Hz), 7.57 (2H, dd, *J*= 8.0, 2.0 Hz), 8.08 (1H, s), 11.37 (1H, s). EIMS (*m/z*): 306 (M$^+$, 31 %), 288 (9), 262 (1), 248 (1), 188 (30), 171 (25), 161 (31), 145 (100), 134 (37), 120 (40). For $C_{20}H_{22}N_2O$ calculated: 78.40 % C, 7.24 % H, 9.14 % N; found: 78.44 % C, 7.25 % H, 9.15 % N.

A3: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.65-2.75 (4H, m), 3.72 (2H, s), 3.80 (3H, s), 6.90-7.05 (5H, m), 7.62 (2H, d, *J*= 6.0 Hz), 8.06 (1H, s), 11.30 (1H, s). EIMS (*m/z*): 322 (M$^+$, 38 %), 188 (21), 177 (22), 171 (15), 150 (56), 145 (100), 135 (26), 131 (26). For $C_{20}H_{22}N_2O_2$ calculated: 74.51 % C, 6.88 % H, 8.69 % N; found: 74.52 % C, 6.90 % H, 8.71 % N.

A4: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.79 (2H, s), 6.90-7.07 (3H, m), 7.94 (2H, d, *J*= 8.8 Hz), 8.27 (2H, d, *J*= 8.8 Hz), 8.23 (1H, s), 11.73 (1H, s). EIMS (*m/z*): 337 (M$^+$, 2 %), 320

TABLE 1. Some characteristics of the compound

Comp.	R_1	R_2	R_3	R_4	YİELD %	M.P. °C	MOL. FORMULA	M.W.
A1	H	H	Cl	H	78	192-194	$C_{19}H_{19}ClN_2O$	326
A2	H	H	CH_3	H	80	164-165	$C_{20}H_{22}N_2O$	306
A3	H	H	OCH_3	H	82	154-156	$C_{20}H_{22}N_2O_2$	322
A4	H	H	NO_2	H	79	205-206	$C_{19}H_{19}N_3O_3$	337
A5	H	H	CN	H	75	211-212	$C_{20}H_{19}N_3O$	317
A6	H	H	$CH(CH_3)_2$	H	80	151-152	$C_{22}H_{26}N_2O$	334
A7	H	H	$OCH_2C_6H_5$	H	85	165-166	$C_{26}H_{26}N_2O_2$	398
A8	H	H	Cl	Cl	88	196-197	$C_{19}H_{18}Cl_2N_2O$	360
A9	H	H	OH	OCH_3	65	191-192	$C_{20}H_{22}N_2O_3$	338
A10	H	H	OH	NO_2	67	197-188	$C_{19}H_{19}N_3O_4$	353
A11	H	H	O----CH_2----O		75	141-142	$C_{20}H_{20}N_2O_3$	336
A12	H	OCH_3	H	OCH_3	73	177-179	$C_{21}H_{24}N_2O_3$	352
A13	H	OCH_3	OCH_3	OCH_3	75	215-217	$C_{22}H_{26}N_2O_4$	382
A14	NO_2	H	H	H	85	201-203	$C_{19}H_{19}N_3O_3$	337
A15	H	NO_2	H	H	86	206-208	$C_{19}H_{19}N_3O_3$	337

TABLE 2. Antituberculosis activity and cytotoxicity of the compounds

Comp.	MABA: $H_{37}Rv$ Data		Cell Titer-Glo: Vero Cell	SI
	IC_{50} (µg/mL)	IC_{90} (µg/mL)	CC_{50} (µg/mL)	(CC_{50} / IC_{90})
A1	98.743	>100	-	-
A2	>100	>100	-	-
A3	>100	>100	-	-
A4	>100	>100	-	-
A5	>100	>100	-	-
A6	>100	>100	-	-
A7	>100	>100	-	-
A8	>100	>100	-	-
A9	>100	>100	-	-
A10	3.072	3.358	>40	>11.9
A11	>100	>100	-	-
A12	>100	>100	-	-
A13	>100	>100	-	-
A14	>100	>100	-	-
A15	>100	>100	-	-

(1), 307 (11), 291 (1), 188 (12), 186 (20), 171 (12), 159 (23), 151 (64), 145 (100), 129 (26). For $C_{19}H_{19}N_3O_3$ calculated: 67.64 % C, 5.68 % H, 12.45 % N; found: 67.64 % C, 5.69 % H, 12.47 % N.

A5: ^{1}H-NMR: δ 1.65-1.75 (4H, m), 2.60-2.75 (4H, m), 3.77 (2H, s), 6.90-7.05 (3H, m), 7.82-7.90 (4H, m), 8.17 (1H, s), 11.77 (1H, s). EIMS (*m/z*): 317 (M^+, 3 %), 188 (13), 187 (18), 186 (14), 171 (12), 159 (18), 145 (100), 131 (78). For $C_{20}H_{19}N_3O$ calculated: 75.69 % C, 6.03 % H, 13.24 % N; found: 75.71 % C, 6.03 % H, 13.27 % N.

A6: ^{1}H-NMR: δ 1.21 (6H, d, *J*= 6.9 Hz), 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 2.91 (1H, dt, *J*= 6.9 Hz), 3.73 (2H, s), 6.90-7.10 (3H, m), 7.31 (2H, d, *J*= 8.2 Hz), 7.60 (2H, dd, *J*= 8.2, 3.8 Hz), 8.09 (1H, s), 11.42 (1H, s). EIMS (*m/z*): 334 (M^+, 28 %), 316 (1), 290 (1), 276 (1), 189 (38), 188 (34), 171 (22), 161 (31), 148 (39), 147 (27), 145 (100), 131 (41). For $C_{22}H_{26}N_2O$ calculated: 79.01 % C, 7.84 % H, 8.38 % N; found: 79.05 % C, 7.87 % H, 8.40 % N.

A7: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.72 (2H, s), 5.15 (2H, s), 6.90-7.10 (5H, m), 7.30-7.50 (5H, m), 7.62 (2H, d, *J*= 8.8 Hz), 8.09 (1H, s), 11.35 (1H, s). EIMS (*m/z*): 398 (M^+, 15 %), 307 (1), 253 (16), 225 (9), 212 (3), 188 (9), 171 (5), 145 (45), 91 (100). For $C_{26}H_{26}N_2O_2$ calculated: 78.36 % C, 6.58 % H, 7.03 % N; found: 78.38 % C, 6.59 % H, 7.00 % N.

A8: ^{1}H-NMR: δ 1.60-1.75 (4H, m), 2.60-2.75 (4H, m), 3.75 (2H, s), 6.90-7.10 (3H, m), 7.48 (1H, d, *J*= 8.5 Hz), 7.68 (1H, s), 7.90-7.97 (1H, m), 8.44 (1H, s), 11.74 (1H, s). EIMS (*m/z*): 360 (M^+, 5 %), 325 (1), 215 (5), 189 (12), 188 (33), 187 (14), 176 (42), 174 (67), 171 (26), 159 (9), 145 (100), 129 (29). For $C_{19}H_{18}Cl_2N_2O$ calculated: 63.17 % C, 5.02 % H, 7.75 % N; found: 63.19 % C, 5.04 % H, 7.71 % N.

A9: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.65-2.80 (4H, m), 3.72 (2H, s), 3.81 (3H, s), 6.82 (1H, d, *J*= 8.1 Hz), 6.90-7.07 (4H, m), 7.26 (1H, s), 7.99 (1H, s), 9.49 (1H, s), 11.30 (1H, s). EIMS (*m/z*): 338 (M^+, 49 %), 320 (12), 294 (1), 193 (36), 188 (25), 171 (17), 166 (47), 165 (40), 145 (100), 129 (28). For $C_{20}H_{22}N_2O_3$ calculated: 70.99 % C, 6.55 % H, 8.28 % N; found: 70.96 % C, 6.59 % H, 8.31 % N.

A10: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.74(2H, s), 6.90-7.05 (3H, m), 7.19 (1H, dd, *J*= 8.7, 2.1 Hz), 7.88 (1H, dt, *J*= 8.7, 2.1 Hz), 7.97 (1H, s), 8.13-8.20 (2H, m), 11.51 (1H, s). EIMS (*m/z*): 353 (M^+, 6 %), 335 (1), 188 (26), 171 (18), 167 (72), 159 (10), 145 (100), 129 (25). For $C_{19}H_{19}N_3O_4$ calculated: 64.58 % C, 5.42 % H, 11.89 % N; found: 64.61 % C, 5.45 % H, 11.92 % N.

A11: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.72 (2H, s), 6.08 (2H, s), 6.90-7.05 (4H, m), 7.12 (1H, dd, *J*= 8.2, 1.6 Hz), 7.25 (1H, d, *J*= 1.6 Hz), 8.02 (1H, s), 11.37 (1H, s). EIMS (*m/z*): 336 (M^+, 51 %), 318 (1), 292 (1), 278 (1), 191 (26), 188 (31), 171 (14), 164 (52), 163 (42), 149 (39), 145 (100), 129 (27). For $C_{20}H_{20}N_2O_3$ calculated: 71.41 % C, 5.99 % H, 8.33 % N; found: 71.44 % C, 5.95 % H, 8.37 % N.

A12: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.74 (2H, s), 3.78 (6H, s), 6.55 (1H, s), 6.93 (2H, s), 6.90-7.05 (3H, m), 8.05 (1H, s), 11.46 (1H, s). EIMS (*m/z*): 352 (M^+, 43 %), 334 (1), 308 (1), 294 (2), 188 (29), 179 (29), 171 (16), 165 (35), 145 (100), 129 (29). For $C_{21}H_{24}N_2O_3$ calculated: 71.57 % C, 6.86 % H, 7.95 % N; found: 71.60 % C, 6.89 % H, 7.99 % N.

A13: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.65-2.77 (4H, m), 3.70 (3H, s), 3.75 (2H, s), 3.82 (6H, s), 6.90-7.05 (5H, m), 8.06 (1H, s), 11.43 (1H, s). EIMS (*m/z*): 382 (M^+, 84 %), 364 (1), 352 (11), 338 (10), 324 (19), 237 (41), 210 (30), 209 (33), 195 (87), 193 (100), 178 (30), 163 (10), 145 (74), 129 (24). For $C_{22}H_{26}N_2O_4$ calculated: 69.09 % C, 6.85 % H, 7.32 % N; found: 69.12 % C, 6.88 % H, 7.35 % N.

A14: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.60-2.75 (4H, m), 3.74 (2H, s), 6.90-7.10 (3H, m), 7.60-7.70 (1H, m), 7.75-7.83 (1H, m), 8.00-8.10 (2H, m), 8.51 (1H, s), 11.77 (1H, s). EIMS (*m/z*): 337 (M^+, 1

%), 320 (10), 303 (2), 246 (1), 201 (10), 188 (10), 186 (12), 171 (20), 151 (35), 145 (100), 130 (30). For $C_{19}H_{19}N_3O_3$ calculated: 67.64 % C, 5.68 % H, 12.45 % N; found: 67.66 % C, 5.71 % H, 12.48 % N.

A15: ^{1}H-NMR: δ 1.65-1.80 (4H, m), 2.62-2.75 (4H, m), 3.78 (2H, s), 6.90-7.10 (3H, m), 7.68-7.75 (1H, m), 8.08-8.15 (2H, m), 8.20-8.25 (1H, m), 8.49 (1H, s), 11.70 (1H, s). EIMS (*m/z*): 337 (M^+, 2 %), 307 (1), 279 (1), 188 (12), 187 (31), 186 (20), 171 (10), 159 (22), 151 (46), 145 (100), 129 (25). For $C_{19}H_{19}N_3O_3$ calculated: 67.64 % C, 5.68 % H, 12.45 % N; found: 67.69 % C, 5.70 % H, 12.43 % N.

Pharmacological evaluation

Primary Screen (Dose Response)

(Determination of a 90% Inhibitory Concentration (IC_{90}))

The initial screen is conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) (14). Compounds are tested in ten 2-fold dilutions, typically from 100 µg/mL to 0.19 µg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose-response curve using a curve-fitting program. Any IC_{90} value of ≤10µg/mL is considered "Active" for antitubercular activity. The "Active" compounds are considered for "Secondary Screening".

Secondary Screen

Determination of Mammalian Cell Cytotoxicity (CC_{50})

The VERO cell cytotoxicity assay (15) is done in parallel with the TB Dose Response assay. After 72 hours exposure, viability is assessed using Promega's Cell Titer-Glo Luminescent Cell Viability Assay (16), a homogeneous method of determining the number of viable cells in culture based on quantitation of the ATP present. Cytotoxicity is determined from the dose-response curve as the CC50 using a curve fitting program. Ultimately, the CC_{50} is divided by the IC_{90} to calculate an SI (Selectivity Index) value. SI values of ≥ 10 are considered for further testing.

RESULTS AND DISCUSSION

The structures of compounds **A1-15** were confirmed by elemental analyses, MS-FAB and ^{1}H- NMR spectral data. All compounds gave satisfactory elemental analysis. The mass spectra (MS (FAB)) of the compounds showed M^+ peaks, in agreement with their molecular formula.

In the 400 MHz ^{1}H-NMR spectra of the compounds, the C_6 and C_7 protons of 5,6,7,8-tetrahydronaphthalene were observed at 1.60-1.80 ppm. The C_5 and C_8 protons of 5,6,7,8-tetrahydronaphthalene were observed at 2.60-2.80 ppm. The CH_2CO protons appeared as singlet at 3.70-3.79 ppm. The N=CH and NH protons were observed at 7.90-8.23 ppm and 11.30-11.77 ppm respectively. All the other aliphatic and aromatic protons were observed at expected regions.

The results of antituberculosis and cytotoxicity screening of newly prepared compounds **A1-15** are expressed in Table 2. The very important result was observed at antituberculosis activity screening for one of the compounds. The compound **A10** showed high antituberculosis activity (IC_{50}: 3.072 µg/mL and IC_{90}: 3.358 µg/mL) and low cytotoxicity (CC_{50}: >40 µg/mL). Because of SI value of the compound **A10** ≥ 10, further tests are in progress.

ACKNOWLEDGEMENTS

Authors are thankful to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in the USA for the in vitro evaluation of antimycobacterial activity and cytotoxicity.

Bazı yeni hidrazon türevlerinin sentezi ve bunların antitüberküloz etkilerinin değerlendirilmesi

ÖZET: Heterosiklik hidrazon yapısı antitüberküloz aktiviteleri nedeniyle medisinal kimyacıların dikkatini çeken, cazip biyolojik olarak aktif önemli bir ilaç sınıfıdır. Bu amaçla, yeni hidrazon türevleri sentezlendi ve antitüberküloz etkinlikleri değerlendirilmiştir. (5,6,7,8-Tetrahidronaftalen-1-il) asetik asit hidraziti ile çeşitli benzaldehitlerin reaksiyonu, 5,6,7,8-tetrahidronaftalen asetik asit benziliden hidrazit türevlerini verdi. Bileşiklerin kimyasal yapıları 1H-NMR, EI-MS spectral verileri ve elemental analiz metodları ile aydınlatıldı. BACTEC 460 radyometrik sistem ve BACTEC 12B ortamından yararlanılarak Mycobacterium tuberculosis H37Rv (ATCC 27294)'e karşı bileşiklerin antitüberküloz aktiviteleri değerlendirilmiştir. Bileşik A10 yüksek antitüberküloz etkinlik (IC50: 3.072 µg/mL ve IC90: 3.358 µg/mL) ve düşük sitotoksisite (CC50: >40 µg/mL) gösterdi.

ANAHTAR KELİMELER: Hidrazon, Antitüberküloz etki, Mycobacterium tuberculosis H37Rv

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