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Synthesis and antituberculosis activity of new 3-alkylsulfanyl-1,2,4-triazole derivatives

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Abstract

The increasing clinical importance of drug-resistant mycobacterial pathogens has lent additional urgency to microbiological research and new antimycobacterial compound development. For this purpose, new alkylsulfanyltriazoles were synthesized and evaluated for antituberculosis activity. The reaction of thienyl-2-acetic acid with thiocarbohydrazide gave the mercaptotriazoles (**II**). The 4-amino-5-(2-thienylmethyl)-3-[1-(2-thienyl)-3-aryl)propion-3-yl]sulfanyl-4H-1,2,4-triazole (**III**) derivatives were synthesized by reacting the mercaptotriazoles with chalcones (**I**). Antituberculosis activities of the synthesized compounds were determined by broth microdilution assay, the Microplate Alamar Blue Assay, in BACTEC12B medium and results were screened *in-vitro*, using BACTEC 460 Radiometric System against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/ml and the tested compounds showed considerable inhibition ranging from 58–84%.

Keywords: *1,2,4-triazole, antituberculosis activity*

Introduction

Tuberculosis (TB) is the leading infections cause of death in the world, with approximately three million patients dying every year. Nearly one third of the world's population is infected with *Mycobacterium tuberculosis* and the World Health Organization (WHO) estimates that about 30 million people will be infected within next the 20 years. Moreover, the resurgence of TB in industrialized countries and the worldwide increase in the prevalence of *Mycobacterium avium* complex (MAC) infections in immunocompromised hosts (often accompanied by other bacterial infections) as well as the appearance of multidrug-resistant (MDR) strains of *M. tuberculosis* have prompted the quest for new drug acting both as antibacterial and antimycobacterial agents, without cross-resistance with known antituberculous agents. Development of resistance to existing drugs is a constant growing phenomenon that has concerned researchers throughout the world, and now has reached alarming levels for certain infectious diseases. This combined with the recent decline in the development of new drugs to combat them, can be anticipated to lead to infectious diseases lacking ready treatment regimens [1–3].

There are two basic approaches to develop a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures, that the TB organism has never been presented with before, for the treatment of multidrug-resistant TB [4].

To pursue this goal, our research efforts are directed to finding new chemical classes of antimycobacterially active agents. The methods of investigation using structure–activity relationships (SAR) enabled us to find some new pharmacophores of the above-mentioned activity. Many studies have been carried out on heterocyclic systems bearing an alkylsulfanyl

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group as a pharmacophore [5–7]. Various heterocyclic rings were taken as a basis to constitute a large series of compounds. Agents with higher activity were recorded among quinoxaline, purine, benzimidazole, benzoxazole, benzothiazole, pyridine and triazole derivatives [7–17]. Special attention was paid to their alkylsulfanyl derivatives. QSAR calculations carried out on various types of heterocycles proved that activity is enhanced by electron withdrawing substituents so that the alkylsulfanyl group bound to an electron deficient carbon atom in various heterocycles is responsible for antimycobacterial activity [7,10,11,12,14,15].

In view of this data, we aimed at the synthesis and antituberculosis evaluation of new 3-alkylsulfanyl-1,2,4-triazole derivatives. We have chosen triazoles, in particular substituted-1,2,4-triazole-3-thiols since their open-chain form consists of thienylthiocarbazide, an analog of the antituberculosis agent Amithiozone [18–21], which are among the various heterocycles that have attracted attention as potential antitubercular agents as the basic heterocyclic moiety.

Experimental

Chemistry

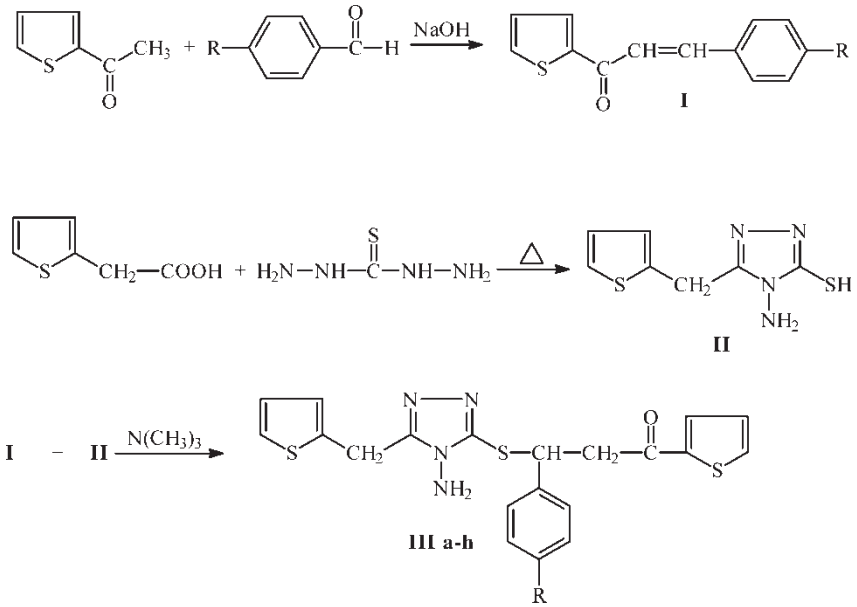
The synthetic route to the required compounds is outlined in Scheme 1. For the synthesis of the title compounds, 4-amino-3-mercapto-5-(2-thienylmethyl)-1,2,4-triazole (**II**) required as starting material was prepared for the first time by the reaction of thienyl-2-acetic acid with thiocarbohydrazide [22–23]. 1-(2-Thienyl)-3-aryl-2-propen-1-one (**I**) was prepared by reacting 2-acetylthiophene with various aromatic aldehydes in the presence of 5% NaOH solution [24]. The reaction of equimolar quantities of these triazole (**II**) with chalcones (**I**) in the presence of triethylamine resulted in the formation of the title compounds (**IIIa–h**) (Table I).

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded using the following instruments. IR: Shimadzu IR-435 spectrophotometer; ^{1}H-NMR: Bruker 250 MHz spectrometer; MS-FAB: VG Quattro Mass spectrometer.

General procedure for synthesis of the compounds

*1-(2-Thienyl)-3-aryl-2-propen-1-one (**I**)*. To a solution of 2-acetylthiophene (20 mmol) in ethanol, an aqueous solution of sodium hydroxide (5%, 10 ml) was added. The resulting solution was heated to 60°C and the appropriate benzaldehyde (20 mmol) was added with constant stirring. The reaction mixture was kept stirring at this temperature for 3–4 h, cooled to room temperature and then allowed to stand overnight. The solid product separated was collected by filtration, dried and recrystallized from ethanol to yield the required propenone.

*4-Amino-3-mercapto-5-(2-thienylmethyl)-1,2,4-triazole (**II**)*. A mixture of thiophene-2-acetic acid (20 mmol) and thiocarbohydrazide (40 mmol) was heated in an oil bath until the contents melted. The mixture was maintained at this temperature for 15–20 min. The product obtained on cooling was heated with dilute Na_2CO_3 solution to remove the



Scheme 1. The general synthesis reactions.

Table I. Some characterizations of the compounds.

Comp.	R	M.P. (°C)	Yield (%)	Molecular Formula	Mol. Weight
IIIa	H	115–117	45	$C_{20}H_{18}N_4OS_3$	426
IIIb	F	100–101	35	$C_{20}H_{17}FN_4OS_3$	444
IIIc	Cl	88–89	40	$C_{20}H_{17}ClN_4OS_3$	460,5
IIId	Br	96–97	45	$C_{20}H_{17}BrN_4OS_3$	505
IIIe	OH	78–80	25	$C_{20}H_{18}N_4O_2S_3$	442
IIIf	NO_2	120–122	55	$C_{20}H_{17}N_5O_3S_3$	471
IIIg	CH_3	80–82	35	$C_{21}H_{20}N_4OS_3$	440
IIIh	OCH_3	110–111	65	$C_{21}H_{20}N_4O_2S_3$	456

unreacted thiophene-2-acetic acid, (if any), washed with water, collected by filtration, and recrystallized from ethanol.

*4-Amino-5-(2-thienylmethyl)-3-[1-(2-thienyl)-3-aryl)propion-3-yl]sulfanyl-4H-1,2,4-triazole (**IIIa–h**).* A mixture of aminomercaptotriazole (**II**) (10 mmol), the appropriate 1-(2-thienyl)-3-aryl-2-propen-1-one (**I**) (10 mmol) and triethylamine (20 ml) were refluxed for 50 h in toluene. The mixture was evaporated to dryness. The residue was washed with water, collected by filtration and recrystallized from methanol.

IIIa–h. IR (KBr) $\upsilon_{max}(cm^{-1})$:3230–3185 (NH), 1695–1655 (C=O), 1610–1450 (C=C and C=N), 750–705 (C—S—C of thiophene).

IIIa. ^{1}H-NMR(250 MHz)(DMSO-d_6) δ (ppm): 3.70–3.85 and 4.30–4.45 (2H, two dd, CO—CH_2), 4.25 (2H, s, NH_2), 5.70 (2H, s, thiophene-CH_2), 6.45 (1H, t, $\mathcal{J}$ = 7.26 Hz, S—CH), 6.70–8.20 (11H, m, aromatic protons). MS (FAB) [M + 1]: *m/z* 427.

IIIc. ^{1}H-NMR(250 MHz)(DMSO-d_6) δ (ppm): 3.70–4.20 (2H, m, CO—CH_2), 4.15 (2H, s, NH_2), 5.75 (2H, s, thiophene-CH_2), 6.40 (1H, t, $\mathcal{J}$ = 7.20 Hz, S—CH), 6.90–8.25 (10H, m, aromatic protons). MS (FAB) [M + 1]: *m/z* 461.

IIIf. ^{1}H-NMR(250 MHz)(DMSO-d_6) δ (ppm): 3.80–3.90 and 4.30–4.40 (2H, two dd, CO—CH_2), 4.20 (2H, s, NH_2), 5.80 (2H, s, thiophene-CH_2), 6.55 (1H, t, $\mathcal{J}$ = 7.15 Hz, S—CH), 6.75–8.30 (10H, m, aromatic protons). MS (FAB) [M + 1]: *m/z* 472.

IIIg. ^{1}H-NMR(250 MHz)(DMSO-d_6) δ (ppm): 2.20 (3H, s, phenyl-CH_3), 3.60–4.15 (2H, m, CO—CH_2), 4.15 (2H, s, NH_2), 5.60 (2H, s, thiophene-CH_2), 6.35 (1H, t, $\mathcal{J}$ = 7.20 Hz, S—CH), 6.60–8.00 (10H, m, aromatic protons). MS (FAB) [M + 1]: *m/z* 441.

IIIh. ^{1}H-NMR(250 MHz)(DMSO-d_6) δ (ppm): 3.65–4.10 (2H, m, CO—CH_2), 4.20 (2H, s, NH_2), 4.30 (3H, s, OCH_3), 5.80 (2H, s, thiophene-CH_2), 6.50 (1H, t, $\mathcal{J}$ = 7.37 Hz, S—CH), 6.80–8.10 (10H, m, aromatic protons). MS (FAB) [M + 1]: *m/z* 457.

Microbiology

In-vitro *evaluation of antimycobacterial activity against Mycobacterium tuberculosis $H_{37}Rv$.* Antituberculotic activities of the compounds were tested at the center of Tuberculosis Antimicrobial Acquisiton and Coordinating Facility (TAACF). Compounds were tested for in-vitro antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/ml, in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence were tested in the BACTEC 460 Radiometric System [25]. Compounds effecting <90% inhibition in the primary screen (i.e., MIC > 6.25 μg/ml) were not generally evaluated further.

BACTEC radiometric method for 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 Mc Farland 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 cntrol 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:

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

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

If a clear susceptibility pattern (the difference of ΔGI of control and the drug sample) was not seen at the

Table II. Antituberculosis activity of the compounds.

Comp.	**IIIa**	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**	**IIIg**	**IIIh**	**Rifampicin**
MIC (μg/ml)	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	0.25
% inhibition	76	74	73	84	58	84	81	64	98

time the control ΔGI was 30, the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

Results and discussion

In the present work, eight new compounds were synthesized. The structures of the obtained compounds were elucidated by spectral data. In the IR spectra, some significant stretching bands due N—H, C═O, C═N and C═C were observed at 3230–3185, 1695–1655 and 1610–1450 cm^{-1} respectively. The specific band for thiophene was observed at 750–705 cm^{-1}. In the ^{1}H-NMR spectra, the signal due to the $COCH_2$ methylene protons, present in all compounds, appeared at 3.60–4.45 ppm, as a double doublet or multiplet. NH_2 protons and S—CH proton were observed at 4.15–4.30 ppm as a singlet and 6.35–6.55 ppm as triplet respectively. All the other aromatic and aliphatic protons were observed in the expected regions. Mass spectra (MS (FAB)) of compounds showed a M + 1 peaks, in agreement with their molecular formula.

The antituberculosis activities of the synthesized compounds were screened in-vitro using a BACTEC 460 radiometric system against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) at 6.25 μg/ml. Rifampicin was used as the test standard. All of the compounds tested showed significant antituberculosis activity as can be inferred from Table II. The compounds **IIId** and **IIIf** which include the nitro- and bromophenyl derivatives respectively showed the highest inhibitions with 84%. Other compounds showed varying inhibition degrees between 58–81%. SAR observation showed that a substitution on phenyl which is a substituent on the alkylsulfanyl moiety, affects the activity. We concluded from our investigations that **IIId** and **IIIf** may be considered promising for the development of new antituberculosis agents.

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References

[1] WHO Web site, http://www.who.org and NIAID Web site, http://www.niaid.nih.gov/factsheets/tb.htm
[2] Snider DE, Castro KG. N Engl J Med 1998;338:1689–1690.
[3] Sbardella G, Mai A, Artico M, Loddo R, Setzu MG, La Colla P. Bioorg Med Chem Lett 2004;14:1537–1541.
[4] Crabb Ch. Bull World Health Organ 2002;80:517–517.
[5] Waisser K, Hladuvkova J, Hrabalek A, Klimesova V, Kubicova L, Kunes J, Palat K, Machacek M, Vinsova J, Buchta V, Jilek P, Odlerova Z. Folia Pharm Univ Carol 1998;21–22:69–81.
[6] Klimesová V, Koci J, Zahajska L. Ces Slov Farm 2002;51:26–36.
[7] Klimesová V, Zahajska L, Waisser K, Kaustová J, Möllman U. Farmaco 2004;59:279–288.
[8] Kunes J, Spulak M, Waisser K, Slosarak M, Janota J. Pharmazie 2000;55:858–859.
[9] Scozzafava A, Mastrolorenzo A, Supuran CT. Bioorg Med Chem Lett 2002;11:1675–1678.
[10] Klimesová V, Kocí J, Pour M, Stachel J, Waisser K, Kaustová J. Eur J Med Chem 2002;37:409–418.
[11] Klimesová V, Kocí J, Waisser K, Kaustová J. Farmaco 2002;57:259–265.
[12] Kocí J, Klimesová V, Waisser K, Kaustová J, Dahse HM, Möllman U. Bioorg Med Chem Lett 2002;12:3275–3278.
[13] Klimesová V, Svoboda M, Waisser K, Pour M, Kaustová J. Farmaco 1999;54:666–672.
[14] Klimesová V, Svoboda M, Waisser K, Kaustová J, Buchta V, Kralova K. Eur J Med Chem 1999;34:433–440.
[15] Klimesová V, Svoboda M, Waisser K, Machacek M, Buchta V, Odlerova Z. Arch Pharm (Pharm Med Chem) 1996;329:438–442.
[16] Mir I, Siddiqui MT. Tetrahedron 1970;26:5235–5238.
[17] Gülerman NN, Doğan HN, Rollas S, Johansson C, Çelik C. Farmaco 2001;56:953–958.
[18] Duca CJ, Rothlauf MV, Scudi JV. Antibiot Chemother 1952;2:16–20.
[19] Nobles WL, Burckhalter JH. J Am Pharm Assoc 1953; 42:176–178.
[20] Caldwell HC, Nobles WL. J Am Pharm Assoc 1956; 45:729–732.
[21] Fujikawa F. Japan 14,685 (1962). Chem Abst 1965: 62: 527.
[22] Masao K. Hiroshi T. Jpn. Pat., 21,420 (1986). Chem Abst 1986; 105: 642097.
[23] Turan-Zitouni G, Kaplancıklı ZA, Kılıç FS, Erol K. Boll Chim Farm 2002;141:192–196.
[24] Dawey W, Tivey DJ. J Chem Soc 1958;80:1230–1236.
[25] Collins L, Franzblau SG. Antimicrob Agents Chemother 1997;41:1004–1009.