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Research Article

ONE-POT SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF 2-AMINO-5-ARYL-5H-THIAZOLO [4,3-b]-1,3,4-THIADIAZOLES

Karigar Asif A.[1], M. Himaja[2]*, Mali Sunil V.[3], K. Jagadeesh Prathap[2], Sikarwar Mukesh S[4]
[1]Department of Pharmaceutical chemistry, Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India
[2]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore 632 014, India
[3]Medicinal Chemistry Division, Piramal Life Sciences, Nirlon Complex, Goregaon East, Mumbai -400 063, India
[4]KLES College of Pharmacy, Ankola, Karnataka, India

*Dr (Mrs).M. Himaja, Professor, Pharmaceutical Chemistry Division, School of Advanced Science VIT University, Vellore-632014. Tamil Nadu, India. Email: dr_himaja@yahoo.com
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ABSTRACT
A series of 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles were synthesized by using aromatic aldehydes, thioglycolic acid and thiosemicarbazide. Equimolar mixtures of aromatic aldehydes with thioglycolic acid and thiosemicarbazide in H_2SO_4 transform into 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles. Structures of all synthesized compounds were confirmed by FTIR, ^{1}H NMR and mass spectral data. Their antitubercular activity has been studied. All the synthesized compounds have shown good antitubercular activity.

KEYWORDS: Aromatic aldehydes, thioglycolic acid, thiosemicarbazide, 2-aryl-(4-oxothiazolidin-3-yl)thiourea, cyclodehydration, 2-amino-5-aryl-5H-thiazolo[4,3-b]-1,3,4-thiadiazoles, Antitubercular activity.

INTRODUCTION
The resistance towards available drugs is rapidly becoming a major worldwide problem. The need to design new compounds to deal with this resistance has become one of the most important areas of research today. Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as "hydrogen binding domain" and "two-electron donor system". It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole, etc. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporins, hence can be used in antibiotic preparations. Thiadiazole is a 5-membered ring system containing two nitrogen and one sulphur atom. They occur in nature in four isomeric forms viz. 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole. The 1,3,4-thiadiazole isomer of thiadiazole series and its dihydro-derivatives provide a bulk of literature on thiadiazole. A glance at the standard reference work shows that more work has been carried out on the 1,3,4-thiadiazole than all other isomers combined. Members of this ring system have found their way into such diverse application as pharmaceuticals, oxidation inhibitors, cyanine dyes, & metal complexing agents. In past decades, thiadiazoles have proved their potential in development of pharmaceutically important organic compounds both of natural and synthetic origin[1]. Thiadiazole analogs deal with a variety of bioactivities viz. antitumor[2,3], anti-HIV[4], antimicrobial[5,6], anticonvulsant[7], antitubercular[8], antiprotozoal[9], anti-inflammatory[10]. Literature is enriched with lot of work on synthesis of potent substituted thiadiazole derivatives with diverse pharmacological activities[11,12] but only few reports have been received on peptide coupling of thiadiazoles. Thus keeping in view the

biological potency of thiadiazole derivatives a series of 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles with an anticipation to get potent agents of more therapeutic efficacy with lesser adverse effects. The wide range of therapeutic values of thiadiazoles prompted us to synthesize the title compounds and screen them for their antitubercular activities.

Tuberculosis is a common and often deadly infectious disease caused by *Mycobacterium tuberculosis* in humans, and it is estimated that one-third of the world's population is currently infected with TB bacillus and annually 2.0 million deaths were found[13]. The treatment of mycobacterial infections especially the tuberculosis, has become an important problem due to the emergence of monodrug and multidrug-resistant strains of *M. tuberculosis*[14]. In this context, there is a need for new drugs to fight against this disease. Recently, N-substituted-2-amino-5-aryl-5H-thiazolo[4,3-b]-1,3,4-thiadiazoles, active against pathogenic fungi of agricultural crops, have become substances, among other 1,3,4-thiadiazoles, that have drawn the attention of researchers[15-17]. The only known method giving rise to that heterocyclic system is based on cyclodehydration of (4-oxothiazolidine-3-yl)thioureas (in concentrated H_2SO_4 medium), which in turn are formed upon cyclodehydration of the products of addition of thioglycolic acid to the azomethine fragment of arylthiosemicarbazones,

MATERIALS AND METHODS

Analytical grade solvents and commercially available reagents were used without further purification. The column chromatography was carried out over silica gel (60-120 mesh), purchased from Sisco Research Laboratories Pvt Ltd. Melting points were determined in DBK, Prog, melting point apparatus Servewell Instruments Pvt Ltd. IR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Shimadzu FT-IR spectrometer. ^1H NMR spectra were recorded on 400-MHz and 500-MHz Bruker spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ relative to TMS, the coupling constants are given in Hz. Mass spectra were recorded using Agilent 1100 MSD spectrometer in electro spray mode.

General procedure of synthesis of compounds 5a—j: An aromatic aldehyde (0.02 mole) and thioglycolic acid (0.02 mole) were mixed, and after 10--15 min. 0.022 mole of thiosemicarbazide was added; then 10 mL of concentrated H_2SO_4 was added in portions upon cooling. The mixture was homogenized and left for 18--24 hours at -20 °C. The reaction mass was treated with 30--50 g ice, the precipitated solid was decanted, water was added, and the obtained suspension was neutralized with 40% NaOH until a weak alkaline reaction. Compounds 3a--j were recrystallized from aqueous dioxane solution.

2-Amino-5-phenyl-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5a): yield 87%, m.p. 156-158^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3472 (-NH_2); 3155(-CH aromatic); 2821 (-CH of thiazole); ^1H NMR spectrum (δ, ppm): 11.42 (s, CH); 8.19 (s, CH); 7.36-7.41 (m, Ph). MS spectrum, *m/z*: 236 $[M+1]^+$.

2-Amino-5-(4-methylphenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5b): yield 88%, m.p. 173-174^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3402 (-NH_2); 3155 (-CH aromatic); 2989 (-CH aliphatic); 2800 (-CH of thiazole); ^1H NMR spectrum (δ, ppm): 10.20 (s, CH); 6.56 (s, CH); 7.18-7.55 (m, Ph); 2.37 (s, Me). MS spectrum, *m/z*: 250 $[M+1]^+$.

2-Amino-5-(4-hydroxyphenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5c): yield 85%, m.p. 226-227^{0}C, yellow crystals. IR spectrum (v/cm^{-1}): 3471 (-NH_2); 3360 (-CH aromatic; 2880 (-CH of thiazole); ^1H NMR spectrum (δ, ppm): 11.14 (s, CH); 7.98 (s, CH); 5.55(s, OH) 7.08-7.74 (m, Ph). MS spectrum, *m/z*: 252 $[M+1]^+$.

2-Amino-5-(4-nitrophenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5d): yield 87.3%, m.p. 158-160 ^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3491 (-NH_2); 3365 (-CH aromatic); 2995 (-CH of thiazole); ^1H NMR spectrum (δ, ppm): 11.14 (s, CH); 7.98 (s, CH); 7.05-7.70 (m, Ph). MS spectrum, *m/z*: 281 $[M+1]^+$.

2-Amino-5-(4-dimethylaminophenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5e): yield 77%, m.p. 209-210^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3440 (-NH_2); 3115 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); ^1H NMR spectrum (δ, ppm): 10.20 (s, CH); 8.27 (s, CH); 7.16-7.30 (m, Ph); 3.77 (s, Me). MS spectrum, *m/z*: 279 $[M+1]^+$.

2-Amino-5-(2-chlorophenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5f): yield 82%, m.p. 214-215^{0}C, yellow crystals. IR spectrum (v/cm^{-1}): 3410 (-NH_2); 3115 (-CH aromatic); 2800 (-CH of thiazole); ^{1}H NMR spectrum (δ, ppm): 11.14 (s, CH); 7.96 (s, CH); 7.08-7.74 (m, Ph). MS spectrum, *m/z*: 271 $[M+1]^+$.

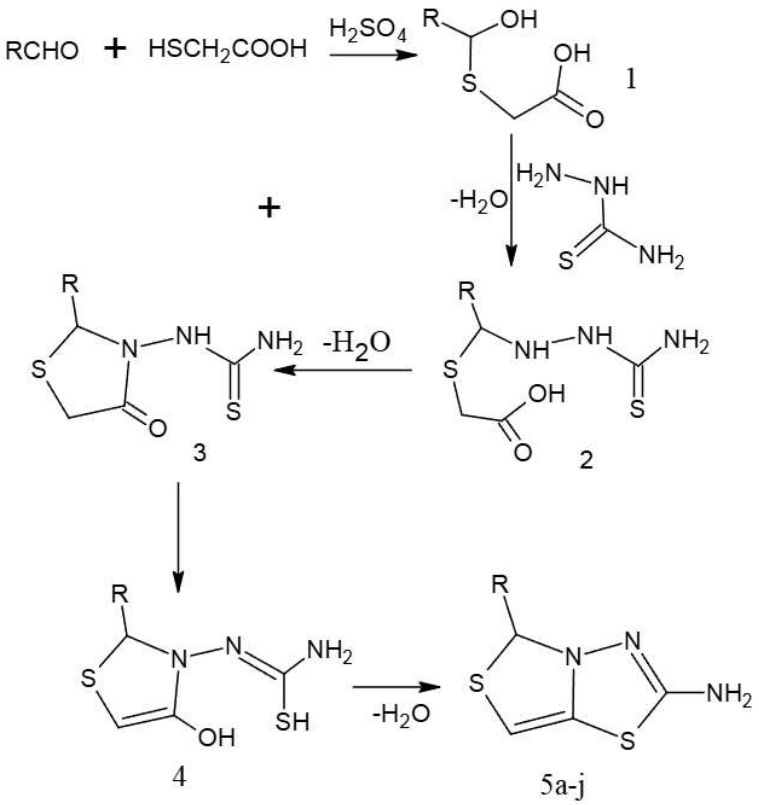
2-Amino-5-(4-chlorophenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5g): yield 91%, m.p. 207-208^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3449 (-NH_2); 3282 (-CH aromatic); 2995 (-CH of thiazole); ^{1}H NMR spectrum (δ, ppm): 9.51 (s, CH); 6.4 (s, CH); 7.28-7.72 (m, Ph). MS spectrum, *m/z*: 271 $[M+1]^+$.

2-Amino-5-(2,4-dichlorophenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5h): yield 89%, m.p. 241-242^{0}C, Brownish yellow crystals. IR spectrum (v/cm^{-1}): 3456 (-NH_2); 3260 (-CH aromatic); 3020 (-CH of thiazole); ^{1}H NMR spectrum (δ, ppm): 9.25 (s, CH); 7.32-7.76 (m, Ph). MS spectrum, *m/z*: 305 $[M+1]^+$.

2-Amino-5-(2-methoxyphenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5i): yield 69%, m.p. 168-169^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3406 (-NH_2); 3288 (-CH aromatic); 3100 (-CH aliphatic); 2800 (-CH of thiazole); ^{1}H NMR spectrum (δ, ppm): 9.79 (s, CH); 6.86 (s, CH); 7.14-7.79 (m, Ph),3.73 (s, OCH_3). MS spectrum, *m/z*: 266 $[M+1]^+$.

2-Amino-5-(3-methoxyphenyl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole (5j): yield 72%, m.p. 212-213^{0}C, yellowish crystals. IR spectrum (v/cm^{-1}): 3417 (-NH_2); 3250 (-CH aromatic); 3000 (-CH aliphatic); 2800 (-CH of thiazole); ^{1}H NMR spectrum (δ, ppm): 9.55 (s, CH); 6.92 (s, CH); 7.12-7.75 (m, Ph); 3.77 (s, OCH_3). MS spectrum, *m/z*: 265 $[M+1]^+$.

Scheme 1



R= Ph (a), 4-MeC_6H_4 (b), 4-HOC_6H_4 (c), 4-$O_2NC_6H_4$ (d), 4-$Me_2NC_6H_4$ (e), 2-ClC_6H_4 (f), 4-ClC_6H_4 (g), 2,4-$Cl_2C_6H_3$ (h), 2-$OMeC_6H_4$ (i), 3-$OMeC_6H_4$ (j)

RESULTS AND DISCUSSION

Chemistry

The synthesis of the finished products from aromatic aldehydes, thiosemicarbazide, and thioglycolic acid involves three steps. We followed the Shukurov et al[18] procedure for one-pot synthesis of 2-amino-5-aryl5 H-thiazolo[4,3-b]-1,3,4-thiadiazoles (5a-j) (Scheme 1) from equimolar quantities of an aromatic aldehyde, thioglycolic acid, and thiosemicarbazide. The interaction between equimolar quantities of aromatic aldehydes and thioglycolic acid proceeds with heat liberation and probably results in semithioacetals of thioglycolic acid (1), which further react with thiosemicarbazide to give functionalized N,S-acetals (2). Thioureas 3 are obtained as a result of cyclodehydration of the latter in concentrated H_2SO_4 medium; they transform into compounds 5a--j through intermediates 4. The structure of compounds 5a--j was confirmed by IR, ^1H NMR and Mass spectroscopy. The IR spectra of these compounds have no absorption band in the region of 1680-1630 cm^{-1}, characteristic of the stretching vibrations of the carbonyl group of the amide fragment in compounds of type 3, which confirms the hydrothiazolo[4,3-b]-1,3,4-thiadiazole structure. There are two absorption bands in the region 3470-3400 cm^{-1} and 3250-3220 cm^{-1} that might be interpreted as asymmetric and symmetric stretching vibrations of the amino group. Four peaks, due to the CH-stretching vibrations of the aromatic ring, were recorded in the interval 3180-2820 cm^{-1}. An intense absorption band is observed in the spectra at 1960 cm^{-1}, which can be assigned to the C-C normal vibrations of the aromatic ring. A group of the absorption bands in the 1600-500 cm^{-1} region is likely to be associated with the 5-phenyl-5H-thiazolo[4,3-b]-1,3,4-thiadiazole system. The methine proton signals and those of the proton in the 5H-position of the thiazole ring are detected in the ^1H NMR spectra at 7.96--8.3 ppm. The resonance lines of the phenyl ring protons are observed at 7.08--8.27 ppm.

Pharmacological Studies

Anti-tubercular activity

The antimycobacterial activity of compounds **5a-j** was assessed against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) using microplate Alamar Blue assay (MABA) [19]. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods [20], and the activity expressed as the minimum inhibitory concentration (MIC) in µg/mL. The MIC was defined as the lowest drug concentration required to complete inhibition of bacterial growth. The MICs of the compounds were depicted in Table 1. Streptomycin and Pyrazinamide was used as standards. All the tested compounds showed better in vitro antitubercular activity at minimum concentrations.

CONCLUSION

We prepared some thiadiazoles **5a-j** in good yields from aromatic aldehydes, thioglycolic acid and thiosemicarbazide. Equimolar mixtures of aromatic aldehydes with thioglycolic acid and thiosemicarbazide in H_2SO_4 transform into 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles and many compounds from this series showed good antitubercular activity at low concentrations. The biological profiles of these new generations of thiadiazoles would represent a fruitful matrix for further development of better antitubercular agents.

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Table 1: Antitubercular activity of 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles

Sl No	Compound	100	50	25	12.5	6.25	3.125	1.6	0.8	0.2	0.1
1	5a	S	S	S	S	R	R	R	R	R	R
2	5b	S	S	S	S	S	R	R	R	R	R
3	5c	S	S	S	S	S	R	R	R	R	R
4	5d	S	S	S	S	R	R	R	R	R	R
5	5e	S	S	S	R	R	R	R	R	R	R
6	5f	S	S	R	R	R	R	R	R	R	R
7	5g	S	S	S	S	S	R	R	R	R	R
8	5h	S	S	S	S	S	R	R	R	R	R
9	5i	S	S	S	R	R	R	R	R	R	R
10	5j	S	S	S	R	R	R	R	R	R	R
11	5k	S	S	S	S	R	R	R	R	R	R
12	5l	S	S	R	R	R	R	R	R	R	R
13	Streptomycin	S	S	S	S	S	R	R	R	R	R
14	Pyrazinamide	S	S	S	S	S	S	R	R	R	R

S- Sensitive, R-Resistant

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