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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of 4-amino-5-phenyl-4*H*-[1,2,4]-triazole-3-thiol derivatives as antimicrobial agents

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Abstract A series of novel 1,2,4-triazole derivatives have been synthesized by condensing the methyl benzoate and methyl salicylate with various substituents; their structures were established on the basis of elemental analysis, Fourier transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (NMR), and mass spectral data. All title compounds were subjected to in vitro antibacterial and antifungal screening against four different bacterial and fungal strains. Preliminary results indicate that some of them exhibit promising activities and deserve further consideration as potential antimicrobials.

Keywords 1,2,4-Traizole · Antibacterial screening · Antifungal screening

Introduction

The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of bacterial and fungal infections and drug side-effects (Fidler, 1998). Therefore, the development of new and different antimicrobial drugs is a very important objective and many research programs are directed towards the design of new antimicrobial agents.

During the last few decades, considerable attention has been devoted to synthesis of 1,2,4-triazole derivatives possessing such comprehensive bioactivities as antibacterial, antifungal (Karabasanagouda *et al.*, 2007; Sztanke *et al.*, 2008),

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antimycobacterial (Klimesová *et al.*, 2004), anti-inflammatory (Mullican *et al.*, 1993), analgesic (Tozkoparan *et al.*, 2005), anticancer (Demirba *et al.*, 2002), antihypertensive (Kakefuda *et al.*, 2002), anticonvulsant (Küçükgüzel *et al.*, 2004), antiviral (El-Essawy *et al.*, 2008), antidepressant (Kane *et al.*, 1988), antiasthmatic (Youichiro *et al.*, 1996), diuretic (Mhasalkar *et al.*, 1970) and hypoglycemic (Blank *et al.*, 1972) activities.

Regarding antimicrobial activity, triazole is structurally similar to imidazole molecule. Although triazole and imidazole act by the same mechanism of action, triazoles possess advantages over imidazoles, which have slow metabolic rate, oral bioavailability, and less effect on human sterol synthesis. For these reasons imidazoles are slowly being replaced by triazole molecules. It was reported that incorporation of various halo substituents into the heterocyclic ring systems augments biological activities considerably (Wang and Shi, 2001) and substitutions at 3-position by chloro-substituted benzene and at 4-position by free -NH_2 group give a broad spectrum of antimicrobial activity due to the ability of halogen to act as polar hydrogen or hydroxy mimic. Substitution of hydrogen by halogen has been a strategy in designing molecules for biological studies (Clerici and Donato, 2001). In view of the above findings, it was thought worthwhile to synthesize a series of eight compounds containing the 1,2,4-triazole moiety with different halogenated and nonhalogenated substituents and study their antimicrobial activities.

Materials and methods

Chemistry

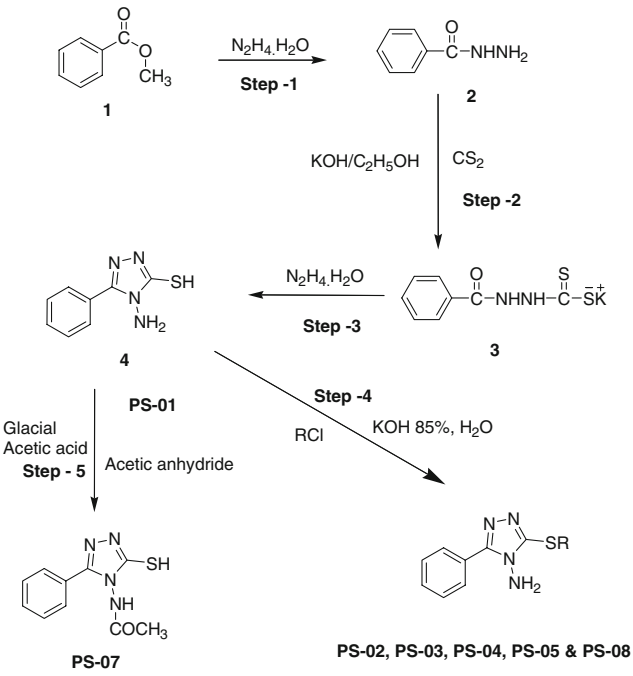
Reaction sequence employed for the synthesis of title compounds is shown in Schemes 1, 2.

Step 1 (Wang and Shi, 2001)

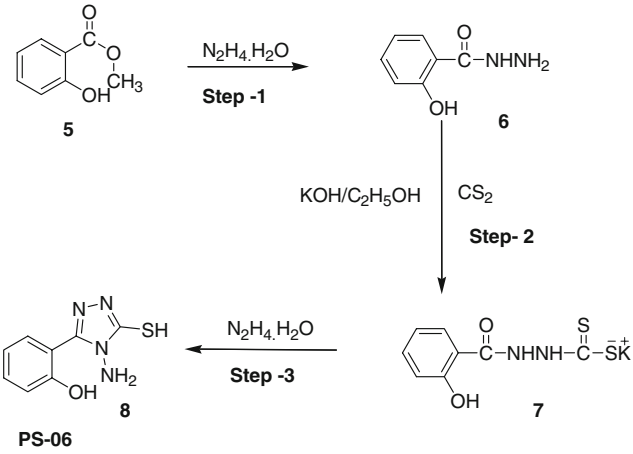
The esters of substituted aromatic acid (0.1 M) (**1** and **5**) were dissolved in 30 ml ethanol, and hydrazine hydrate (0.1 M) was added dropwise to the mixture with stirring. The resulting mixture was allowed to reflux for 6 h. Excess ethanol was distilled out and the contents were allowed to cool. The crystals formed were filtered, washed thoroughly with water, and dried. The completion of the reaction was monitored by thin layer chromatography (TLC) by using silica gel-G coated plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed with ultraviolet (UV) light.

Step 2

KOH (0.15 M) was dissolved in absolute ethanol (200 ml). To the above solution, aryl acid hydrazide (0.1 M) (**2** and **6**) was added and the solution cooled on ice. To this, carbon disulfide (0.15 M) was added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 15 h. It was then



Scheme 1 Synthesis of substituted 1,2,4-triazole (PS01, PS02, PS03, PS04, PS05, PS07, and 08)



Scheme 2 Synthesis of 2-(4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-phenol (PS06)

diluted with anhydrous ether. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether (100 ml) and dried under vacuum. The potassium salt thus obtained was in quantitative yield and was used in the next step without further purification.

Step 3

A suspension of potassium dithiocarbazinate (0.1 M) (**3** and **7**) in water (5 ml) and hydrazine hydrate (15 ml, 0.3 M) was refluxed for 30 min with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide gas (lead acetate paper and odor). A homogeneous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (100 ml). On acidification with concentrated hydrochloric acid, the required triazole **4** (PS01) and **8** (PS06) was precipitated out. It was filtered, washed thoroughly with cold water, and recrystallized from ethanol. The completion of the reaction was monitored with TLC by using silica gel-G coated plates by using ethyl acetate and petroleum ether (1:1) as eluent and observed with UV light.

Step 4

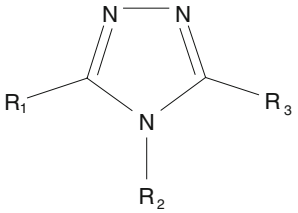
The appropriate (Clerici and Donato, 2001) triazole (0.1 mol) (PS01) was suspended in water (9 ml), and 0.1 mol of KOH (85% solution) was added under stirring at room temperature. After a few minutes (5–10 min), the solution was brought to 0°C in an ice bath, and halo-substituted aromatic compound (0.1 mol) was dropped in with vigorous stirring. The reaction mixture was checked by TLC (Et_2O as eluant). When reaction was complete, concentrated sulfuric acid (10 ml) was added, and from the crude mixture a white precipitate was formed slowly (PS02, PS03, PS04, PS05, and PS08). This was filtered, washed with water, and crystallized from ethanol.

Step 5

Acetic anhydride (0.19 ml, 0.002 mole) was added (Clerici and Donato, 2001; Afaf *et al.*, 2000) to a boiling solution of 4-amino-5-phenyl-4*H*-[1,2,4]-triazole-3-thiol (0.001 mole) (PS01) in glacial acetic acid (3 ml). After refluxing for 2 h, the reaction mixture was cooled and poured into ice-cold water. The formed precipitate was filtered off and recrystallized from ethanol to give the title compound (PS07).

TLC was used to reach the completion of reaction and purity of the compounds synthesized. Melting points were determined in open glass capillary tubes using Thiels tube containing liquid paraffin and were uncorrected. IR spectra were obtained in KBr discs on a Shimadzu-8400S FTIR spectrophotometer. Elemental analysis was performed using EL 11/carlo Ebra 1108 analyzer. ^{1}H NMR spectra were recorded on a FT NMR (500 MHz); MeOD was used as a solvent. Chemical shifts are reported as δ (ppm). The fast atom bombardment (FAB) mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. Elemental analysis was within $\pm$ 0.4% of theoretical values. Substitution pattern and characterization data of the synthesized compounds are reported in Tables 1, 2, respectively. The spectral data are presented in Table 3.

Table 1 Substitutions on the 1,2,4-triazole ring of compounds PS01–08



Compd.	R_1	R_2	R_3
PS01	$-C_6H_5$	$-NH_2$	–SH
PS02	$-C_6H_5$	$-NH_2$	$-S(C_6H_4)4-SO_2Cl$
PS03	$-C_6H_5$	$-NH_2$	$-S(C_6H_4)4-Br$
PS04	$-C_6H_5$	$-NH_2$	$-S(C_6H_4)4-CCl_3$
PS05	$-C_6H_5$	$-NH_2$	$-S(C_6H_4)4-Cl$
PS06	$-(C_6H_4)2-OH$	$-NH_2$	–SH
PS07	$-C_6H_5$	$-NHCOCH_3$	–SH
PS08	$-C_6H_5$	$-NH_2$	$-(C_6H_3)-2,4-(NO_2)_2$

Table 2 Characterization of the title compounds (PS01–08)

Compd No.	Formula (m. wt.)	M. P. (°C)	Yield (%)	% Analysis (calculated)/found C
PS01	$C_8H_5N_4S$ (189.19)	198–200	72	(49.98) 49.94
PS02	$C_{14}H_{11}ClN_4O_2S_2$ (366.85)	230–232	67	(45.84) 45.74
PS03	$C_{14}H_{11}BrN_4S$ (347.24)	208–210	64	(48.43) 48.40
PS04	$C_{15}H_{11}Cl_3N_4S$ (385.70)	122–124	68	(46.71) 46.68
PS05	$C_{14}H_{11}ClN_4S$ (302.78)	223–225	64	(55.53) 55.50
PS06	$C_8H_8N_4OS$ (208.22)	202–205	74	(46.14) 46.05
PS07	$C_{10}H_{10}N_4OS$ (234.28)	192–196	72	(51.27) 51.25
PS08	$C_{14}H_{10}N_6O_4S$ (358.34)	234–236	69	(46.93) 46.86

Biological activities

Antibacterial activities

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* DH5α, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa MTCC 3363*, and *Pseudomonas fluoroscens ATCC 3458* (recultured) bacterial strains by paper disc diffusion method (Chandrasekharan *et al.*, 2005). Muller–Hinton agar medium (38 g/l) was sterilized by autoclave at 121°C for 15 min and then poured into a Petri disc to a depth of 3–4 mm and allowed to solidify. Plates were dried and 0.1 ml standardized inoculum suspension of the microorganism [10^5 colony-forming units (CFU) /ml] was poured and uniformly

Table 3 Spectral data for the newly synthesized compounds

Compound	Spectral data
PS01	IR: (KBr, cm^{-1}): 3412, 3070, 2667, 1640; ^1H NMR (MeOD): δ5.6 (s, 2H, NH_2) and 7.25–7.6 (m, 5H, Ar–H and SH); MS: m/z 193 (M^++1).
PS02	IR: (KBr, cm^{-1}): 3450, 3070, 1640, 648; ^1H NMR (MeOD): δ5.6(s, 2H, NH_2), 7.1–7.8 (m, 9H, Ar–H); MS: m/z 366 (M^+), 368 (M^++2).
PS03	IR: (KBr, cm^{-1}): 3490, 3050, 760, 560; ^1H NMR (MeOD): δ6.9(s, 2H, NH_2); MS: m/z 346 (M^+), 348 (M^++2).
PS04	IR: (KBr, cm^{-1}): 3445, 3070, 1630, 1014, 627; ^1H NMR (MeOD): δ7.2–7.75 (m, 11H, Ar–H and NH_2); MS: m/z 384 (M^+), 386 (M^++2), 388 (M^++4), 390 (M^++6).
PS05	IR: (KBr, cm^{-1}): 3490, 1014, 760; ^1H NMR (MeOD): δ6.85 (s, 2H, NH_2), 7.38 (m, 9H, Ar–H); MS: m/z 304 (M^++2).
PS06	IR: (KBr, cm^{-1}): 3400, 3312, 2667; ^1H NMR (MeOD): δ7.25–7.6 (m, 5H, Ar–H and SH), 6.85 (s, 2H, NH_2), 9.5 (s, 1H, OH); MS: m/z 208 (M^+).
PS07	IR: (KBr, cm^{-1}): 3210, 2667; ^1H NMR (MeOD): δ2.2 (s, 3H, $COCH_3$), 3.3 (bs, 1H, NH), 7.2–8.5 (m, 6H, Ar–H and SH); MS: m/z 234 (M^+).
PS08	IR: (KBr, cm^{-1}): 3312, 3047, 1511, 1325, 630; ^1H NMR (MeOD): δ7.15 (s, 2H, NH_2), 7.6–8.4 (m, 8H, Ar–H); MS: m/z 358 (M^+).

spread. Then the paper impregnated with the test and standard compound was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 30°C for 24 h. Then, after this incubation, the minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ceftriaxone as standard. Zone of inhibition were determined for PS01–08 and the results are summarized in Table 4.

Antifungal activities

Newly prepared compounds were screened for antifungal activity against *Aspergillus niger* ATCC 4578, *Sacharomyces cerevisiae* 180, *Trichoderma* sp. ATCC 5248, and *Fusarium monaliforme* NCIM 1276 by the same method as in the antibacterial screening but the medium was replaced by potato dextrose agar medium (39 g/l). Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ketoconazole as standard. Zones of inhibition were determined for PS01–08 and the results are summarized in Table 5.

Results and discussion

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds (PS01–08) showed moderate to good inhibition at 5–20 μg/ml in

Table 4 Antibacterial activity of compounds PS01–08

Compd.	MIC in μg/ml and zone of inhibition (mm)			
	E. coli	*B. subtilis*	*P. aeruginosa*	*P. fluoroscens*
PS01	12.5 (16–20)	12.5 (16–20)	12.5 (16–20)	12.5 (16–20)
PS02	15.0 (<10)	15.0 (<10)	15.0 (<10)	15.0 (<10)
PS03	10.0 (16–20)	10.0 (16–20)	10.0 (16–20)	10.0 (16–20)
PS04	5.0 (14–20)	5.0 (14–20)	5.0 (16–22)	5.0 (14–18)
PS05	10.0 (16–20)	10.0 (16–20)	10.0 (16–20)	10.0 (16–20)
PS06	12.5 (16–20)	12.5 (16–20)	12.5 (16–24)	12.5 (16–20)
PS07	15.0 (<10)	15.0 (<10)	15.0 (<10)	15.0 (<10)
PS08	15.0 (<10)	15.0 (<10)	15.0 (<10)	15.0 (<10)
Ceftriaxone	5.0 (16–20)	5.0 (16–20)	5.0 (18–25)	5.0 (16–20)

MIC values were evaluated using concentration range 5–20 μg/ml

Escherichia coli (*E. coli*) DH5α, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 3363, *Pseudomonas fluoroscens* (*P. fluoroscens*) ATCC 3458

Table 5 Antifungal activity of compounds PS01–08

Compound	MIC in μg/ml and zone of inhibition (mm)			
	A. niger	*S. cerevisiae*	*T.* sp.	*F. monaliforme*
PS01	12.5 (16–22)	12.5 (16–22)	12.5 (16–22)	12.5 (16–22)
PS02	15.0 (<12)	15.0 (<12)	15.0 (<12)	15.0 (<12)
PS03	10.0 (16–22)	10.0 (16–22)	10.0 (16–22)	10.0 (16–22)
PS04	5.0 (14–18)	5.0 (16–20)	5.0 (22–26)	5.0 (18–22)
PS05	10.0 (16–22)	10.0 (16–22)	10.0 (16–22)	10.0 (16–22)
PS06	12.5 (16–22)	12.5 (16–22)	12.5 (16–22)	12.5 (16–22)
PS07	15.0 (<12)	15.0 (<12)	15.0 (<12)	15.0 (<12)
PS08	15.0 (<12)	15.0 (<12)	15.0 (<12)	15.0 (<12)
Ketoconazole	5.0 (16–20)	5.0 (18–24)	5.0 (25–32)	5.0 (20–26)

MIC values were evaluated using concentration range 5–20 μg/ml

Aspergillus niger (A. niger) ATCC 4578, *Sacharomyces cerevisiae (S. cerevisiae)* 180, *Trichoderma* sp. *(T.* sp.*)* ATCC 5248, *Fusarium monaliforme (F. monaliforme)* NCIM 1276

ethanol. The screening results indicate that all of the compounds tested exhibited significant antibacterial and antifungal activities when compared with the reference drugs. It was observed that the compound containing chloro-substituted group in 3-position with free NH_2 group in 4-position of 1,2,4-triazole (PS04) shows maximum bactericidal as well as fungicidal activity as compared with other compounds, whereas PS03 and PS05 showed good activity. This good activity is attributed to presence of pharmacologically active 4-chloro, 4-bromo, and 4-trichloromethyl groups attached to phenyl ring of the triazole ring. The compounds with free NH_2 groups at the 4-position (PS01 and PS06) showed moderate activity as compared

with PS04. The other compounds (PS02, PS07, and PS08) showed lower fungicidal effects compared with their bactericidal effects.

Conclusions

We report successful synthesis and antimicrobial activity of a new 1,2,4-triazole moiety. The antimicrobial activity study revealed that all the tested compounds showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that presence of 4-chloro, 4-bromo, and 4-carbon trichloride groups attached to phenyl ring to the triazole ring of the title compounds is responsible for good antimicrobial activity.

The field is further open for study of these compounds with respect to toxicity, chronic toxicity, pharmacokinetics, and clinical studies to establish these molecules as drugs in the market.

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