Journal of Enzyme Inhibition and Medicinal Chemistry, April 2009; 24(2): 406–412

Design, synthesis and *in vitro* microbiological evaluation of 6,6-dimethyl-7,9-diaryl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones - A new series of 'tailor-made' compounds

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(*Received 18 December 2007; accepted 31 March 2008*)

Abstract

Some novel 'tailor-made' compounds, 6,6-dimethyl-7,9-diaryl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones **23–27** have been studied for their *in vitro* antibacterial activity against *Staphylococcus aureus, β-Heamolytic streptococcus, Vibreo cholerae, Salmonella typhii, Shigella felxneri, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and anti-fungal activity against *Aspergillus flavus*, *Mucor*, *Rhizopus* and *Microsporum gypsuem*. Compounds **24** and **25** exerted potent antibacterial activity against *S. aureus*, *β-H. streptococcus*, *E. coli* and *P. aeruginosa* whereas all compounds **23–27** exerted strong *in vitro* antifungal activity against *A. flavus*, *Mucor* and *Rhizopus*.

Keywords: *6, 6-dimethyl-7, 9-Diaryl-1, 2, 4, 8-tetraazaspiro[4.5]decan-3-thiones,* m-*Chloroperbenzoic acid, Synthesis, Antibacterial activity, Antifungal activity*

Introduction

Synthesis of bioactive compounds in the field of organic chemistry received significant attention resulting in substantial advances both in the synthetic and medicinal aspects. Bioactive heterocyclic ring systems having 2,6-diaryl-piperidine-4-one nucleus with different substituents at 3- and 5-positions of the ring have aroused great interest due to their wide variety of biological properties such as antiviral, antitumour [1,2], central nervous system [3], local anesthetic [4], anticancer [5], antimicrobial activity [6] and their derivative piperidine are also biologically important and act as neurokinin receptor antagonists [7], analgesic and anti-hypertensive agents [8].

The 1,2,4-triazole nucleus [9–12] has been incorporated in a wide variety of therapeutically interesting drugs including H1/H2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, anti anxiety agents, sedatives, analgesics, and anti convulsants.

Due to an increase in the number of immunocompromised hosts, [13], over the past decades, the incidence of systemic microbial infections has been increasing dramatically. The increasing incidence of bacterial resistance to a large number of antibacterial agents such as glycopeptides (vancomycin, inhibition cell walls synthesis), sulfonamide drugs (inhibitors of tetrahydrofolate synthesis), β-lactam antibiotics (penicillins and cephalosporins), nitroimidazoles and quinolones (DNA inhibitors), tetracyclins, chloramphenicol and macrolides (erythromycin, inhibiting protein synthesis) is becoming a major concern [14]. For the past several years, vancomycin has been considered the last line of defense agent against Gram-positive infections and no alternative drugs for treating diseases that have become resistant to vancomycin [15]. Patients undergoing organ transplants, anticancer chemotherapy or long treatment with antimicrobial agents and patients with AIDS are immuno suppressed and very susceptible to life threatening systemic fungal infections like

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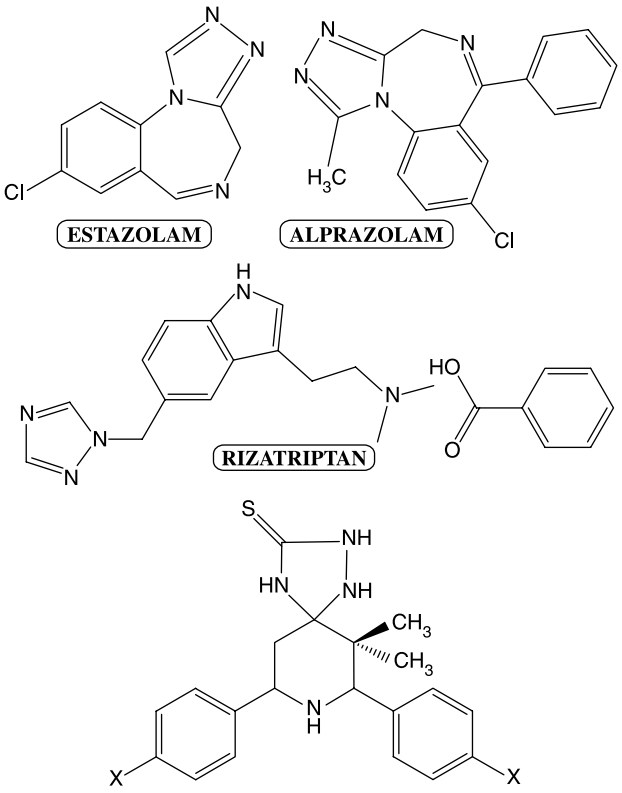
ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802188099



Scheme 1. Some 1,2,4-triazole based antibacterial and antifungal drugs.

Candidiasis, *Cryptococcosis* and *Aspergillosis*. Antifungal azoles, fluconazole and itraconazole which are strong inhibitors of lanosterol 14α-demethylase (cytochrome *P*45014DM) and orally active have been widely used in antifungal chemotherapy. Reports are available on the developments of resistance to currently available antifungal azoles in *Candida spp.*, as well as clinical failures in the treatment of fungal infections [16–19]. Furthermore, most of the present antifungal drugs are not effective against invasive *Aspergillosis* and the only drug of choice in such patients is the injectable amphotericin B. Some examples of 1,2,4-triazole based antibacterial and antifungal drugs are estazolam [20], alprazolam [21] and rizatriptan [22]. It is known from Scheme 1 that some clinically useful drugs contain a 1,2,4-triazole moiety.

These observations places new emphasis on the need to search for alternative new and more effective antimicrobial agents with a broad spectrum. Recently, we exploited the synthesis of 2,6-diarylpiperidin-4-one derivatives [23–25] with a view to incorporate various other bioactive heterocyclic nucleus such as 1,2,3-selenadiazoles, 1,2,3-thiadiazoles, diazepans intact for evaluation of associated antibacterial and antifungal activities. In the interest of above, we planned to synthesize a system, which comprises both piperidine and triazolidin-3-thione components together to give a compact structure like title piperidinyl spiro-1,2,4-triazolidin-3-thiones.

Experimental

Chemistry

Performing TLC assessed the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Nicolet-Avatar–330 FT-IR spectrophotometer and note worthy absorption values (cm^{-1}) alone are listed. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer using DMSO-*d* as solvent. The ESI + ve MS spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis was obtained on Carlo Erba 1106 CHN analyzer.

By adopting the literature procedure [26], 3,3-dimethyl-2,6-diarylpiperidin-4-ones were prepared **13–17**.

General method of preparation of 3,3-dimethyl-2,6-diarylpiperidin-4-one thiosemicarbazones ***18–22***. A mixture of 3,3-dimethyl-2,6-diarylpiperidin-4-one (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (40 mL) was refluxed on a steam bath for 2 h and was concentrated to one-third of its original volume. After cooling, the mixture was poured over crushed ice. The solid product thus obtained was filtered off and recrystallized twice from ethanol to give 3,3-dimethyl-2,6-diarylpiperidin-4-one thiosemicarbazones as crystalline solid.

Typical procedure for the synthesis of 6,6-dimethyl-7,9-diphenyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thione ***23***. A solution of 3,3-dimethyl-2,6-diphenylpiperidin-4-one thiosemicarbazone **18** (0.005 mol) in dichloromethane (50 mL) was treated with *m*-chloroperbenzoic acid (0.01 mol) and stirred for 1 h at (0–5)°C. The reaction mixture was decomposed with an ice-cold solution of sodium hydrogen carbonate solution. The organic layer was washed with brine solution, and then with excess of water and dried over anhydrous sodium sulfate. After evaporation of the solvent under reduced pressure, a gummy mass was obtained, which was solidified on treatment of petroleum ether (bp40–60). Final purification of 7,9-diphenyl-6,6-dimethyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thione was done by column chromatography using silica gel (100–200 mesh), with ethyl acetate -Petroleum ether (bp40–60) in the ratio (3:7) as eluent. IR (KBr) (cm^{-1}): 3424, 3366, 3146, 3030, 2971, 2928, 1289, 764, 701; ^{1}H NMR (δ ppm): 0.94 (s, 3H, CH_3 at C-6), 1.15 (s, 3H, CH_3 at C-6), 2.63 (s, 1H, H_8), 2.13-2.19 (dd, 1H, H_{10a}), 3.22–3.29 (dd, 1H, H_{10e}), 3.66 (s, 1H, H_2) 3.77–3.83 (dd, 1H, H_{9a}, $J_{9a,10e} = 2.92$, $J_{9a,10a} = 17.12$), 7.13 (s, 1H, H_1), 7.26–7.58 (m, 10H, H_{arom}), 8.32 (s, 1H, H_4); ^{13}C NMR (δ ppm): 20.8, CH_3 at C-6, 21.3 CH_3 at C-6,

32.6, C-10, 42.4, C-6, 59.9, C-9, 69.7, C-7, 79.1, C-5, 126.7–128.9 $-C_{arom}$,140.4, 144.1 *ipso*-C, 179.0 C-3.

The compounds **24–27** were synthesized correspondingly.

6,6-dimethyl-7,9-bis(4-methyphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione ***24***. IR (KBr) (cm^{-1}): 3429, 3315, 3102, 3022, 2972, 2924, 1246, 817, 745; ^1H NMR (δ ppm): 1.02 (s, 3H, CH_3 at C-6), 1.08 (s, 3H, CH_3 at C-6), 2.07 (s, 6H, CH_3 at arom. ring) 2.49 (s, 1H, H_8), 2.18–2.27 (dd, 1H, H_{10a}), 2.94–3.10 (dd, 1H, H_{10e}), 3.61 (s, 1H, H_2) 3.67–3.71 (dd, 1H, H_{9a}, $J_{9a,10e} = 2.12$, $J_{9a,10a} = 15.52$), 7.08 (s, 1H, H_1), 7.10–1.42 (m, 8H, H_{arom}), 8.50 (s, 1H, H_4); ^{13}C NMR (δ ppm): 20.6, CH_3 at C-6, 21.1 CH_3 at C-6, 22.7, CH_3 at arom. ring 32.3, C-10, 42.2, C-6, 59.8, C-9, 69.5, C-7, 79.0, C-5, 126.5–128.7 $-C_{arom}$, 136.1, 137.5, 141.1, 159.7 *ipso*-C, 180.0 C-3.

6,6-dimethyl-7,9-bis(4-methoxyphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione ***25***. IR (KBr) (cm^{-1}): 3427, 3314, 3246, 3158, 2969, 2929, 1246, 832, 750; ^1H NMR (δ ppm): 0.92 (s, 3H, CH_3 at C-6), 1.16 (s, 3H, CH_3 at C-6), 2.49 (s, 1H, H_8), 2.13–2.31 (dd, 1H, H_{10a}), 3.47–3.51 (dd, 1H, H_{10e}), 3.59 (s, 1H, H_2) 3.69–3.74 (dd, 1H, H_{9a}, $J_{9a,10e} = 2.16$, $J_{9a,10a} = 16.04$), 3.87 (s, 6H, OCH_3 at arom. ring), 7.13 (s, 1H, H_1), 7.33–7.97 (m, 8H, H_{arom}), 8.30 (s, 1H, H_4); ^{13}C NMR (δ ppm): 20.8, CH_3 at C-6, 22.7 CH_3 at C-6, 32.7, C-10, 42.6, C-6, 55.0, 54.9, OCH_3 at arom. ring, 59.7, C-9, 69.1, C-7, 79.0, C-5, 126.6–129.8 $-C_{arom}$, 132.5, 136.2, 158.4, 159.9 *ipso*-C, 179.0 C-3.

6,6-dimethyl-7,9-bis(4-fluorophenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione ***26***. IR (KBr) (cm^{-1}): 3427, 3369, 3254, 3149, 2975, 2931, 1291, 823, 748; ^1H NMR (δ ppm): 1.12, (s, 3H, CH_3 at C-6), 1.17 (s, 3H, CH_3 at C-6), 2.60 (s, 1H, H_8), 2.31–2.39 (dd, 1H, H_{10a}), 3.53–3.58 (dd, 1H, H_{10e}), 3.68–3.76 (dd, 1H, H_{9a}, $J_{9a,10e} = 2.15$, $J_{9a,10a} = 16.38$), 7.18 (s, 1H, H_1), 7.37–7.81 (m, 8H, H_{arom}), 8.37 (s, 1H, H_4); ^{13}C NMR (δ ppm): 21.5, CH_3 at C-6, 22.3 CH_3 at C-6, 32.8, C-10, 42.8, C-6, 60.1, C-9, 69.9, C-7, 80.6, C-5, 127.8–129.3 $-C_{arom}$, 137.9, 141.6, 158.3, 158.74 *ipso*-C, 180.2 C-3.

6,6-dimethyl-7,9-bis(4-chlorophenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione ***27***. IR (KBr) (cm^{-1}): 3425, 3368, 3251, 3147, 2972, 2929, 1289, 824, 745; ^1H NMR (δ ppm): 1.16, (s, 3H, CH_3 at C-6), 1.19 (s, 3H, CH_3 at C-6), 2.61 (s, 1H, H_8), 2.30–2.38 (dd, 1H, H_{10a}), 3.51–3.57 (dd, 1H, H_{10e}), 3.69–3.74 (dd, 1H, H_{9a}, $J_{9a,10e} = 2.16$, $J_{9a,10a} = 16.40$), 7.20 (s, 1H, H_1), 7.43–7.83 (m, 8H, H_{arom}), 8.39 (s, 1H, H_4); ^{13}C NMR (δ ppm): 21.4, CH_3 at C-6, 22.6 CH_3 at C-6, 32.6, C-10, 42.4, C-6, 60.8, C-9, 69.8, C-7, 80.1, C-5, 128.0–129.8 $-C_{arom}$, 137.7, 141.4, 158.0, 158.4 *ipso*-C, 180.0 C-3.

Microbiology

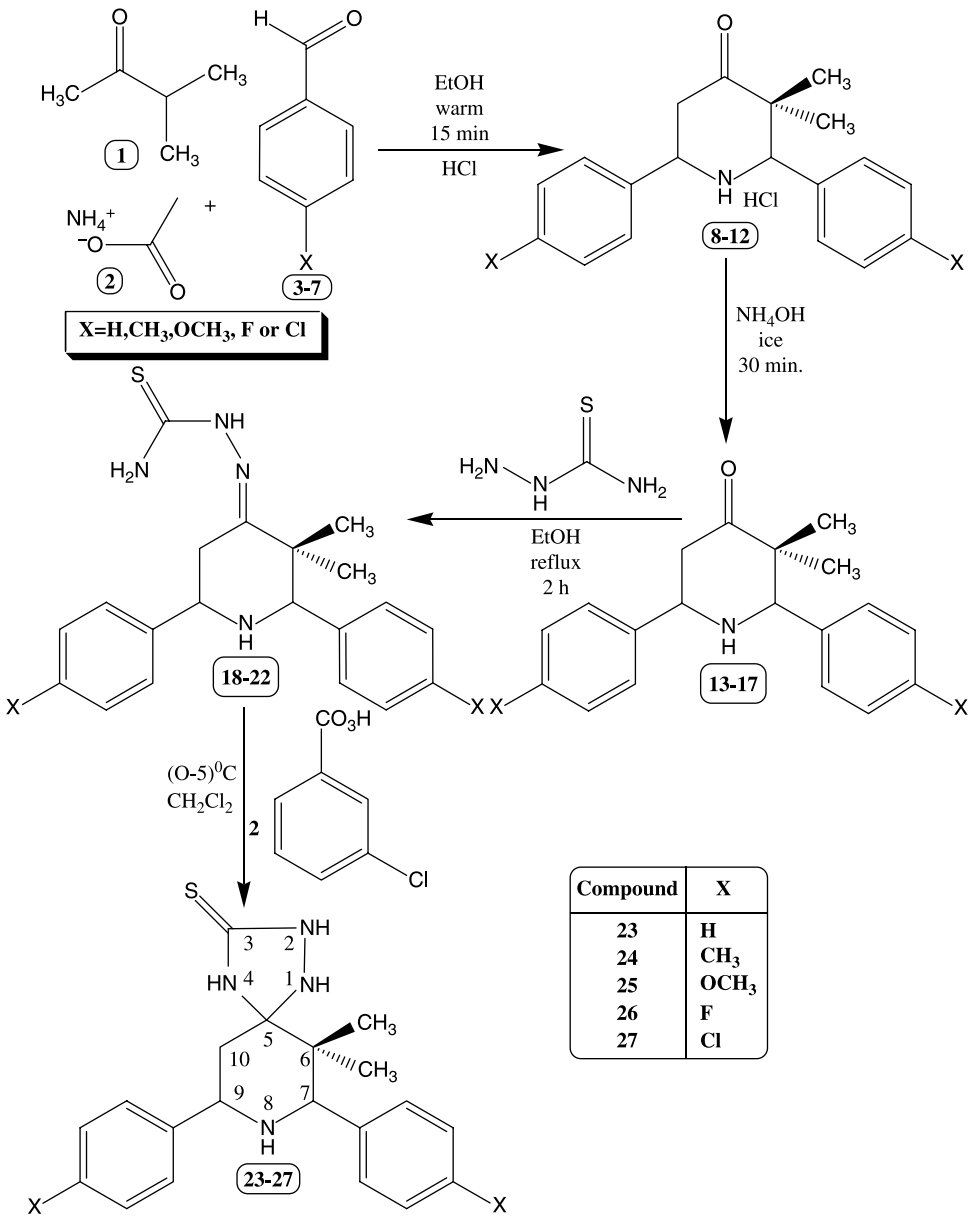
Materials. All the clinically isolated bacterial strains namely Staphylococcus aureus, β-Heamolytic streptococcus, Vibreo cholerae, Salmonella typhii, Shigella felxneri, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and fungal strains namely Aspergillus flavus, Mucor, Rhizopus and Microsporum gypsuem are obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity. The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB) (Hi-media, Mumbai) for fungi and nutrient broth (NB) (Hi-media, Mumbai) for bacteria by two-fold serial dilution method [27]. The respective test compounds **23–27** were dissolved in dimethylsulfoxide to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7 days old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4–10^5 cfu/mL. The final inoculums size was 10^5cfu/mL for antibacterial assay and $1.1–1.5 \times 10^2$ cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 72–96 h for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacteria studies and Fluconazole was used as standard for fungal studies.

Results and discussion

Chemistry

1,2,4-triazolidin-3-thione and their derivatives can be conveniently synthesized from aldehyde/ketone thiosemicarbazones and also from substituted thiosemicarbazide by cyclization using suitable reagents [28]. Earlier MnO_2 and H_2O_2 had been used for the synthesis of such compounds by the oxidative cyclization of thiosemicarbazones but the use of *m*-Chloroperbenzoic acid has provided better results. The schematic representation and the analytical data of compounds

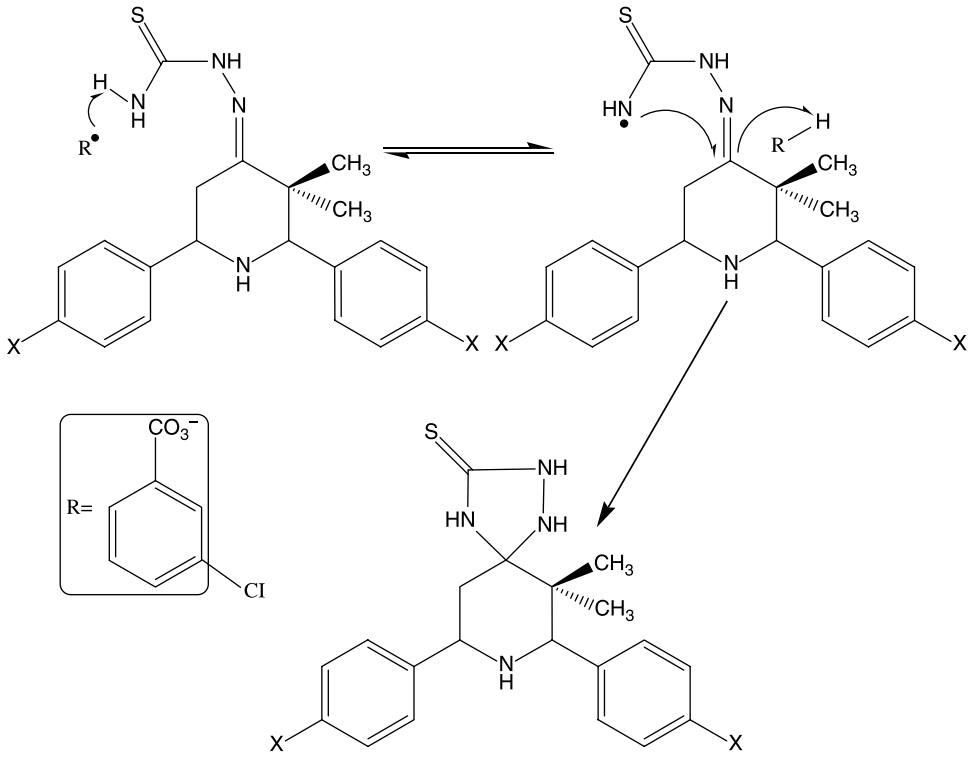


Scheme 2. Synthetic route for the formation of 7,9-diaryl-6,6-dimethyl-1,2,4,8-tetraazaspiro[4,5]-decan-3-thlones.

23–27 are given in Scheme 2 and Table I, respectively. Cyclocondensation reaction of respective ketone, aldehyde and ammonium acetate in the ratio of 1:2:1, respectively afforded the formation of 2,6-diaryl-3,3-dimethyl-piperidin-4-ones **13–17**. 2,6-diaryl-3,3-dimethyl-piperidin-4-ones are converted into their thiosemicarbazones **18–22** and are eventually oxidative cyclized with *m*-chloroperbenzoic acid at 0–5°C to afford 7,9-diaryl-6,6-dimethyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones **23–27**. The importance of the title compounds is due to their diverse potential, broad-spectrum biological activity. The structure of the newly

Table I. Physical and analytical data for 7,9-diaryl-6,6-dimethyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones **(23–27)**.

Compound	Yield (%)	m.p° C	Elemental analysis (%) C Found (calculated)	H Found (calculated)	N Found (calculated)	m/z (M^+) Molecular formula
23	55	119	68.10 (68.15)	6.82 (6.86)	15.85 (15.89)	353 $C_{20}H_{24}N_4S$
24	45	110	69.39 (69.44)	7.36 (7.42)	14.68 (14.72)	381 $C_{22}H_{28}N_4S$
25	50	112	64.01 (64.05)	6.80 (6.84)	13.54 (13.58)	413 $C_{22}H_{28}N_4O_2S$
26	60	127	61.78 (61.83)	5.66 (5.71)	14.38 (14.42)	389 $C_{20}H_{22}F_2N_4S$
27	50	121	56.97 (57.01)	5.21 (5.26)	13.25 (13.30)	421 $C_{20}H_{22}Cl_2N_4S$



Scheme 3. Proposed free radical mechanism for the conversion of thiosemicarbozones to the spiro-1,2,4-triazolidin-3-thiones.

synthesized compounds **23–27** is confirmed by melting point, elemental analysis, MS, FT-IR, one-dimensional NMR (^{1}H & ^{13}C) spectroscopic data. A free radical mechanism (Scheme 3) has been proposed for the conversion of thiosemicarbazones to the piperidinyl spiro-1,2,4-triazolidin-3-thiones.

Antibacterial activity

New spiro-1,2,4-triazolidin-3-thione derivatives, 7,9-diaryl-6,6-dimethyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones **23–27** were tested for their antibacterial activity *in vitro* against *S. aureus*, *β-H.streptococcus*, *V. cholerae*, *S. typhii*, *S. felxneri*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. Ciprofloxacin was used as standard drug. Minimum inhibitory concentration (MIC) in μg/mL values is reproduced in Table II. Compounds **24** and **25** exerted potent antibacterial activity against *S. aureus*, *β-H. streptococcus*, *E. coli* and *P. aeruginosa*.

Antifungal activity

The *in vitro* antifungal activity of piperidinyl spiro-1,2,4-triazolidin-3-thiones **23–27** was studied against the fungal strains *viz.*, *A.flavus, Mucor, Rhizopus* and *M.gypsuem*. Fluconazole was used as a standard drug. Minimum inhibitory concentration (MIC) in μg/mL values is reproduced in Table III. All the synthesized compounds **23–27** exerted strong *in vitro* antifungal activity against *Aspergillus flavus, Mucor* and *Rhizopus*.

Conclusion

The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesized piperidinyl spiro-1,2,4-triazolidin-3-thiones **23–27** are clearly known from Tables II and III. A close inspection of the *in vitro* antibacterial and antifungal activity profile in differently electron donating

Table II. *In vitro* antibacterial activity (MIC) values for compounds 23–27.

Micro organisms	Minimum Compound 23	Inhibitory Compound 24	Concentration Compound 25	(MIC) Compound 26	in μg/mL Compound 27	Ciprofloxacin
S.aureus	100	12.5	6.25	100	50	25
β-H. streptococcus	200	6.25	12.5	100	200	50
V. cholerae	100	200	100	200	100	50
S. typhii	200	100	200	100	100	50
S. felxneri	100	200	100	100	50	25
E. coli	100	12.5	12.5	50	100	25
K. pneumonia	100	100	100	100	50	50
P. aeruginosa	200	6.25	6.25	50	100	25

Table III. *In vitro* antifungal activity (MIC) values for compounds 23–27.

Micro organisms	Minimum Compound 23	Inhibitory Compound 24	Concentration Compound 25	(MIC) Compound 26	in μg/mL Compound 27	Fluconazole
A. flavus	25	50	50	12.5	6.25	50
Mucor	50	12.5	50	25	12.5	50
Rhizopus	50	25	12.5	12.5	6.25	25
M. gypsuem	100	50	50	100	100	25

(CH_3 and OCH_3) functional group substituted phenyl rings of novel piperidinyl spiro-1,2,4-triazolidin-3-thiones **24** and **25** exerted strong anti-bacterial activity against the tested bacterial strains *viz*. *S. aureus*, *β-H.streptococcus*, *E. coli* and *P. aeruginosa*. Results of the anti-fungal activity study show that the nature of substituents on the phenyl ring viz., methyl, methoxy, fluoro and chloro functions at the *para* positions of the aryl moieties are determinant for the nature and extent of the anti-fungal activity of all the synthesized compounds **23–27** over fungal strains namely *A.flavus, Mucor and Rhizopus*. The method of action of these compounds is unknown. These observations may promote a further development of our research in this field. Further development of this group of piperidinyl spiro-1,2,4-triazolidin-3-thiones may lead to compounds with better pharmacological profile than standard antibacterial and antifungal drugs.

Acknowledgements

Authors are thankful to NMR Research Centre, Indian Institute of Science, Bangalore for recording spectra. Two of our authors namely J.Thanusu and V.Kanagarajan are highly thankful for Annamalai University authorities for providing financial support in the form of Research Fellowship.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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