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Synthesis and study of antibacterial and antifungal activities of novel 8-methyl-7,9-diaryl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones

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Abstract

Some novel spiropiperidinyl-1,2,4-triazolidin-3-thiones have been synthesized and studied for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans*, *Candida-6, Candida-51*, *Aspergillus niger* and *Aspergillus flavus*. Compounds **30**–**32** exhibited potent in vitro antibacterial activity against *E. coli* and *P. aeruginosa* whereas the same set of compounds exerted potent in vitro antifungal activity against *Candida-6*, *A. niger* and *A. flavus*.

Keywords: Piperidin-4-one; 1,2,4-Triazolidin-3-thiones; Hydrogen peroxide; Antibacterial activity; Antifungal activity

1. Introduction

Over the past decades, the incidence of systemic microbial infections has been increasing dramatically due to an increase in the number of immuno-compromised hosts [1]. 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 [2]. 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 [3].

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 Candidiasis, Cryptococcosis and Aspergillosis. Antifungal azoles, fluconazole and itraconazole which are strong inhibitors of lanosterol 14α-demethylase (cytochrome $P450_{14DM}$) 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 [4–7]. 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-triaole based antibacterial and antifungal drugs are estazolam [8,9], alprazolam [10] and rizatriptane [11].

These observations places new emphasis on the need of as well as 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 [12–20] with a view to incorporate various other bioactive heterocyclic nucleus such as triazolidin-3-thione, benzimidazole, benzoxazole, Δ^2-1,3,4-thiadiazoline intact for evaluation of associated antibacterial and antifungal activities and also as a reagent for effecting functional group interconversion.

Synthesis of molecules, which are novel still resembling known biologically active molecules by virtue of the presence of some critical structural features, is an essential component of the search for new leads in drug designing programme. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a

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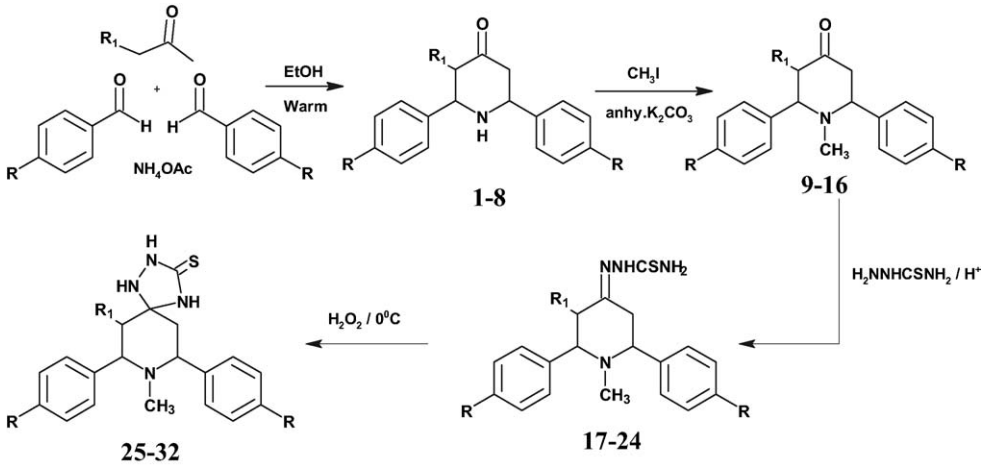


Scheme 1.

number of biologically active and medicinally useful molecules. In the interest of above, we planned to synthesize a system, which combines both bioactive piperidine and triazolidin-3-thione components together to give a compact structure like title compounds.

2. Chemistry

Triazoles and their derivatives can be conveniently synthesized from aldehyde/ketone thiosemicarbazones and also from substituted thiosemicarbazides by cyclization using suitable reagents [21].

Earlier MnO_2 [22], $FeCl_3{\cdot}6H_2O$ [23], H_2O_2 [24] and *m*-CPBA [25] were used to effect cyclization of steroidal and non-steroidal ketone thiosemicarbazones into corresponding 1,2,4-triazolidin-3-thiones. In our earlier communication [12], we found and reported that the use of MnO_2, $FeCl_3{\cdot}6H_2O$ to effect cyclization of 2,6-diaryl piperidin-4-one thiosemicarbazones results in mixture of 1,2,4-triazolidin-3-thiones and -3-ones. Use of *m*-CPBA was not also so successful as that resulted in mixture of 1,2,4-triazolidin-3-thione and *N*-hydroxy derivative along with other non-separable impurities. But cyclization using hydrogen peroxide at 0 °C results in exclusive formation of 1,2,4-triazolidin-3-thione in good yields.

The schematic representation and analytical data for the synthesized compounds **25**–**32** are furnished in Scheme 1 and Table 1, respectively. Condensation of respective ketone, aldehyde and ammonium acetate in the ratio of 1:2:1, respectively, afforded the formation of piperidin-4-ones 1–8. *N*-alkylation of piperidin-4-ones 1–8 using methyl iodide in the presence of anhydrous potassium carbonate as a base resulted in 1-methylpiperidin-4-ones. Use of methyl iodide over dimethyl sulfate for *N*-alkylation is found to be superior in terms of yield, handling and easy workup. 1-Methylpiperidones are converted into their thiosemicarbazones and are eventually cyclized into spiro-1,2,4-triazolidin-3-thiones using H_2O_2 at 0 °C.

Table 1
Analytical data of compounds **25**–**32**[a]

Entry	R_1	R	Yield (%)	M.p. (°C)
25	H	H	84	116
26	CH_3	H	76	109-110
27	H	CH_3	80	127-128
28	CH_3	CH_3	72	114-115
29	H	Cl	82	148
30	CH_3	Cl	73	129-130
31	H	OCH_3	80	104
32	CH_3	OCH_3	70	91

[a] The micro analysis values for C, H and N were within ± 4% of the theoretical values.

To comprehend structure activity relationship well, numberings of the target compound are done (Fig. 1).

Conversion of thiosemicarbazones into spiro-1,2,4-triazolidin-3-thiones involves a free radical mechanism (Scheme 2) and is consistent with a one already reported for similar cyclization [25]. The mode of cyclization takes up stereo chemical preferences.[1]

[1] There are three stereogenic centers in the case of compounds **25**, **27**, **29** and **31**. So normally one can expect eight stereoisomers. There are four stereogenic centers in the case of compounds **26**, **28**, **30** and **32**. Hence expectation of 16 stereoisomers is quite normal. But it had been well proved that mostly and favorably 2,6-diarylpiperidin-4-one ring adopts chair conformation with all its substituents at equatorial disposition [26]. Moreover equatorial disposition of phenyl group at C_2 and C_6 makes the chair conformation more rigid thereby preventing interconvertion of one chair from into another. During the mode of cyclization, trigonal (sp^2 carbon) carbon is converted into tetrahedral carbon (sp^3 carbon). Hence in the case of compounds **25**–**32**, only two diastereoisomers are now possible having NH.CS... group either at axial or equatorial orientation. Here only one diastereoisomer, in which NH.CS... group is *cis* with respect to 6α-H, was selectively obtained as evidenced by spectral data, which could be explained on the basis that during cyclization, the heterocyclic ring was preferably closed in such a way that NH.CS... group became equatorially attached to the C_5 to avoid steric effects due to C_6–CH_3 group and high-energy situation.

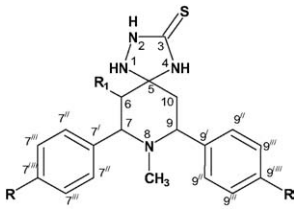
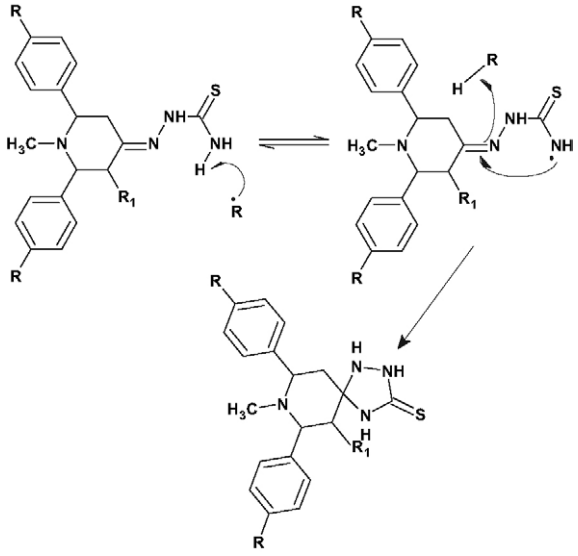


Fig. 1.



Scheme 2.

3. Results and discussion

Structure activity relationship results.

3.1. Antibacterial activity

All the synthesized novel spiro-piperidinyl heterocycles **25–32** were tested for their antibacterial activity in vitro against *Staphylococcus aureus* (NCLM-2492), *Bacillus subtilis* (NCLM-2439), *Escherichia coli* (NCLM-2345) and *Pseudomonas aeruginosa* (NCLM-2035). Penicillin and Streptomycin were used as standard drugs whose minimum inhibitory concentration (MIC) values were provided in Table 2.

Table 2
In vitro antibacterial activity of compounds **25–32**

Entry	MIC in μg ml^{-1}			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
25	100	100	50	100
26	100	100	50	100
27	100	100	50	50
28	100	50	50	100
29	25	50	50	25
30	25	25	25	25
31	50	25	25	25
32	25	25	25	25
Penicillin	12.5	25	50	50
Streptomycin	50	12.5	12.5	25

In general all the synthesized novel spiro-triazolidin-3-thiones **25–32** exerted a wide range of modest antibacterial activity in vitro against the tested organisms.

Compounds **25** and **26** without any substituent at para position of the aryl moieties at C_7 and C_9 positions of the six membered heterocyclic ring exhibited antibacterial activity in vitro at 100 μg ml^{-1} against all the tested organisms except *E. coli*. They inhibit *E. coli* at a MIC of 50 μg ml^{-1}. It is obvious from the Table 2 that substitution of methyl group for hydrogen at C_6 position in **25** (compound **26**) did not change the activity.

Introduction of methyl groups at the para position of the aryl moieties at C_7 and C_9 in **25** (compound **27**) produced twice the activity against *P. aeruginosa* whereas introduction of another methyl group at C_6 position in addition to the above methyl group in **25** (compound **28**) exhibited double the activity compared to **27** against *B. subtilis* alone. From this it is clear that methyl group modification at both the para position of the aryl moieties at C_7, C_9 and at C_6 position of heterocyclic ring did not result in enhancing the activity against rest of the bacterial strains *S. aureus* and *E. coli*.

Replacement of methyl group present at the para position of the aryl moieties at C_7 and C_9 of **27** (compound **29**) by chloro functionalities yielded good improvement in activity against all the tested organisms except *E. coli*. Substitution of methyl group at C_6 in **29** (compound **30**) for hydrogen exhibited remarkable antibacterial activity against all the tested organisms.

Instead of chloro functionalities, substitution of methoxy groups in **29** (compound **31**) results twofold increase in activity against *B. subtilis* and *E. coli* except *P. aeruginosa* whereas against *S. aureus*, the antibacterial activity was reduced by about 50%. Introduction of methyl group at C_6 position of **31** (compound **32**) exerted maximum activity at a MIC of 25 μg ml^{-1} against all the tested bacterial strains.

3.2. Antifungal activity

The in vitro antifungal activity of the synthesized novel spiro heterocyclic compounds **25–32** was studied against the fungal strains viz., *Candida albicans* (NCLM-C27), *Candida-6* (NCLM-C27), *Candida-51* (NCLM-C27), *Aspergillus niger* (NCLM-590) and *Aspergillus flavus* (NCLM-539). Amphotericin B was used as a standard drug whose MIC values are provided in Table 3.

Generally all the synthesized compounds exerted a wide range of modest in vitro antifungal activity against all the tested organisms except **25**, which failed to show activity against *Candida-6*, *Candida-51* and *A. flavus* and 26 against *Candida-51* even at a high concentration of 200 μg ml^{-1}.

The compound **25** without any substituent at the para position of the aryl groups present at C_2 and C_6 positions of the six membered heterocyclic moiety did not show in vitro antifungal activity even at a maximum concentration of 200 μg ml^{-1} against *Candida-6*, *Candida-51* and *A. flavus* while against *C. albicans* and *A. niger* registered activity at a

Table 3
In vitro antifungal activity of compounds **25**–**32**

Entry	MIC in μg ml^{-1}				
	C. albicans	*Candida-6*	*Candida-51*	*A. niger*	*A. flavus*
25	100	–	–	50	–
26	100	200	–	50	200
27	200	100	200	100	200
28	200	100	200	50	200
29	25	50	100	25	100
30	50	25	50	25	50
31	25	25	50	25	25
32	25	25	25	25	50
Amphotericin B	25	25	25	50	50

'–' No inhibition at 200 μg ml^{-1}.

MIC of 100 and 50 μg ml^{-1}, respectively. Introduction of methyl group at C_6 in **25** (compound **26**) did not enhance activity against all the tested fungal strains except *Candida-6* and *A. flavus* for which the inhibition is observed only at 200 μg ml^{-1}. This compound also failed to show antifungal activity against *Candida-51* even at 200 μg ml^{-1}.

By the introduction of methyl groups at the para position of the aryl moieties at C_7 and C_9 of **25** (compound **27**), antifungal activity was suppressed to half against *C. albicans* and *A. niger* whereas the activity was improved against *Candida-6* and *Candida-51*. Against *A. flavus* compound **27** recorded no change in activity when compared to **26**. Due to the substitution of methyl group at C_6 in **27** (compound **28**) the MIC's for *C. albicans*, *Candida-6, Candida-51* and *A. flavus* were not changed when compared to compound **27** whereas against *A. niger*, inhibition was observed at 50 μg ml^{-1}.

Due to the replacement of methyl groups by chloro functionalities at aryl moieties in compound **27** (compound **28**), a marked improvement in activity was noticed when compared to **27**. This antifungal activity of **29** was significantly promoted against *C. albicans*. Introduction of methyl group at C_6 position of compound **29** (compound **30**) caused a further enhancement in activity against *Candida-6, Candida-51* and *A. flavus* whereas against *A. niger*, there was no change in activity. The antifungal activity of **30** was reduced by 50% due to the introduction of methyl group at C_6 position of the heterocyclic moiety in **29**.

Substitution of methoxy groups in place of chloro functions in **29** and **30** led to compounds **31** and **32**. These two compounds exerted good antifungal activity in vitro at a MIC of 25–50 μg ml^{-1} against all the tested organisms. The compound **31** showed improved activity against *C. albicans* and *A. flavus* whereas against remaining fungal strain no change in activity was observed compared to that of compound **30**.

On comparison with **31**, enhanced activity was observed by methyl group modification at C_6 position in **31** (compound **32**) against *Candida-51* while against rest of the organisms no change in activity was resulted with the exception of *A. flavus*, which recorded diminished antifungal activity.

4. Conclusion

A close examination of the in vitro antibacterial and antifungal activity profile in differently substituted novel spirotriazolidinethiones against the tested bacterial strains viz. *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* and the fungal strains viz. *C. albicans*, *Candida-6*, *Candida-51*, *A. niger* and *A. flavus*, respectively, provides a better structure activity relationship correlate, which may be summarized as follows.

The compounds with chloro (or) methoxy functions at the para position of the aryl moieties present at C_7 and C_9 positions of the six membered heterocyclic ring along with/without methyl group at C_6 play an important role in eliciting its antibacterial and antifungal potency.

Furthermore, among the novel spiroheterocycles tested, the compounds with methoxy group at the para position of the aryl moieties at C_7 and C_9 positions of heterocyclic moiety was found to be superior in inhibiting all the bacterial strains than chloro modification at the same position. But the introduction of methyl group at C_6 position in the above said compounds exerted an improved inhibition against all the tested bacterial strains particularly against *E. coli*.

The novel spiropiperidinyl heterocycles with chloro functions at the para position of the aryl moieties along with methyl group modification at C_6 position and the compounds with methoxy functions in place of chloro functionalities along with/without methyl modification at C_6 position exhibited well pronounced inhibition against *A. niger* and *Candida-6* fungal strains.

From this study it is evident that compounds **30**–**32** are showing promising activity when compared to penicillin towards inhibiting *E. coli* and *P. aeruginosa* bacterial strains. Similarly, the same set of compounds is also comparatively better than amphotericin B against *A. niger* in terms of activity.

Results of this study show that the nature of substituent on the phenyl ring is determinant, besides a methyl a group at C_6, for the nature and extent of the activity of the synthesized compounds, which might have influences on their inhibiting mechanism of actions. The effect of the C_6 methyl group on SAR may be due to its effect on stereo chemical preferences

on the piperidine ring. The method of action of these compounds is unknown. Theses observations may promote a further development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection.

5. Experimental

5.1. Microbiology

5.1.1. Materials

The bacterial strains *E. coli* (NCLM-2345), *P. aeruginosa* (NCLM-2035), *S. aureus* (NCLM-2492), *B. subtilis* (NCLM-2439) and antifungal strains *C. albicans* (NCLM-C27), *Candida-6* (NCLM-C27), *Candida-51* (NCLM-C27), *A. niger* (NCLM-590), *A. flavus* (NCLM-539) are procured from National Chemical Laboratory, Pune, India.

5.1.2. 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 the twofold serial dilution method [28]. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg ml^{-1} stock solutions. 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 10^2–10^5 cfu ml^{-1}. The final inoculum size was 10^5 cfu ml^{-1} for antibacterial assay and $1.1–1.5 \times 10^2$ cfu ml^{-1} for antifungal assay. Testing was performed at pH 7.4 ± 0.2. 0.2 ml of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on till six such dilution were obtained. A set of assay tubes containing only inoculated broth was kept as control and likewise solvent controls were also sun simultaneously. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The MICs were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Penicillin, streptomycin and amphotericin B were used as standards.

5.2. Chemistry

The reactions and the purity of the products were assessed by performing TLC. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Perkin–Elmer 297 IR spectrophotometer and note worthy absorption values (reciprocal centimeter) alone are listed. ^{1}H-NMR spectra were recorded at 400 MHz on Bruker amx 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. ^{13}C-NMR spectra were recorded at 125 MHz on Brucker amx 400 MHz spectrophotometer using $CDCl_3$. The mass spectra were recorded on a VG analytical 7070E instrument equipped with VG 11-250 data acquisition system. Satisfactory micro analysis were obtained on Carlo Erba 1106 and Perkin Elmer models 240 CHN analyzer.

By adopting the literature precedent [27], 2,6-diarylpiperidin-4-ones 1-8 were prepared by the condensation of appropriate ketones, aldehydes and ammonium acetate in 1:2:1 ratio.

5.2.1. General method of preparation of 1-methyl-2,6-diarylpiperidin-4-one thiosemicabazones (**17–24**)

A mixture of 1-methyl-2,6-diarylpiperidin-4-one (0.01 mol) and thiosemicarbazide (0.01 mol) in methanol (45 ml) was refluxed for 3 h in acidic medium 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 methanol to give 1-methyl-2,6-diarylpiperidin-4-one thiosemicarbazone as crystalline solid.

5.2.2. 8-Methyl-7,9-diphenyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thiones (**25**)

A solution of 1-methyl-2,6-diphenylpiperidin-4-one thiosemicarbazone 17 (0.005 mol) in chloroform (40 ml) was treated with excess of hydrogen peroxide (30%, 20 ml) and stirred for 3 h at 0 °C. After the completion of reaction, the organic layer was separated and dried over anhydrous sodium sulfate. The solvents were removed under reduced pressure. The crude product thus obtained was purified over silica gel column (pet ether/ethyl acetate, 5:2) and the product 25 was obtained as needles when it was recrystallized from methanol.

IR (KBr) (cm^{-1}): 3338, 3316 (N–H), 2361, 1609, 1490, 1452, 1346, 1258 (C=S), 1210, 1067, 1026, 938, 905, 856, 809, 752, 695, 664, 602, 593, 555, 525; Mass (*m*/*z*): 338(M^+), 323, 294, 278, 263, 209, 194, 179, 159, 145, 133, 120, 105 (100%), 91, 77, 65, 51; ^{1}H-NMR (δ ppm): 3.60(dd, ^3J = 12.11 Hz; 3.23 Hz, 2H, H_{7a}, H_{9a}); 2.20–2.40 (m, 4H, H_{6a}, H_{10a}, H_{6e}, H_{10e}); 7.28–7.50 (m, 10H, aryl protons); 8.45 (b.s, 2H, NHCSNH); 7.13 (s, 1H, NH at 1); 1.63 (s, 3H, NCH_3); ^{13}C-NMR (δ ppm): 66.034 (C_7, C_9); 39.349 (C_6, C_{10}); 77.789(C_5); 180.984 (*C*=S); 41.010 (NCH_3); 124.934, 126.714, 127.263, 147.824 (aryl carbons).

The compounds **26–32** were synthesized similarly.

*5.2.2.1. 6,8-Dimethyl-7,9-diphenyl-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**26**).* IR (KBr) (cm^{-1}): 3424, 3341, 3310 (N–H), 2358, 1567, 1490, 1452, 1376, 1350, 1265 (C=S), 1073, 842, 790, 751, 696, 665, 632, 524; Mass (*m*/*z*): 352(M^+), 337, 308, 292, 277, 223, 207, 194, 179, 159, 146, 132, 118 (100%), 104, 91, 77, 65, 55, 51; ^{1}H-NMR (δ ppm):

3.61(dd, ^{3}J = 12.35 Hz; 3.01 Hz, 1H, H_{9a}); 3.19(d, ^3J = 11.10 Hz, 1H, H_{7a}); 2.22–2.47 (m, 3H, H_{6a}, H_{10a}, H_{10e}); 7.17–7.46 (m, 10H, aryl protons); 8.40 (b.s, 2H, NHCSNH); 6.90 (s, 1H, NH at 1); 1.59 (s, 3H, NCH_3); 0.81 (d, J = 6.53 Hz, 3H, CH_3 at 6); ^{13}C-NMR (δ ppm): 72.212 (C_7); 65.888 (C_9); 39.561 (C_6); 37.897 (C_{10}); 78.398(C_5); 181.683 (*C*=S); 42.621 (NCH_3); 124.620, 126.131, 126.471, 127.290, 127.572, 139.976, 146.436 (aryl carbons); 12.940 (CH_3 at 6).

*5.2.2.2. 8-Methyl-7,9-bis(p-methylphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**27**).* IR (KBr) (cm^{-1}): 3365, 3306 (N–H), 2345, 1602, 1502, 1463, 1341, 1265 (C=S), 1210, 1026, 998, 920, 895, 821, 800, 764, 692, 671, 613, 542, 524, 490; Mass (*m*/*z*): 366(M$^+$), 351, 322, 306, 291, 237, 217, 207, 173, 147, 120, 118 (100%), 91, 65, 50; ^{1}H-NMR (δ ppm): 3.63(dd, ^3J = 12.12 Hz; 3.24 Hz, 2H, H_{7a}, H_{9a}); 2.15–2.38 (m, 4H, H_{6a}, H_{10a}, H_{6e}, H_{10e}); 7.25, 7.35 (2d, 8H, aryl protons); 8.47 (b.s, 2H, NHCSNH); 7.13 (s, 1H, NH at 1); 2.42 (s, 6H, aryl CH_3); 1.62 (s, 3H, NCH_3); ^{13}C-NMR (δ ppm): 65.326 (C_7, C_9); 38.447 (C_6, C_{10}); 77.814(C_5); 180.518 (*C*=S); 40.982 (NCH_3); 127.848, 128.526, 136.976, 146.436 (aryl carbons); 20.582 (aryl CH_3).

*5.2.2.3. 6,8-Dimethyl-7,9-bis(p-methylphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**28**).* IR (KBr) (cm^{-1}): 3426, 3312 (N–H), 2346, 1581, 1498, 1472, 1420, 1381, 1320, 1268 (C=S), 1201, 1132, 1026, 963, 890, 526, 772, 720, 680, 626; Mass (*m*/*z*): 380(M$^+$), 365, 336, 320, 305, 251, 237, 235, 173, 160, 146, 132 (100%), 118, 91, 65, 51; ^{1}H-NMR (δ ppm): 3.59(dd, ^3J = 12.34 Hz; 3.02 Hz, 1H, H_{9a}); 3.20(d, ^3J = 11.09 Hz, 1H, H_{7a}); 2.22–2.46 (m, 3H, H_{6a}, H_{10a}, H_{10e}); 7.28–7.42 (m, 8H, aryl protons); 8.39 (b.s, 2H, NHCSNH); 6.91 (s, 1H, NH at 1); 2.45 (s, 6H, aryl CH_3); 1.58 (s, 3H, NCH_3); 0.82(d, J = 6.53 Hz, 3H, CH_3 at 6); ^{13}C-NMR (δ ppm): 71.478 (C_7); 66.104 (C_9); 39.618 (C_6); 38.028 (C_{10}); 78.434(C_5); 181.414 (*C*=S); 41.432 (NCH_3); 128.314, 128.522, 129.779, 130.615, 135.679, 137.851, 138.648, 146.135 (aryl carbons); 20.995 (aryl CH_3); 12.889 (CH_3 at 6).

*5.2.2.4. 8-Methyl-7,9-bis(p-chlorophenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**29**).* IR (KBr) (cm^{-1}): 3328, 3296 (N–H), 2396, 1601, 1451, 1435, 1376, 1246 (C=S), 1185, 1072, 1036, 978, 923, 891, 820, 800, 673, 606, 593, 560; Mass (*m*/*z*): 406(M$^+$), 391, 362, 346, 331, 277, 247, 237, 193, 166, 139 (100%), 111, 95, 75, 65, 50; ^{1}H-NMR (δ ppm): 3.64(dd, ^3J = 12.12 Hz; 3.23 Hz, 2H, H_{7a}, H_{9a}); 2.18–2.42 (m, 4H, H_{6a}, H_{10a}, H_{6e}, H_{10e}); 7.31, 7.37 (2d, 8H, aryl protons); 8.47 (b.s, 2H, NHCSNH); 7.08 (s, 1H, NH at 1); 1.59 (s, 3H, NCH_3); ^{13}C-NMR (δ ppm): 65.195 (C_7, C_9); 38.251 (C_6, C_{10}); 77.771(C_5); 180.622 (*C*=S); 40.532 (NCH_3); 128.251, 128.404, 133.201, 146.744 (aryl carbons).

*5.2.2.5. 6,8-Dimethyl-7,9-bis(p-chlorophenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**30**).* IR (KBr) (cm^{-1}): 3433, 3327, 3293 (N–H), 2393, 1586, 1486, 1472, 1360, 1346, 1242 (C=S), 1090, 982, 882, 801, 710, 693, 620; Mass (*m*/*z*): 420(M$^+$), 405, 376, 360, 291, 277, 247, 193, 180, 166, 140, 138 (100%), 111, 75, 65, 55, 50; ^{1}H-NMR (δ ppm): 3.61(dd, ^3J = 12.35 Hz; 3.01 Hz, 1H, H_{9a}); 3.21(d, ^3J = 11.14 Hz, 1H, H_{7a}); 2.22–2.50 (m, 3H, H_{6a}, H_{10a}, H_{10e}); 7.33–7.45 (m, 8H, aryl protons); 8.45 (b.s, 2H, NHCSNH); 6.86 (s, 1H, NH at 1); 1.60 (s, 3H, NCH_3); 0.81(d, J = 6.56 Hz, 3H, CH_3 at 6); ^{13}C-NMR (δ ppm): 71.317 (C_7); 65.892 (C_9); 39.433 (C_6); 37.852 (C_{10}); 78.461(C_5); 181.427 (*C*=S); 41.014 (NCH_3); 127.287, 128.725, 128.992, 131.360, 131.987, 133.921, 138.923, 146.446 (aryl carbons); 12.931 (CH_3 at 6).

*5.2.2.6. 8-Methyl-7,9-bis(p-methoxyphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**31**).* IR (KBr) (cm^{-1}): 3361, 3304 (N–H), 2361, 1596, 1474, 1442, 1370, 1268 (C=S), 1230, 1082, 1029, 943, 914, 881, 838, 796, 678, 620, 586, 543; Mass (*m*/*z*): 398(M$^+$), 383, 354, 338, 323, 267, 239, 189, 163, 134 (100%), 117, 107, 91, 75, 65, 50; ^{1}H-NMR (δ ppm): 3.62(dd, ^3J = 12.09 Hz; 3.20 Hz, 2H, H_{7a}, H_{9a}); 2.14–2.38 (m, 4H, H_{6a}, H_{10a}, H_{6e}, H_{10e}); 6.89, 7.34 (2d, 8H, aryl protons); 8.44 (b.s, 2H, NHCSNH); 7.18 (s, 1H, NH at 1); 3.93 (s, 6H, aryl OCH_3); 1.61 (s, 3H, NCH_3); ^{13}C-NMR (δ ppm): 65.347 (C_7, C_9); 38.474 (C_6, C_{10}); 77.760(C_5); 180.671 (*C*=S); 40.631 (NCH_3); 115.545, 127.841, 141.900, 158.539 (aryl carbons); 54.516 (aryl OCH_3).

*5.2.2.7. 6,8-Dimethyl-7,9-bis(p-methoxyphenyl)-1,2,4,8-tetraazaspiro[4.5]decan-3-thione (**32**).* IR (KBr) (cm^{-1}): 3416, 3372, 3298 (N–H), 2367, 1580, 1491, 1463, 1390, 1340, 1262 (C=S), 1118, 1020, 941, 846, 797, 660, 618; Mass (*m*/*z*): 412(M$^+$), 397, 368, 352, 337, 283, 267, 247, 239, 189, 162, 148 (100%), 135, 117, 107, 75, 65, 55, 50; ^{1}H-NMR (δ ppm): 3.60(dd, ^3J = 12.36 Hz; 2.97 Hz, 1H, H_{9a}); 3.20(d, ^3J = 11.14 Hz, 1H, H_{7a}); 2.20–2.47 (m, 3H, H_{6a}, H_{10a}, H_{10e}); 7.35–7.37, 6.83–6.85 (m, 8H, aryl protons); 8.47 (b.s, 2H, NHCSNH); 6.98 (s, 1H, NH at 1); 3.94 (s, 6H, aryl OCH_3); 1.59 (s, 3H, NCH_3); 0.80(d, J = 6.57 Hz, 3H, CH_3 at 6); ^{13}C-NMR (δ ppm): 71.476 (C_7); 66.033 (C_9); 39.615 (C_6); 38.115 (C_{10}); 78.450(C_5); 181.468 (*C*=S); 41.142 (NCH_3); 114.381, 115.892, 128.012, 130.952, 134.743, 141.683, 157.051, 159.236 (aryl carbons); 54.793 (aryl OCH_3); 13.008 (CH_3 at 6).

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