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# SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF NOVEL [N-ACETYL-2,6-DIARYLPIPERIDIN-4-YL]-5-SPIRO-4-ACETYL-2-(ACETYLAMINO)-$\Delta^2$-1,3,4-THIADIAZOLINES

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**Abstract.** Some novel spiropiperidinyl thiadiazolines have been synthesized and their bacterial activity against *Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klepsiella pneumoniae* and antifungal activity against *Cryptococcus neoformans, Candida-6, Candida-51, Aspergillus niger* and *Aspergillus flavus* were evaluated. Compound **23** exhibited potent antibacterial activity *in vitro* against *Klepsiella pneumoniae* while compound **24** exerted potent antifungal activity *in vitro* against *Cryptococcus neoformans*.

## Introduction

2,6-Disubstituted piperidines form a biologically important class of compounds due to their diverse pharmacological activities and their presence in a wide variety of alkaloids.[1] The piperidine nucleus can frequently be recognized in the structure of numerous naturally occuring alkaloids and synthetic compounds with interesting biological and pharmacological properties. As a consequence, the development of general methods for the synthesis of piperidine derivatives has been the subject of considerable synthetic efforts.[2,3]

The 1,3,4-Thiadiazoline nucleus is also a biologically active heterocyclic ring, which is associated with wide range of pharmacological activities[4]. 1,3,4-Thiadiazolines / 1,3,4-thiadiazoles are shown to exhibit antibacterial[5], diuretic[5], antifungal[6], antiinflammatory[7], herbicidal[8], antiviral[9],

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plant growth regulatory[10], hypotensive[11], CNS depressant[11] and carbonic anhydrase inhibitory[12] activities.

An essential component of the search for new leads in a drug-design programme is the synthesis of molecules, which are novel and resemble known biologically active molecules by virtue of the presence of certain pharmacophoric groups. Certain small heterocylic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules. Recently our group exploited the synthesis of several 2,6-diarylpiperidin-4-one derivatives with an attempt to incorporate various other bioactive heterocyclic systems such as 1,2,4-triazolidin-3-thione, 1,3,4-thiadiazoline, benzimidazole and benzoxazole due to their biological importance and also for their effect on functional group interconversion[13-20].

Hence the scope of the present investigation is the synthesis of a new, novel system, which combines both bioactive piperidine and $\Delta^2$-1,3,4-thiadiazoline components to give a compounds of expected antibacterial and antifungal activities. Several substituted analogues have been synthesized to evaluate the *in vitro* antibacterial and antifungal structure activity relationship.

## Results and Discussion

### Chemistry

The synthesized compounds are shown in **Scheme 1**. The piperidin-4-one thiosemicarbazones, obtained from the corresponding piperidin-4-ones,[25] upon cyclization under acetylating condition using acetic anhydride, afforded the formation of [N-acetyl-2,6-diarylpiperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazolines. Acylation of aldehyde / ketone thiosemicarbazones followed by peracid oxidation has been reported as a route to 1,3,4-thiadiazoles[21]. The intermediate acylation products were once considered to be the $N^4$,S-diacyl derivatives. Since the discovery that the diacyl compounds are infact the cyclized 4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazolines[22] and not the $N^4$, S-diacyl derivatives as previously thought,

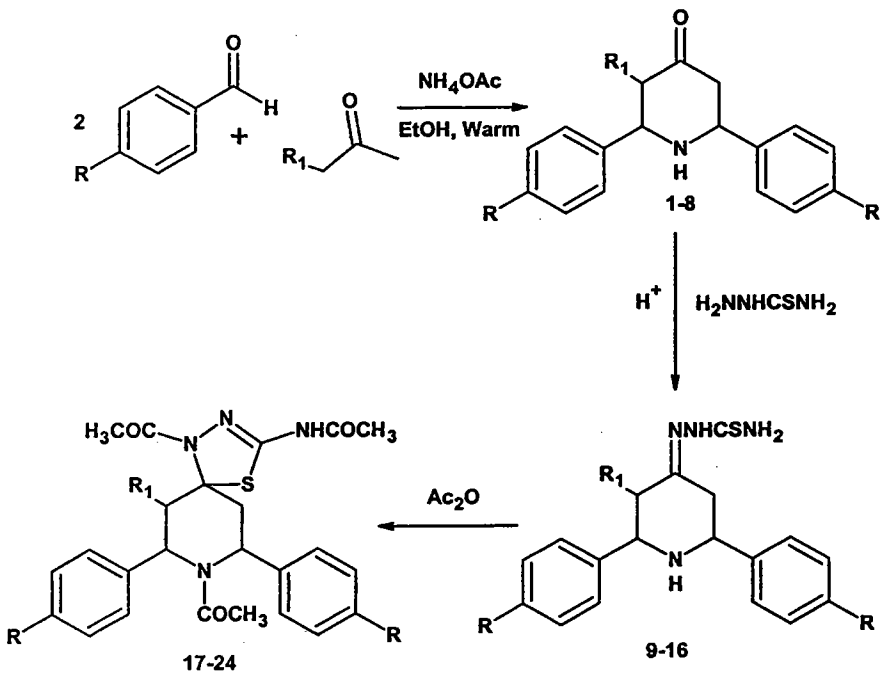
only a few reports are available for the construction of $\Delta^2$-1,3,4-thiadiazolines under acetylating conditions.[23] The present investigation also adds to the fact that the cyclization of heterocyclic ketone thiosemicarbazones under acylation condition results in the formation of $\Delta^2$-1,3,4-thiadiazolines.

The mechanism for the formation of $\Delta^2$-1,3,4-thiadiazolines may be explained on the basis of hard and soft acid and base principle. The harder acetylating reagents react with the harder nitrogen atom rather than softer the sulfur atom and this acylation favours the cyclization of thiosemicarbazones exclusively to $\Delta^2$-1,3,4-thiadiazolines **Scheme 2.**

**Pharmacology**

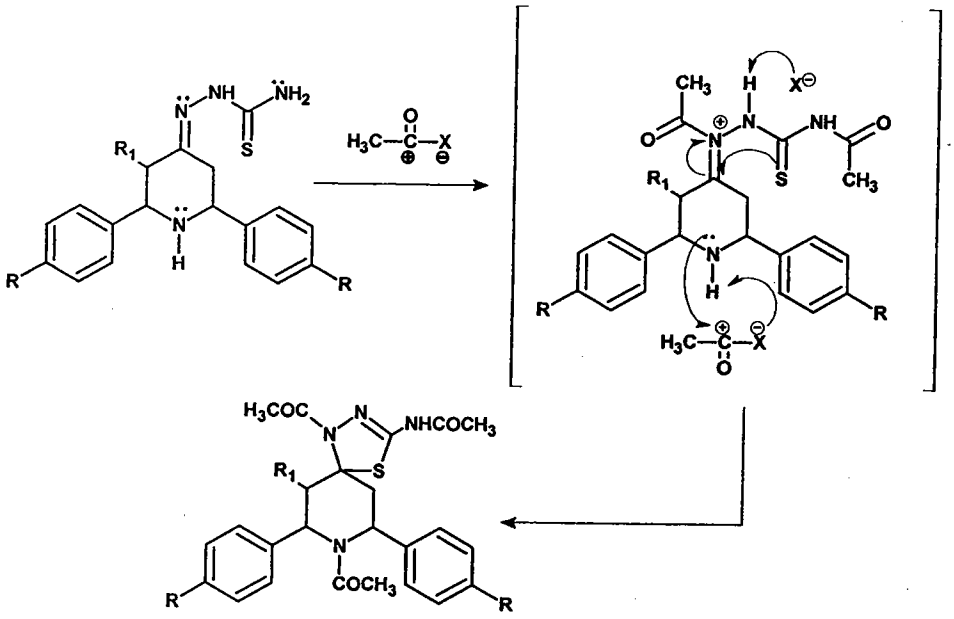
***In vitro* antibacterial and antifungal sensitivity**

The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB) for fungi and Nutrient broth (NB) for bacteria by the two-fold serial dilution method[24]. The test compounds were dissolved in DMSO (dimethylsulphoxide) to obtain 1 mg/mL stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar at 37°C while fungal spores from 24 h-7 days old Sabourauds agar 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 per mL. 0.2 mL of the solution of each compound was added to 1.8 mL of seeded broth to form the first dilution. One mL of the first concentration was diluted with a further 1 mL of the seeded broth to give the second dilution and so on till six dilutions were obtained. A set of assay tubes containing only seeded broth were kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in BOD incubators at 37°C for bacteria and 28°C 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. Penicillin, Streptomycin and Amphotericin B were used as standards.




Scheme 1

| R | $R_1$ | Compd. |
|---|---|---|
| H | H | 1, 9, 17 |
| H | $CH_3$ | 2, 10, 18 |
| $CH_3$ | H | 3, 11, 19 |
| $CH_3$ | $CH_3$ | 4, 12, 20 |
| Cl | H | 5, 13, 21 |
| Cl | $CH_3$ | 6, 14, 22 |
| $OCH_3$ | H | 7, 15, 23 |
| $OCH_3$ | $CH_3$ | 8, 16, 24 |



Scheme 2

## Results

### 1. Antibacterial Activity

All the synthesized novel spiropiperidinyl-$\Delta^2$-1,3,4-thiadiazolines **17-24** were tested for their antibacterial activities *in vitro* against *Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klepsiella pneumoniae*. Penicillin and Streptomycin were used as standard drugs whose minimum inhibitory concentration (MIC) values were furnished in **Table 2.**

In general all the synthesized novel spiropiperidinyl-$\Delta^2$-1,3,4-thiadiazolines exerted a wide range of modest *in vitro* antibacterial activity against all the tested organisms except **17,** which failed to show effects against *S.faecalis* and **17,18** which both failed against *P.aeruginosa* and *K.pneumoniae*. Compounds **17, 18,** without any substituent at the *para* position of the aryl moieties at $C_2$ and $C_6$ positions of the six-membered heterocyclic moiety exhibited antibacterial activity *in vitro* at 100 μg/mL against *B. subtilis* whereas against *E.coli* it showed a MIC of 50 μg/mL. Replacement of hydrogen by a methyl group at $C_3$ position in **17,** compound **18** did not

change the activity against all the tested organisms but against *S. faecalis*, a slight improvement in activity resulted.

Introduction of methyl groups at both the *para* positions of the aryl moieties at $C_2$ and $C_6$ in **17**, compound **19** yielded small improvements in the activity against most test organisms while showing decreased activity against *E.coli*. Introduction of another methyl group at $C_3$ position of the six-membered piperidine ring along with the above methyl group in **17**, compound **20** exhibited double the activity against all the tested bacterial strains compared to **19**. Further more **20** showed good improvement in activity against *K.pneumoniae*.

Replacement of methyl group of the *para* position of the aryl moieties at $C_2$ and $C_6$ by chloro functionalities showed further improvement in the activity. The compounds **21** and **22** showed MIC in the range of 12.5 μg/mL to 50 μg/mL against all the tested organisms, particularly against *S.faecalis* and *K.pneumoniae* which exhibited maximum activity. Replacement of hydrogen by methyl group at $C_3$ in **21**, compound **22** results in double the activity compared to **21** against all except *B. subtilis* and *P. aeruginosa*.

**Table :1 Chemical data for compounds 17-24[a]**

| Compd. | Yield (%) | m.p[b] (°C) | Molecular Formula |
|---|---|---|---|
| **17** | 74 | 180-84 | $C_{24}H_{26}N_4O_3S$ |
| **18** | 61 | 164-67 | $C_{25}H_{28}N_4O_3S$ |
| **19** | 71 | 187-90 | $C_{26}H_{30}N_4O_3S$ |
| **20** | 69 | 169-72 | $C_{27}H_{32}N_4O_3S$ |
| **21** | 70 | 209-12 | $C_{24}H_{24}N_4O_3SCl_2$ |
| **22** | 67 | 203-06 | $C_{25}H_{26}N_4O_3SCl_2$ |
| **23** | 73 | 166-69 | $C_{26}H_{30}N_4O_5S$ |
| **24** | 66 | 155-58 | $C_{27}H_{32}N_4O_5S$ |

[a]The elemental analysis value for C, H and N were within ±0.4 the theoritical values

[b]Decomposition point

Introduction of methoxy groups in the place of chloro functionalities in **21** and **22**, compounds **23** and **24** respectively, resulted in decreased activity against *P. aeruginosa*, whereas there was an improvement in the activity against *B. subtilis* and *K. pneumoniae*. Compounds **23** and **24** exhibited MIC ranging from 6.25 μg/mL to 25 μg/mL against all the tested organisms except *P. aeruginosa*. On comparison with **23**, introduction of methyl group at $C_6$ position in **23**, compound **24**, resulted in double the activity against *P. aeruginosa* and *K. pneumoniae*. Compound **24** showed maximum antibacterial activity at a MIC of 6.25 μg/mL against *K. pneumoniae*.

**Table :2 *In vitro* antibacterial activity of compounds 17-24**

| Compd. | Minimum inhibitory concentration (MIC) in μg/mL | | | | |
|---|---|---|---|---|---|
| | *S.faecalis* | *B.subtilis* | *E.coli* | *P.aeruginosa* | *K.pneumoniae* |
| **17** | - | 100 | 50 | - | - |
| **18** | 100 | 100 | 50 | - | - |
| **19** | 100 | 100 | 100 | 100 | 100 |
| **20** | 50 | 100 | 50 | 50 | 25 |
| **21** | 25 | 50 | 50 | 25 | 25 |
| **22** | 12.5 | 50 | 25 | 25 | 12.5 |
| **23** | 25 | 25 | 25 | 50 | 12.5 |
| **24** | 25 | 25 | 25 | 25 | 6.25 |
| Penicillin | 12.5 | 25 | 50 | 50 | 12.5 |
| Streptomycin | 50 | 12.5 | 12.5 | 25 | 25 |

'-' no inhibition at 100 μg/mL

## 2. Antifungal activity

The *in vitro* antifungal activity of fungal strains viz., *Cryptococcus neoformans, Candida-6, Candida-51, Aspergillus niger* and *Aspergillus flavus*. Amphotericin B was used as a standard drug whose minimum inhibitory concentration values are furnished in **Table 3**.

Generally all the synthesized novel spiropiperidinyl-$\Delta^2$-1,3,4- thiadiazolines **(17-24)** exhibited a wide range of modest *in vitro* antifungal activity against the tested organisms. Compounds **17** and

**18** without any substituent at $C_2$ and $C_6$ positions of the six-membered piperidine moiety, exerted no significant growth inhibition of strains *C. neoformans, Candida-6* and *Candida-51* even at a high concentration of 100 μg/mL. Compound **17**, against *A.flavus*, showed no noticeable fungal growth inbibition at this concentration. Replacement of hydrogen by a methyl group at $C_3$ position in **17**, compound **18**, did not change the activity against all the tested organisms except *A. niger.*

**Table 3: *In vitro* antifungal activity of compounds 17-24**

| Compd. | Minimum inhibitory concentration (MIC) in μg/mL against fungi | | | | |
|---|---|---|---|---|---|
| | *C.neoformans* | *Candida- 6* | *Candida- 51* | *A.niger* | *A.flavus* |
| **17** | - | - | - | 100 | - |
| **18** | - | - | - | 50 | 100 |
| **19** | - | 50 | 100 | 50 | - |
| **20** | - | 50 | 100 | 50 | 100 |
| **21** | 25 | 50 | 50 | 25 | 50 |
| **22** | 12.5 | 25 | 25 | 25 | 50 |
| **23** | 12.5 | 25 | 50 | 25 | 25 |
| **24** | 6.25 | 50 | 25 | 25 | 25 |
| Amphotercin B | 25 | 25 | 25 | 50 | 50 |

'-' no inhibition at 100 μg/mL

Due to the introduction of methyl groups at the *para* position of the aryl moieties at $C_2$ and $C_6$ in **17**, compound **19**, no significant activity improvement was observed against *C.neoformans* and *A. flavus*. But good improvement in activity was observed against *Candida-6, Candida-51* and *A. niger* by this modification. Replacement of hydrogen at $C_3$ in **19** by a methyl group, compound **20**, did not show appreciable change in the activity against the tested organisms. But compound **19**, which lacked any significant inhibition, even at a high concentration of 100 μg/mL against *C.neoformans* and *A. flavus*, showed inhibition at this concentration against *A. flavus* by the methyl modification.

Replacement of methyl groups in **19** by chloro functions, compound **21,** caused further improvement in the activity against all the tested fungi. With the exception of *A. niger*, the introduction of methyl group at $C_3$ in compound **22,** resulted in a two-fold increase in activity. Against *C. neoformans* compound **22** exerted maximum activity.

Introduction of methoxy groups in the place of chloro functionalities in **21** and **22** led to compounds **23** and **24**. These two compounds showed promising antifungal activities inferred in terms of their MICs over rest of the compounds. Compound **24** is more potent against *C. neoformans* as evidenced by its growth inhibition at a MIC of 6.25 μg/mL. The antifungal activity of compound **24** was reduced by fifty percent when compared to **23** due to the introduction of methyl group at the $C_3$ position. Increased activity against *C. neoformans* with no significant change in activity against the other organisms are observed by the introduction of a methyl group at $C_3$ position.

**Conclusion**

An examination of the *in vitro* antibacterial and antifungal activity profile of different substituted spiropiperidinyl-$\Delta^2$-1,3,4-thiadiazolines against the bacterial strains viz., *Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klepsiella pneumoniae* and the fungal strains viz., *Cryptococcus neoformans, Candida-6, Candida-51, Aspergillus niger* and *Aspergillus flavus*, respectively provide a structure activity correlate, which may be summarized as follows. Compound **23** exhibited potent *in vitro* antibacterial activity against *Klepsiella pneumoniae* while compound **24** exerted potent *in vitro* antifungal activity against *Cryptococcus neoformans.*

The compounds with chloro or methoxy functions at the *para* position of the aryl moieties present at $C_2$ and $C_6$ positions of the six membered piperidine moiety exhibited pronounced inhibition of the above described bacterial and fungal strains. Introduction of a methyl group at $C_3$ along with the methoxy or chloro functions at the *para* position of the aryl moieties exhibited more pronounced inhibition of some of the above described bacterial strains while inhibition of some fungal strains was reduced by this modification. This would indicate that the chloro/methoxy functions at the *para* position of the aryl moieties at $C_2$ and $C_6$ along with or without methyl

group at $C_3$ play an important role in eliciting biological response. Thus, in the future this class of compounds may be used as leads to generate better drugs to combat bacterial and fungal infections.

**Experimental**

TLC was performed to monitor the reaction progress and purity of products. Melting points were recorded in open capillaries and were uncorrected. IR spectra were recorded in Perkin-Elmer 297 spectrophotometer in KBr pellets and only noteworthy absorption levels (reciprocal centimeter) are listed. $^1H$ - NMR spectra were recorded at 400 MHz on Bruker amx 400 MHz spectrophotometer in $CDCl_3$ using TMS as internal standard and $^{13}C$-NMR spectra were recorded at 100 MHz on Bruker amx 400 MHz spectrophotometer in $CDCl_3$. Mass spectra were recorded on a VG analytical 7070E instrument equipped with VG 11-250 data acquisition system. Satisfactory microanalysis was obtained on Carlo Erba 1106 and Perkin Elmer models 240 CHN analyzer according the precedent literature.[25] 2,6-Diarylpiperidin-4-ones **(1-8)** were prepared by the condensation of appropriate ketones, aldehydes and ammonium acetate in 1:2:1 ratio.

**2,6-Diphenylpiperidin-4-one thiosemicarbazone (9)**

To a boiling solution of 2,6-diphenylpiperidin-4-one (0.01 mol) in methanol (45 mL) and a few drops of conc. HCl, the methanolic solution of thiosemicarbazide (0.01 mol) was added dropwise with stirring. The reaction mixture was refluxed for 3 h on a water bath. After cooling, the solid product was filtered off and recrystallized from methanol to give 2,6-diphenylpiperidin-4-one thiosemicarbazone. The compounds **10-16** were prepared under similar conditions.

**[N-Acetyl-2,6-diphenylpiperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (17 )**

2,6-Diphenylpiperidin-4-one thiosemicarbazone(**9**) (0.025 mol) was treated with freshly distilled acetic anhydride (35 mL) and the mixture was refluxed for 6-7 h on a water bath (90-100°C). The removal of solvent from the cooled reaction mixture in *vacuo* gave a solid mass. The resulting solid mass was purified over neutral alumina column (ethanol-ethyl acetate, 4:1) and recrystallized from methanol to yield **17.** IR (KBr) ($cm^{-1}$): 3244 (N-H), 1692, 1631 (C=O), 1621 (C=N), 1232 (C-N), 719 (C-S); Mass (m/z): 407 (M-43), 322, 304, 289, 264, 261, 247, 235, 185, 157, 128, 115, 91, 77, 59, 43(100%); $^1H$ NMR (δ ppm) : 5.21(dd, $^3J$ = 11.91 Hz; 2.92 Hz, 2H,

$H_{2a}$, $H_{6a}$); 2.20 – 2.36 (m, 4H, $H_{3a}$, $H_{3e}$, $H_{5a}$, $H_{5e}$); 8.9 (s, 1H, NH); 7.10-7.31 (m, 10H, aryl protons); 1.96 (s, 9H, $NCOCH_3$); $^{13}C$ NMR (δ ppm) : 63.395 ($C_2$, $C_6$); 43.759 ($C_3$, $C_5$); 39.694 ($C_4$); 140.046 (C=N); 23.010, 23.139, 24.319 (three $NCOCH_3$); 169.233, 169.868, 172.640 (three $NCOCH_3$); 126.696, 127.472, 129.187, 142.210 (aryl carbons).

The compounds **18-24** were prepared under similar conditions.

**[N-Acetyl-3-methyl-2,6-diphenylpiperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (18 )** IR (KBr) ($cm^{-1}$) : 3246 (N-H), 1691, 1633 (C=O), 1620 (C=N), 1230 (C-N), 717 (C-S); Mass (m/z): 421(M-43), 406, 380, 364, 347, 323, 303, 289, 264, 261, 247, 222, 185, 174, 157, 128, 115, 104, 91, 77, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.82(dd, $^3J$ = 12.02 Hz; 2.67 Hz, 1H, $H_{6a}$); 5.25(d, $^3J$ = 10.86 Hz, 1H, $H_{2a}$); 2.20 – 2.53 (m, 3H, $H_{3a}$, $H_{5a}$, $H_{5e}$); 8.8 (s, 1H, NH); 7.20 (m, 10H, aryl protons); 1.97-1.98 (s, 9H, $NCOCH_3$); 1.1 (d, J = 6.57 Hz, 3H, $CH_3$ at 3); $^{13}C$ NMR (δ ppm) : 63.386 ($C_2$); 58.123 ($C_6$); 41.671($C_3$); 43.759 ($C_5$); 39.650 ($C_4$); 141.614 (C=N); 14.783 ($CH_3$ at 3); 22.937, 23.098, 24.288 (three $NCOCH_3$); 168.453, 168.697, 169.377 (three $NCOCH_3$); 126.633, 127.156, 128.054, 128.390, 128.559, 129.145, 143.710, 145.412 (aryl carbons).

**[N-Acetyl-2,6-*bis*(*p*-methylphenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (19)** IR (KBr) ($cm^{-1}$) : 3246 (N-H), 1690, 1635 (C=O), 1622 (C=N), 1235 (C-N), 721 (C-S); **Mass (m/z):** 435 (M-43), 393, 351, 324, 277, 235, 223, 207, 186, 118, 116, 91, 75, 65, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.30(dd, $^3J$ = 11.87 Hz; 2.90 Hz, 2H, $H_{2a}$, $H_{6a}$); 2.10 – 2.28 (m, 4H, $H_{3a}$, $H_{3e}$, $H_{5a}$, $H_{5e}$); 8.6 (s, 1H, NH); 7.24, 7.36 (2d, 8H, aryl protons); 2.43 (s, 6H, aryl $CH_3$); 1.95 (s, 9H, $NCOCH_3$); $^{13}C$ NMR (δ ppm) : 59.342 ($C_2$, $C_6$); 43.340 ($C_3$, $C_5$); 39.669 ($C_4$); 139.646 (C=N); 22.937, 23.130, 24.123 (three $NCOCH_3$); 168.132, 168.437, 170.641 (three $NCOCH_3$); 20.574 (aryl $CH_3$); 127.852, 128.468, 136.974, 146.438 (aryl carbons).

**[N-Acetyl-3-methyl-2,6-*bis*(*p*-methylphenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (20)** IR (KBr) ($cm^{-1}$) : 3248 (N-H), 1691, 1638 (C=O), 1624 (C=N), 1236 (C-N), 722 (C-S); Mass (m/z): 449 (M-43), 407, 365, 338, 324, 291, 235, 223, 207,186, 158, 132, 118, 116, 91, 75, 65, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.36(dd, $^3J$ = 12.00 Hz; 2.68 Hz, 1H, $H_{6a}$); 4.88(d, $^3J$ = 10.84 Hz, 1H, $H_{2a}$); 2.17 – 2.32 (m, 3H, $H_{3a}$, $H_{5a}$, $H_{5e}$); 8.5 (s, 1H, NH); 7.29-7.43 (m, 8H, aryl protons); 2.46 (s, 6H, aryl $CH_3$); 1.93 (s, 9H, $NCOCH_3$); 0.99 (d, J = 6.54 Hz, 3H, $CH_3$ at 3); $^{13}C$ NMR (δ ppm) : 65.504 ($C_2$); 60.074 ($C_6$); 44.410($C_3$); 42.571 ($C_5$); 40.159

($C_4$); 140.447 (C=N); 11.999 ($CH_3$ at 3); 22.878, 23.213, 24.012 (three $NCOCH_3$); 167.872, 168.123, 169.342 (three $NCOCH_3$); 20.987 (aryl $CH_3$); 128.310, 128.522, 129.779, 130.610, 135.679, 137.852, 138.644, 146.134 (aryl carbons).

**[N-Acetyl-2,6-*bis*(*p*-chlorophenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (21)** IR (KBr) ($cm^{-1}$) : 3242 (N-H), 1696, 1642 (C=O), 1630 (C=N), 1235 (C-N), 723 (C-S); Mass (m/z): 475 (M-43), 433, 391, 364, 350, 275, 263, 247, 186, 158, 138, 116, 91, 77, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.35(dd, $^3J$ = 11.87 Hz; 2.89 Hz, 2H, $H_{2a}$, $H_{6a}$); 2.02 – 2.26 (m, 4H, $H_{3a}$, $H_{3e}$, $H_{5a}$, $H_{5e}$); 8.5 (s, 1H, NH); 7.32, 7.38 (2d, 8H, aryl protons); 1.95 (s, 9H, $NCOCH_3$); $^{13}C$ NMR (δ ppm) : 59.211 ($C_2$, $C_6$); 43.109 ($C_3$, $C_5$); 39.629 ($C_4$); 139.752 (C=N); 22.892, 23.212, 24.180 (three $NCOCH_3$); 168.234, 169.432, 170.321 (three $NCOCH_3$); 128.252, 128.405, 133.204, 146.742 (aryl carbons).

**[N-Acetyl-3-methyl-2,6-*bis*(*p*-chlorophenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (22)** IR (KBr) ($cm^{-1}$) : 3243 (N-H), 1692, 1631 (C=O), 1623 (C=N), 1234 (C-N), 724 (C-S); Mass (m/z): 489 (M-43), 447, 405, 364, 331, 275, 247, 186, 158, 138, 116, 111, 91, 75, 65, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.40(dd, $^3J$ = 12.00 Hz; 2.67 Hz, 1H, $H_{6a}$); 4.90(d, $^3J$ = 10.85 Hz, 1H, $H_{2a}$); 2.09 – 2.37 (m, 3H, $H_{3a}$, $H_{5a}$, $H_{5e}$); 8.40 (s, 1H, NH); 7.34-7.47 (m, 8H, aryl protons); 1.93 (s, 9H, $NCOCH_3$); 0.90 (d, J = 6.57 Hz, 3H, $CH_3$ at 3); $^{13}C$ NMR (δ ppm) : 65.343 ($C_2$); 59.863 ($C_6$); 44.190 ($C_3$); 42.361 ($C_5$); 40.139 ($C_4$); 140.532 (C=N); 12.040 ($CH_3$ at 3); 22.789, 23.136, 24.432 (three $NCOCH_3$); 167.678, 168.432, 169.686 (three $NCOCH_3$); 127.288, 128.726, 128.990, 131.364, 131.989, 133.922, 138.920, 146.445 (aryl carbons).

**[N-Acetyl-2,6-*bis*(*p*-methoxyphenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (23)** IR (KBr) ($cm^{-1}$) : 3251 (N-H), 1699, 1628 (C=O), 1620 (C=N), 1235 (C-N), 722 (C-S); Mass (m/z): 467 (M-43), 425, 383, 356, 342, 309, 267, 255, 239, 186, 158, 134, 116, 107, 91, 77, 65, 59, 43 (100%); $^1H$ NMR (δ ppm) : 5.30(dd, $^3J$ = 11.84 Hz; 2.86 Hz, 2H, $H_{2a}$, $H_{6a}$); 2.00 – 2.26 (m, 4H, $H_{3a}$, $H_{3e}$, $H_{5a}$, $H_{5e}$); 8.90 (s, 1H, NH); 6.89, 7.35 (2d, 8H, aryl protons); 3.93 (s, 6H, aryl $OCH_3$); 1.93 (s, 9H, $NCOCH_3$); $^{13}C$ NMR (δ ppm) : 59.363 ($C_2$, $C_6$); 43.362 ($C_3$, $C_5$); 39.619 ($C_4$); 139.806 (C=N); 22.912, 23.314, 24.213 (three $NCOCH_3$); 168.341, 169.532, 170.324 (three $NCOCH_3$); 54.618 (aryl $OCH_3$); 115.544, 127.837, 141.903, 158.541 (aryl carbons).

**[N-Acetyl-3-methyl-2,6-*bis*(*p*-methoxyphenyl)piperidin-4-yl]-5-spiro-4-acetyl-2-(acetylamino)-$\Delta^2$-1,3,4-thiadiazoline (24)** IR (KBr) ($cm^{-1}$) : 3249 (N-H), 1698, 1640 (C=O), 1628 (C=N), 1235 (C-N), 723 (C-S); Mass (m/z): 481 (M-43), 439, 397, 370, 356, 323, 267, 255, 186, 158, 148, 134, 116, 107, 91, 75, 65, 43 (100%); $^1$H NMR (δ ppm) : 5.41(dd, $^3$J = 12.02 Hz; 2.63 Hz, 1H, $H_{6a}$); 4.85(d, $^3$J = 10.89 Hz, 1H, $H_{2a}$); 2.07 – 2.30 (m, 3H, $H_{3a}$, $H_{5a}$, $H_{5e}$); 8.72 (s, 1H, NH); 6.85-6.87, 7.36-7.38 (m, 8H, aryl protons); 3.94 (s, 6H, aryl $OCH_3$); 1.93 (s, 9H, $NCOCH_3$); 0.81 (d, J = 6.38 Hz, 3H, $CH_3$ at 3); $^{13}$C NMR (δ ppm) : 65.501 ($C_2$); 59.972 ($C_6$); 44.403($C_3$); 42.654 ($C_5$); 40.119 ($C_4$); 140.566 (C=N); 12.124 ($CH_3$ at 3); 22.846, 23.492, 24.532 (three $NCOCH_3$); 168.542, 169.431, 170.642 (three $NCOCH_3$); 54.895 (aryl $OCH_3$); 114.383, 115.890, 128.005, 130.955, 134.746, 157.266, 159.234 (aryl carbons).

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