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Research Article

### SYNTHESIS, DOCKING AND ANTITUBERCULAR ACTIVITY OF SOME NEWER SCHIFF BASES

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#### ABSTRACT

Schiff bases are important class of heterocyclic compounds due to their versatile biological activity. A novel series of 5-(substituted) phenyl-*N*-[(1*E*)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine (Va-n) derivatives were synthesized in good yield by the condensation of 2-amino-5-substituted acid-1,3,4-thiadiazole (IV a, b) with various selected aromatic aldehydes. The structures of the synthesized compounds were confirmed by FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, MASS data and elemental analysis. Docking studies has been carried out by using Hex software for all the newly synthesized compounds. All the synthesized compounds were tested against *Mycobacterium tuberculosis* using Microplate Alamar Blue Assay method. Preliminary results indicated that most of the compounds tested in this study demonstrated comparable activity against *Mycobacterium tuberculosis* as compared with standard Streptomycin, Pyrazinamide and Ciprofloxacin. Among the series, (V a-n), compound V k, V l, V m and V n showed good antitubercular activity compared to standard Streptomycin, Ciprofloxacin and Pyrazinamide and the remaining compounds showed similar activity compared to standard streptomycin. The structure and biological activity relationship of titled compounds showed that the presence of pharmacophoric moieties like 1,3,4-thiadiazole nucleus and the substituent's like 2-methoxy benzaldehyde, 4-methoxy benzaldehyde, 2-hydroxy benzaldehyde and 4-hydroxy benzaldehyde attached to it will increases the activity.

**Keywords:** Tuberculosis, Schiff base, Thiosemicarbazide, 1,3,4-thiadiazole, Nicotinic acid, Isonicotinic acid, Docking.

#### INTRODUCTION

It is well established that slight alterations in the structure of certain Schiff bases are able to bring drastic changes to yield better drug with less toxicity to the host. It is observed that chemical modification not only alters physicochemical properties but also pharmacological activities<sup>1</sup>. Discovery of newer and more potent analogs of molecules with already established activities form a key part of research in the pharmaceutical field. Bringing about slight modifications in the parent compound often serves to enhance the activity of the compound and also in most cases eliminates adverse effects or toxicity associated with the parent drug<sup>2</sup>. Schiff base heterocycles are still the most prescribed compounds used in medicine. They are considered as an important contribution of science to humanity<sup>3</sup>. Biological activity of these heterocycles has helped the medicinal chemist to plan, organize and implement newer approaches towards the discovery of newer drugs. In view of the general observation that pharmacological activity is invariably associated with a large variety of

heterocyclic compounds, the investigation of some Schiff base heterocycles derivatives has been undertaken. Derivatives of these compounds are reported to possess a wide spectrum of biological activities which include anti-inflammatory, analgesic, antitumour, antihypertensive, anticonvulsant and antimicrobials<sup>4-9</sup>. Owing to the importance and established physiological activity of these, it was thought to synthesize and investigate compounds with comparable structures. Owing to the importance and established pharmacological activity of these compounds we are directed our attention towards synthesis and docking of some new Schiff base derivatives with object of screening them for antitubercular activity.

#### MATERIALS AND METHODS

##### Chemistry:

All Chemicals used in the synthesis of the compounds described were purchased from Loba chemicals, Ozone chemicals, S.D. fine chemicals Ltd. and Spectrochem Ltd. They were nicotinic acid, isonicotinic acid, thiosemicarbazide, phosphoryl oxy trichloride, chloroacetyl chloride, ethanol,

triethyl amine and various aromatic aldehydes, sulphuric acid, potassium hydroxide etc. These are either AR/LR grade or the best possible pharma grade available were used here. Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. FT-IR spectra in KBr disk were recorded from 4000 to 400  $\text{cm}^{-1}$  on Avatar 330 FT-IR spectrometer equipped with DTGS detector.  $^1\text{H}$  NMR spectra were recorded on 400-MHz and 500-MHz Bruker spectrometer in  $\text{DMSO}-d_6$  or  $\text{CDCl}_3$  using TMS as an internal standard. Mass spectra were recorded using Agilent 1100 MSD spectrometer in electro spray mode. Compounds were checked for their purity by TLC on silica gel G plates and spots were located by exposing to UV light and iodine vapours. The following materials that were either AR/LR grade or the best possible pharma grade available were used as supplied by the manufacturer.

#### Chemical synthesis:

##### General method for the synthesis of Schiff bases<sup>10-12</sup>

2-amino-5-substituted acid-1,3,4-thiadiazoles (**IV a, b**) are prepared according to literature procedure, i.e., by the reaction of thiosemicarbazide with substituted acids. The physico-chemical data of (**IV a, b**) are presented in Table 1. The 5-pyridin-3-yl-1,3,4-thiadiazol-2-amines (**IV a, b**) was dissolved in ethanol and was reacted with aromatic aldehydes to yield schiff bases (**V a-n**). These schiff bases (**V a-n**) were then characterized by the elemental analysis, IR, NMR and MASS spectral studies. The IR spectra of Schiff bases show the prominent band at 1630  $\text{cm}^{-1}$  for the azomethine group.

##### General method for the synthesis of 5-(substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine (**V a-n**)

2-amino-5-substituted acid-1,3,4-thiadiazole (**IV a, b**) required as the starting material was prepared by the reaction of thiosemicarbazide with substituted acids. 2-amino-5-substituted acid-1,3,4-thiadiazole (**IV a, b**) on condensation with various selected aromatic aldehydes furnished schiff bases, 5-(substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine (**V a-n**) as shown in scheme I. The physico-chemical parameters of the synthesized compounds are depicted in Table 1.

#### DOCKING STUDIES

Docking study was done with *HEX*-software for the newly synthesized compounds to dock with the antitubercular protein (2YES) from Protein Data Base (PDB). *Hex* is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can also calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. In *Hex*'s docking calculations, each molecule is modeled using 3D expansions of real orthogonal spherical polar basis functions to encode both surface shape and electrostatic charge and potential distributions. *Hex* is also very easy to use. However, to use *Hex* most effectively, sometimes require some thought when setting up the calculation, especially when setting up the starting orientations of the proteins to be docked.

**Molecular docking involves the following steps using Hex 5.1 software:**

Identified a target protein 2YES from the Protein data Bank. Downloaded PDB FILE (text) and saved it in Example Folder of *Hex* 5.1. Drawn all the ligands using chemsketch. Generated 3-D view (SDF format), convert it into MOL file. Converted into PDB format by using Swiss PDB viewer and saved it. Open *Hex* 5.1 software, selected appropriate protein and ligand and performed docking. The docking results of the synthesized compounds *Va-n* are depicted in Table 2.

#### ANTITUBERCULAR ACTIVITY<sup>13</sup>

**Method:** Microplate Alamar Blue Assay (MABA)

**Preparation of Inoculums:** 100  $\mu\text{l}$  of the Middle brook 7H9 broth

**Requirements:** 96 wells plate, Para film (all are sterilized by dry heat).

**Nutrient Medium:** 25 $\mu\text{l}$  of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80

**Working procedure** Stock solutions of the synthesized compounds and standard drug used were prepared in sterilized deionized water and taken in the concentration of 0.1 to 100  $\mu\text{l}/\text{ml}$ .

The anti-mycobacterial activities of compounds *V a-n* were assessed against *M. tuberculosis* using Microplate Alamar Blue Assay<sup>14</sup> (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method<sup>15-16</sup>. Briefly, 200 $\mu\text{l}$  of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100  $\mu\text{l}$  of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2  $\mu\text{g}/\text{ml}$ . Plates were covered and sealed with Para film and incubated at 37°C for five days. After this time, 25 $\mu\text{l}$  of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The antitubercular activities of the synthesized compounds are depicted in Table 3.

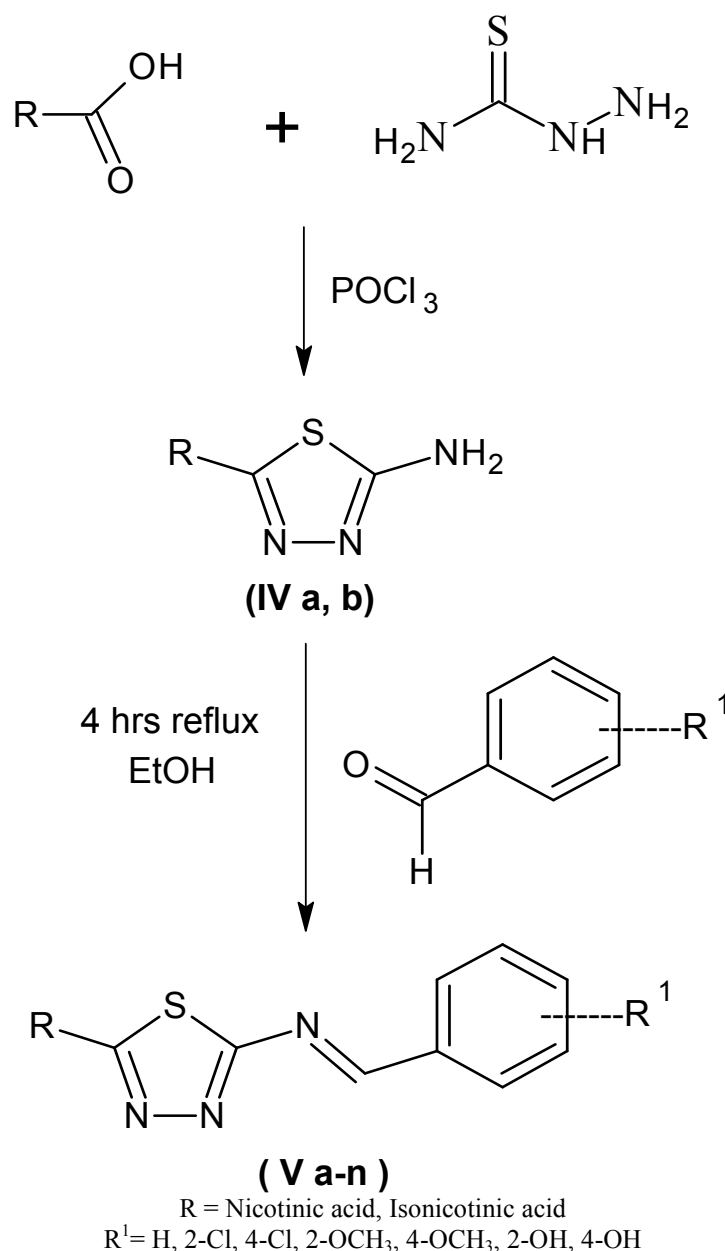
## RESULTS AND DISCUSSION

A new series of 5-(substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine (**V a-n**) were synthesized in good yield according to reaction scheme I. All the newly synthesized compounds were screened for the antitubercular activity by MABA (Microplate Alamar Blue Assay) method. Pyrazinamide, Streptomycin and Ciprofloxacin drugs are used as standards and activity of all newly synthesized compounds were measured against it. Docking studies has been carried out by using *Hex* software for all the compounds. All the derivatives were docked with target protein for tuberculosis (2YES) to predict the antitubercular activity. The result of the docking studies clearly confirms that the synthesized compounds *V k*, *V l*, *V m* and *V n* are active against the tuberculosis i.e. methoxy and hydroxyl derivatives with isonicotinic acid showed potent antitubercular activity. The structures of the synthesized

compounds were confirmed by FT-IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FABMS data and elemental analysis. The antimycobacterial activities of compounds V a-n were assessed against *M. tuberculosis*H<sub>37</sub>Rv (ATCC 27294) using Microplate Alamar Blue Assay (MABA). This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with BACTEC radiometric methods and the activity expressed as the minimum inhibitory concentration (MIC) in  $\mu\text{g/ml}$ . The MICs of the compounds were carried out and showed good antitubercular activity compared to standards Pyrazinamide

streptomycin and ciprofloxacin. In a series V a-n, compound V k, V l, V m and V n showed good antitubercular activity compared to standard Streptomycin, Ciprofloxacin and Pyrazinamide and the remaining compounds showed similar activity compared to standard streptomycin. The structure and biological activity relationship of title compounds showed that the presence of 1,3,4-thiadiazole nucleus and the substituent's like 2-methoxy benzaldehyde, 4-methoxy benzaldehyde, 2-hydroxy benzaldehyde and 4-hydroxy benzaldehyde attached to it will increase the activity.

SCHEME 1



Reaction sequence for the synthesis of 5-(substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine (V a-n)

**Table 1: Physico-chemical data of 5-(Substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine(V a-n)**

Compound	R	R <sup>1</sup>	Yield (%)	M. P. (°C)	R <sub>f</sub> <sup>*</sup>	Molecular Formula
V a	Nicotinic acid	H	64	195	0.65	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> S
V b	Nicotinic acid	2-Cl	56	180	0.74	C <sub>14</sub> H <sub>9</sub> ClN <sub>4</sub> S
V c	Nicotinic acid	4-Cl	48	183	0.68	C <sub>14</sub> H <sub>9</sub> ClN <sub>4</sub> S
V d	Nicotinic acid	2-OCH <sub>3</sub>	66	202	0.66	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> OS
V e	Nicotinic acid	4-OCH <sub>3</sub>	51	210	0.74	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> OS
V f	Nicotinic acid	2-OH	45	209	0.81	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> OS
V g	Nicotinic acid	4-OH	65	233	0.67	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> OS
V h	Isonicotinic acid	H	58	180	0.66	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> S
V i	Isonicotinic acid	2-Cl	58	175	0.62	C <sub>14</sub> H <sub>9</sub> ClN <sub>4</sub> S
V j	Isonicotinic acid	4-Cl	47	220	0.81	C <sub>14</sub> H <sub>9</sub> ClN <sub>4</sub> S
V k	Isonicotinic acid	2-OCH <sub>3</sub>	63	204	0.69	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> OS
V l	Isonicotinic acid	4-OCH <sub>3</sub>	67	185	0.61	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> OS
V m	Isonicotinic acid	2-OH	45	167	0.80	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> OS
V n	Isonicotinic acid	4-OH	48	241	0.85	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> OS

\* Benzene: Methanol: Ammonia (75:25:0.25) as a mobile phase and iodine vapors as visualizing agent.

## SPECTRAL DATA

### *N*-[(1E)-phenyl methylene]-5-pyridin-3-yl-1,3,4-thiadiazol-2-amine (V a)

The following spectral data were recorded for compound V a: FTIR (KBr) cm<sup>-1</sup>: 3115 (Ar C-H), 2960 (C-H), 2880 (C-H thiazole), 1590 (-N=CH), 702 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.14 (s, 1H, -N=CH), 7.30-7.68 (m, 9H, Ar-H); MS spectrum, *m/z*: 267[M+1]<sup>+</sup>; CHN analysis calculated for C<sub>14</sub>H<sub>10</sub>N<sub>4</sub>S: C (63.14), H (3.78), N (21.04). Found: C (63.05), H (3.51) and N (20.81).

### *N*-[(1E)-(2-chlorophenyl)methylene]-5-pyridin-3-yl-1,3,4-thiadiazol-2-amine (V b)

The following spectral data were recorded for compound V b: FTIR (KBr) cm<sup>-1</sup>: 3110 (Ar C-H), 2890 (C-H), 2810 (C-H thiazole), 1598 (-N=CH), 682 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.04 (s, 1H, -N=CH); 7.40-7.75 (m, 8H, Ar-H); MS spectrum, *m/z*: 301 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>14</sub>H<sub>9</sub>ClN<sub>4</sub>S: C (55.91), H (3.02), N (18.63). Found: C (55.05), H (3.08) and N (18.81).

### *N*-[(1E)-(4-chlorophenyl)methylene]-5-pyridin-3-yl-1,3,4-thiadiazol-2-amine(V c)

The following spectral data were recorded for compound V c: FTIR (KBr) cm<sup>-1</sup>: 3095 (Ar C-H); 2930 (C-H); 2820 (C-H thiazole), 1582 (-N=CH), 710 (C-S-C); <sup>1</sup>H NMR chemical shifts at (400 MHz, CDCl<sub>3</sub>, δ ppm): 8.20 (s, 1H, N=CH), 7.15-7.72 (m, 8H, Ar-H); MS spectrum, *m/z*: 301 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>14</sub>H<sub>9</sub>ClN<sub>4</sub>S: C (55.91), H (3.02), N (18.63). Found: C (55.05), H (3.08) and N (18.81).

### *N*-[(1E)-(2-methoxyphenyl)methylene]-5-pyridin-3-yl-1,3,4-thiadiazol-2-amine (V d)

The following spectral data were recorded for compound V d: FTIR (KBr) cm<sup>-1</sup>: 3157 (Ar C-H), 2929 (C-H), 2850 (C-H thiazole), 1598 (-N=CH), 705 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.37 (s, 1H, N=CH), 6.86-7.52 (m, 8H, Ar-H), 1.45 (s, 3H, CH<sub>3</sub>); MS spectrum, *m/z*: 297 [M+1]<sup>+</sup>; CHN analysis calculated for C<sub>15</sub>H<sub>12</sub>N<sub>4</sub>OS: C (60.79), H (4.08), N (18.91). Found: C (60.01), H (4.04) and N (18.39).

### *N*-[(1E)-(4-methoxyphenyl)methylene]-5-pyridin-3-yl-1,3,4-thiadiazol-2-amine (V e)

The following spectral data were recorded for compound V e: FTIR (KBr) cm<sup>-1</sup>: 3108 (Ar C-H), 2950(C-H), 2870 (C-H thiazole), 1585 (-N=CH), 712 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.39 (s, 1H, N=CH), 7.25-7.63 (m, 8H, Ar-H), 2.45 (s, 3H, CH<sub>3</sub>); MS spectrum, *m/z*: 297 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>15</sub>H<sub>12</sub>N<sub>4</sub>OS: C (60.79), H (4.08), N (18.91). Found: C (60.01), H (4.04) and N (18.30).

### 2-[(E)-[(5-pyridin-3-yl-1,3,4-thiadiazol-2-yl)imino]methyl]phenol (V f)

The following spectral data were recorded for compound V f: FTIR (KBr) cm<sup>-1</sup>: 3548 (O-H), 3005 (Ar C-H), 2890 (C-H), 2750 (C-H thiazole), 1583 (-N=CH), 712 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.36 (s, 1H, N=CH), 7.22-7.68 (m, 8H, Ar-H), 5.26 (s, 1H, OH); MS spectrum, *m/z*: 283 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>14</sub>H<sub>10</sub>N<sub>4</sub>OS: C (59.56), H (3.57), N (19.85). Found: C (60.01), H (3.71) and N (19.30).

### 4-[(E)-[(5-pyridin-3-yl-1,3,4-thiadiazol-2-yl)imino]methyl]phenol (V g)

The following spectral data were recorded for compound V g: FTIR (KBr) cm<sup>-1</sup>: 3545 (O-H); 3005 (Ar C-H); 2955 (C-H); 2875 (C-H thiazole); 1515 (-N=CH), 695 (C-S-C). <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.19 (s, 1H, N=CH); 7.18-7.34 (m, 8H, Ar-H); 5.28 (s, 1H, OH); MS spectrum, *m/z*: 283 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>14</sub>H<sub>10</sub>N<sub>4</sub>OS: C (59.56), H (3.57), N (19.85). Found: C (60.01), H (3.71) and N (19.30).

### *N*-[(1E)-phenyl methylene]-5-pyridin-4-yl-1,3,4-thiadiazol-2-amine (V h)

The following spectral data were recorded for compound V h: FTIR (KBr) cm<sup>-1</sup>: 3005 (Ar C-H), 2965 (C-H), 2885 (C-H thiazole), 1550 (-N=CH), 702 (C-S-C); <sup>1</sup>H NMR chemical shifts at ( 400 MHz, CDCl<sub>3</sub>, δ ppm): 8.22 (s, 1H, N=CH), 7.05-7.45 (m, 9H, Ar-H), MS spectrum, *m/z*: 267 [M+1]<sup>+</sup>. CHN analysis calculated for C<sub>14</sub>H<sub>10</sub>N<sub>4</sub>S: C (63.14), H (3.78), N (21.04). Found: C (63.21), H (3.74) and N (21.24).



***N*-[(1*E*)-(2-chlorophenyl)methylene]-5-pyridin-4-yl-1,3,4-thiadiazol-2-amine (V i)**

The following spectral data were recorded for compound V i: FTIR (KBr)  $\text{cm}^{-1}$ : 3005 (Ar C-H), 2955 (C-H), 2875 (C-H thiazole), 1535 (-N=CH), 780 (C-S-C);  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 8.30 (s, 1H, N=CH), 7.10-7.38 (m, 8H, Ar-H); MS spectrum,  $m/z$ : 301  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{14}\text{H}_9\text{ClN}_4\text{S}$ : C (55.91), H (3.02), N (18.63). Found: C (54.21), H (3.04) and N (18.28).

***N*-[(1*E*)-(4-chlorophenyl)methylene]-5-pyridin-4-yl-1,3,4-thiadiazol-2-amine (V j)**

The following spectral data were recorded for compound V j: FTIR (KBr)  $\text{cm}^{-1}$ : 3111 (Ar C-H), 2962 (C-H), 2875 (C-H thiazole), 1583 (-N=CH), 714 (C-S-C);  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 9.39 (s, 1H, N=CH), 7.17-7.72 (m, 8H, Ar-H); MS spectrum,  $m/z$ : 301  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{14}\text{H}_9\text{ClN}_4\text{S}$ : C (55.91), H (3.02), N (18.63). Found: C (54.11), H (3.14) and N (18.03).

***N*-[(1*E*)-(2-methoxyphenyl)methylene]-5-pyridin-4-yl-1,3,4-thiadiazol-2-amine (V k)**

The following spectral data were recorded for compound V k: FTIR (KBr)  $\text{cm}^{-1}$ : 3120 (Ar C-H), 2965 (C-H), 2892 (C-H thiazole), 1585 (-N=CH), 718 (C-S-C);  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 9.28 (s, 1H, N=CH), 7.80-7.95 (m, 8H, Ar-H), 3.15 (s, 3H, -OCH<sub>3</sub>), 1.42-1.47 (s, 6H, CH<sub>3</sub>); MS spectrum,  $m/z$ : 297  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{15}\text{H}_{12}\text{N}_4\text{OS}$ : C (60.79), H (4.08), N (18.91). Found: C (59.71), H (4.14) and N (18.29).

***N*-[(1*E*)-(4-methoxyphenyl)methylene]-5-pyridin-4-yl-1,3,4-thiadiazol-2-amine (V l)**

The following spectral data were recorded for compound V l: FTIR (KBr)  $\text{cm}^{-1}$ : 3171.17 (Ar C-H), 2844.28 (C-H), 2727.68 (C-H thiazole), 1571.20 (-N=CH), 760.23 (C-S-C);  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.52-7.54 (d, 4H, Ar-H), 7.40 (s, 1H, N=CH), 7.18-7.21 (d, 4H, Ar-H), 2.61 (s, 3H, -OCH<sub>3</sub>);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  32.65, 43.74, 130.60, 134.86, 140.93, 144.85, 150.86, 153.55, 160.17, 160.68, 163.23, 164.40; MS spectrum,  $m/z$ : 297  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{15}\text{H}_{12}\text{N}_4\text{OS}$ : C (60.79), H (4.08), N (18.91). Found: C (59.88), H (4.04) and N (18.61).

**2-[(*E*)-[(5-pyridin-4-yl-1,3,4-thiadiazol-2-yl)imino]methyl]phenol (V m)**

The following spectral data were recorded for compound V m: FTIR (KBr)  $\text{cm}^{-1}$ : 3543 (O-H); 3008 (Ar C-H); 2957 (C-H); 2875 (C-H thiazole); 1515 (-N=CH), 696 (C-S-C).  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 8.16 (s, 1H, N=CH); 7.17-7.34 (m, 8H, Ar-H); 5.27 (s, 1H, OH); MS spectrum,  $m/z$ : 283  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{14}\text{H}_{10}\text{N}_4\text{OS}$ : C (59.56), H (3.57), N (19.85). Found: C (59.81), H (3.64) and N (19.63).

**4-[(*E*)-[(5-pyridin-4-yl-1,3,4-thiadiazol-2-yl)imino]methyl]phenol (V n)**

The following spectral data were recorded for compound V n: FTIR (KBr)  $\text{cm}^{-1}$ : 3541 (O-H); 3005 (Ar C-H); 2957 (C-H); 2875 (C-H thiazole); 1514 (-N=CH), 698 (C-S-C).  $^1\text{H}$  NMR chemical shifts at (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 8.17 (s, 1H, N=CH); 7.17-7.33 (m, 8H, Ar-H); 5.26 (s, 1H, OH); MS spectrum,  $m/z$ : 283  $[\text{M}+1]^+$ . CHN analysis calculated for  $\text{C}_{14}\text{H}_{10}\text{N}_4\text{OS}$ : C (59.56), H (3.57), N (19.85). Found: C (59.12), H (3.38) and N (19.21).

**Table 2: Docking results of 5-(Substituted) phenyl-*N*-[(1*E*)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine(V a-n)**

Compound	R	R <sup>1</sup>	PDB CODE	E SCORE (KJ / Mol)
V a	Nicotinic acid	H	2YES	-137.71
V b	Nicotinic acid	2-Cl	2YES	-138.92
V c	Nicotinic acid	4-Cl	2YES	-141.32
V d	Nicotinic acid	2-OCH <sub>3</sub>	2YES	-144.35
V e	Nicotinic acid	4-OCH <sub>3</sub>	2YES	-143.88
V f	Nicotinic acid	2-OH	2YES	-144.61
V g	Nicotinic acid	4-OH	2YES	-142.21
V h	Isonicotinic acid	H	2YES	-139.82
V i	Isonicotinic acid	2-Cl	2YES	-141.36
V j	Isonicotinic acid	4-Cl	2YES	-143.33
V k	Isonicotinic acid	2-OCH <sub>3</sub>	2YES	-148.58
V l	Isonicotinic acid	4-OCH <sub>3</sub>	2YES	-149.08
V m	Isonicotinic acid	2-OH	2YES	-146.18
V n	Isonicotinic acid	4-OH	2YES	-147.13

**Table 3: Antitubercular activity of 5-(Substituted) phenyl-*N*-[(1*E*)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine(V a-n)**

Compound	R	R <sup>1</sup>	MIC ( $\mu\text{g/mL}$ ) <i>M. tuberculosis</i> H <sub>37</sub> Rv
V a	Nicotinic acid	H	50
V b	Nicotinic acid	2-Cl	25
V c	Nicotinic acid	4-Cl	25
V d	Nicotinic acid	2-OCH <sub>3</sub>	12.5
V e	Nicotinic acid	4-OCH <sub>3</sub>	12.5
V f	Nicotinic acid	2-OH	12.5

<b>V g</b>	Nicotinic acid	4-OH	12.5
<b>V h</b>	Isonicotinic acid	H	25
<b>V i</b>	Isonicotinic acid	2-Cl	12.5
<b>V j</b>	Isonicotinic acid	4-Cl	12.5
<b>V k</b>	Isonicotinic acid	2-OCH <sub>3</sub>	6.25
<b>V l</b>	Isonicotinic acid	4-OCH <sub>3</sub>	6.25
<b>V m</b>	Isonicotinic acid	2-OH	6.25
<b>V n</b>	Isonicotinic acid	4-OH	6.25
Pyrazinamide			3.125
Streptomycin			6.25
Ciprofloxacin			3.125

## CONCLUSION

A new series 5-(Substituted) phenyl-N-[(1E)-(substituted) phenyl methylene]-1,3,4-thiadiazol-2-amine V a-n were synthesized in good yields as shown in table and were evaluated for antitubercular activities. Docking studies has been carried out by using Hex software for all the derivatives. The structures of the synthesized compounds were confirmed by FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, FABMS data and elemental analysis. All the derivatives of V a-n were analyzed for their physicochemical properties as shown in tables. All the derivatives of V a-n were docked with target protein for tuberculosis (2YES) to predict the antitubercular activity. The results of the docking study for all the derivatives are shown in table. The result of the docking studies clearly confirms that the synthesized compounds V k, V l, V m and V n are active against the tuberculosis i.e. methoxy and hydroxyl derivatives with isonicotinic acid showed potent antitubercular activity. The antimycobacterial activities of compounds V a-n were assessed against *M. tuberculosis*H<sub>37</sub>Rv (ATCC 27294) using Microplate Alamar Blue Assay (MABA). This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with BACTEC radiometric methods and the activity expressed as the minimum inhibitory concentration (MIC) in µg/ml. The MICs of the compounds were carried out and showed good antitubercular activity compared to standards pyrazinamide streptomycin and ciprofloxacin. In the series V a-n, compound V k, V l, V m and V n showed good antitubercular activity compared to standard Streptomycin, Ciprofloxacin and Pyrazinamide and the remaining compounds showed similar activity compared to standard streptomycin. The structure and biological activity relationship of title compounds showed that the presence of 1,3,4-thiadiazole nucleus and the substituent's like 2-methoxy benzaldehyde, 4-methoxy benzaldehyde, 2-hydroxy benzaldehyde and 4-hydroxy benzaldehyde attached to it will increase the activity.

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