ISSN 2348-232X

Unique Research Journal of Chemistry

Available online: www.ujconline.net

Research Article

NEWER SCHIFF BASES AS ANTIBACTERIALS AND ANTIFUNGALS

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Received: 14-10-2014; Revised: 12-11-2014; Accepted: 10-12-2014

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ABSTRACT

Schiff bases are important class of heterocycles due to their wide biological activities. A new series of Schiff bases were synthesized with good yields and the structures were confirmed by by FT-IR, ^{1}H NMR, ^{13}C NMR, MASS data and elemental analysis. All the synthesized compounds were screened for their antibacterial and antifungal activity. The results of antibacterial study for **3a-l** series revealed that the compounds **3b**, **3f, 3h, 3i** and **3j** showed good antibacterial activity against four organisms tested but exhibited less activity than the standard drugs. The remaining compounds showed moderate activity. Results of antifungal activity showed that compounds **3f**, **3i** & **3l** showed better activity against *C. albicans* and *A. niger*. The remaining compounds exhibited less activity than the standard drug.

Keywords: Schiff base, Thiosemicarbazide, 1,3,4-thiadiazole, Antibacterial activity, Antifungal activity

INTRODUCTION

In past decades thiadiazole Schiff bases have proved their potential in development of pharmaceutically important organic compounds both of natural and synthetic origin[1]. Schiff base heterocycles are still the most prescribed compounds used in medicine. They are considered as an important contribution of science to humanity. 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. Thiadiazole Schiff base analogs deal with a variety of bioactivities viz. antitumor[2,3], anti-HIV[4], antimicrobial[5,6], anticonvulsant[7], antitubercular[8], antiprotozoal[9], anti-inflammatory[10]. Literature is enriched with lot of work on synthesis of potent substituted thiadiazole derivatives with diverse pharmacological activities[11,12]. On the other side literature survey revealed that 1,3,4-thiadiazole, 2-azetidinones and 4-thiazolidinones are also associated with pharmacological activities like antimicrobial, antiviral, anesthetic, anticonvulsant , etc. These findings prompted us to synthesize 2-azetidinones and 4-thiazolidinones derivatives of 2-amino-5-aryl-5H-thiazolo[4,3-b]-1,3,4-thiadiazoles. Each of the prepared analogues has been tested for their antitubercular, antibacterial and antifungal activity activities and the results are reported in this paper.

MATERIALS AND METHODS

Analytical grade solvents and commercially available reagents were used without further purification. All chemicals were obtained from spectrochem Ltd (Mumbai, India). The column chromatography was carried out over silica gel (60-120 mesh), purchased from Sisco Research Laboratories Pvt Ltd. Melting points were determined in DBK, Prog, melting point apparatus Servewell Instruments Pvt Ltd. FTIR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Shimadzu FT-IR spectrometer. ^{1}H NMR spectra were recorded on 400-MHz and 500-MHz Bruker spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ relative to TMS, the coupling constants are given in Hz. Mass spectra were recorded using Agilent 1100 MSD spectrometer in electro spray mode.

Preparation of 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazole (1): 2-amino-5-aryl-5H-thiazolo[4,3-b]-l,3,4-thiadiazoles are prepared according to literature procedure[18]. An aromatic aldehyde (0.02 moles) and thioglycolic acid (0.02 moles) were mixed, and after 10--15 min. 0.022 moles of thiosemicarbazide was added; then 10 mL of concentrated H_2SO_4 was added in portions upon cooling. The mixture was homogenized and left for 18-24 hours at -20 °C. The reaction mass was treated with 30--50 g ice, the precipitated solid was decanted, water was added, and the obtained suspension was neutralized with 40% NaOH until a weak alkaline reaction. Synthesized compounds were recrystallized from aqueous dioxane solution.

General procedure for the synthesis of compounds 3a-3l:
To a stirred solution of compound 1(0.01 mole) in ethanol (50ml) containing sulphuric acid (2ml) was added appropriate aromatic aldehyde (0.01 mole) and the mixture refluxed for 4-6 hours on a water bath. The separated solid was filtered and recrystallised from ethanol to give the title compounds.

RESULTS AND DISCUSSION

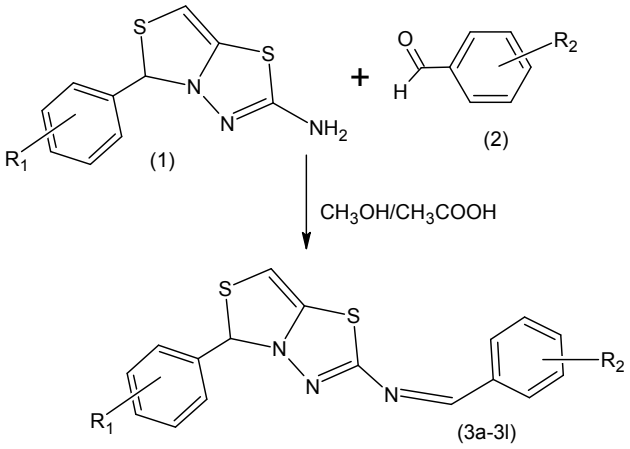
A new series of 2-amino-5-aryl-5 H-thiazolo[4,3-b]- 1,3,4-thiadiazole derivatives of amino acids and peptides **3a-3j** was synthesized with good yields and the structures were confirmed by IR, ^{1}H NMR and mass spectral data. The synthesis of compounds **3a-3j** is mentioned in Scheme **1** and their physicochemical data is described in Table **1**.

IR spectra of peptide derivatives **3a-3j** showed Amide I and Amide II bands at 1662-1639 cm^{-1} and 1538-1531 cm^{-1} indicating formation of peptide bonds and successfulness of coupling reaction. Four peaks, due to the CH-stretching vibrations of the aromatic ring, were recorded in the interval 3180--2820 cm^{-1}. This fact was further confirmed by appearance of broad singlet at 9.32–6.96 ppm (for imino proton of CONH moiety), methine proton signals and those of the proton in the 5H-position of the thiazole ring are detected in the ^{1}H NMR spectra at 7.96--8.3 ppm. The resonance lines of the phenyl ring protons are observed at 7.08--8.27 ppm. in ^{1}H NMR spectra of compounds **3a-3j**. Mass spectra of peptide ester derivatives showed molecular ion peaks along with isotopic peaks at *m/z* values, consistent with their respective molecular formulas.

Pharmacological Studies

Antibacterial and Antifungal activity

Compounds **3a-3l** were screened for antibacterial and antifungal activities using the Disc Diffusion method[23] by measuring the zone of inhibiton. A 24 h culture of bacterial strains of *S. aureus* ATCC 12598, *E. fecalis* ATCC 35550, *K.pneumonia* ATCC 29665 and *E. coli* ATCC 25922 were cultivated in Brain heart infusion agar medium and the fungal strains of *A. niger* ATCC 9029, and *C. albicans* ATCC 2091 were cultivated in Sabouraud agar medium respectively. All the compounds were tested at different concentration level. Dimethyl formamide was used as a solvent and as control. Ciprofloxacin and Fluconazole were used as a standard for comparison of the results. The diameter of zone of inhibition was measured in millimeter (mm) after 24h incubation at 37^{0}C. The Antibacterial activity results are tabulated in **Table 2** and Antifungal in **Table 3**.



SCHEME 1

Table 1: Physical and analytical data of synthesized Compounds

Sr. No	R_1	R_2	Yield (%)	M.P. (^{o}C)	Mol. formula	Analysis % found (Calculated)		
						C	H	N
3a	-C_6H_5	-C_6H_5	62	140	$C_{17}H_{13}N_3S_2$	63.13	4.05	12.99
3b	-C_6H_5	2-ClC_6H_4	58	144	$C_{17}H_{12}ClN_3S_2$	57.05	3.38	11.74
3c	-C_6H_5	4-ClC_6H_4	63	109	$C_{17}H_{12}ClN_3S_2$	57.05	3.38	11.74
3d	4-$CH_3C_6H_4$	-C_6H_5	71	114	$C_{18}H_{15}N_3S_2$	64.06	4.48	12.45
3e	4-$CH_3C_6H_4$	2-$CH_3C_6H_4$	78	132	$C_{19}H_{17}N_3S_2$	64.92	4.87	11.95
3f	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	76	156	$C_{19}H_{17}N_3S_2$	64.92	4.87	11.95
3g	4-OHC_6H_4	-C_6H_5	72	179	$C_{17}H_{13}N_3OS_2$	60.15	3.86	12.38
3h	4-OHC_6H_4	2-OHC_6H_4	75	186	$C_{17}H_{13}N_3O_2S_2$	57.45	3.69	11.82
3i	4-OHC_6H_4	4-OHC_6H_4	78	154	$C_{17}H_{13}N_3O_2S_2$	57.45	3.69	11.82
3j	4-$(CH3)_2NC_6H_4$	-C_6H_5	76	156	$C_{19}H_{18}N_4S_2$	62.27	4.95	15.29
3k	4-$(CH3)_2NC_6H_4$	2-$OCH_3C_6H_4$	72	179	$C_{20}H_{20}N_4OS_2$	60.58	5.08	14.13
3l	4-$(CH3)_2NC_6H_4$	4-$OCH_3C_6H_4$	66	141	$C_{20}H_{20}N_4OS_2$	60.58	5.08	14.13

Table 2: Antibacterial activity of synthesized compounds

Compound	*S. aureus*	*E. fecalis*	*K. pneumoniae*	*E. coli*
3a	8	6	7	6
3b	12	10	8	12
3c	10	9	8	7
3d	9	8	8	14
3e	14	11	8	8
3f	11	9	9	7
3g	10	8	7	6
3h	11	8	8	8
3i	13	10	9	8
3j	9	8	7	6
3k	10	9	8	7
3l	12	10	8	8
DMSO	-	-	-	-
Ciprofloxacin	26	28	30	32

Table 3: Antifungal activity of synthesized compounds

Compound	*A. niger*	*C. albicans*
3a	6	7
3b	8	8
3c	8	9
3d	8	7
3e	9	7
3f	10	10
3g	7	8
3h	9	9
3i	10	10
3j	6	7
3k	8	9
3l	10	9
DMSO	-	-
Fluconazole	26	24

SPECTRAL DATA

5-phenyl-*N*-[(1*E*)-phenylmethylene][1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3a).**

The following spectral data were recorded for compound **3a:** FTIR (KBr) cm^{-1}: 3115 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1590 (-N=CH), 702 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 11.14 (s, 1H, CH); 8.14 (s, 1H, -N=CH); 7.98 (s, 1H, CH); 7.30-7.68 (m, 10H, Ar-H); MS spectrum, *m/z*: 324$[M+1]^+$.

N-[(1*E*)-(2-chlorophenyl)methylene]-5-phenyl[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3b).**

The following spectral data were recorded for compound **3b:** FTIR (KBr) cm^{-1}: 3115 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1598 (-N=CH), 682 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 11.14 (s, 1H, CH); 8.14 (s, 1H, -N=CH); 7.98 (s, 1H, CH); 7.30-7.68 (m, 9H, Ar-H). MS spectrum, *m/z*: 359$[M+1]^+$.

N-[(1*E*)-(4-chlorophenyl)methylene]-5-phenyl[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3c).**

The following spectral data were recorded for compound **3c:** FTIR (KBr) cm^{-1}: 3115 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1582 (-N=CH), 702 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 11.14 (s, 1H, CH); 8.14 (s, 1H, N=CH); 7.98 (s, 1H, CH); 7.15-7.72 (m, 9H, Ar-H); MS spectrum, *m/z*: 358$[M+1]^+$.

5-(4-methylphenyl)-*N*-[(1*E*)-phenylmethylene][1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3d).**

The following spectral data were recorded for compound **3d:** FTIR (KBr) cm^{-1}: 3120 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1582 (-N=CH), 705 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 10.16 (s, 1H, CH); 8.39 (s, 1H, N=CH); 7.86 (s, 1H, CH); 7.25-7.63 (m, 9H, Ar-H); 2.34 (s, 3H, CH_3). MS spectrum, MS spectrum, *m/z*: 339 $[M+1]^+$.

5-(4-methylphenyl)-*N*-[(1*E*)-(2-methylphenyl)methylene][1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3e).**

The following spectral data were recorded for compound **3e:** FTIR (KBr) cm^{-1}: 3110 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1590 (-N=CH), 712 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 10.16 (s, 1H, CH); 8.39 (s, 1H, N=CH); 7.86 (s, 1H, CH); 7.25-7.63 (m, 8H, Ar-H); 2.45 (s, 6H, CH_3). MS spectrum, MS spectrum, *m/z*: 353 $[M+1]^+$.

5-(4-methylphenyl)-*N*-[(1*E*)-(4-methylphenyl)methylene][1,3] thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3f).**

The following spectral data were recorded for compound **3f:** FTIR (KBr) cm^{-1}: 3110 (Ar C-H); 2960 (C-H); 2880 (C-H thiazole); 1585 (-N=CH), 720 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 10.16 (s, 1H, CH); 8.39 (s, 1H, N=CH); 7.86 (s, 1H, CH); 7.25-7.63 (m, 8H, Ar-H); 2.35 (s, 6H, CH_3). MS spectrum, MS spectrum, *m/z*: 352 $[M+1]^+$.

4-(2-{[(1*E*)-phenylmethylene]amino}[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-5-yl)phenol **(3g).**

The following spectral data were recorded for compound **3g:** FTIR (KBr) cm^{-1}: 3545 (O-H); 3005 (ArC-H); 2955 (C-H); 2875 (C-H thiazole); 1515 (-N=CH), 695 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.63 (s, 1H, CH); 7.83 (s, 1H, CH); 8.19 (s, 1H, N=CH); 7.18-7.34 (m, 9H, Ar-H); ; 5.28 (s, 1H, OH). MS spectrum, *m/z*: 341 $[M+1]^+$.

2-[(*E*)-{[5-(4-hydroxyphenyl)[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]imino}methyl]phenol **(3h).**

The following spectral data were recorded for compound **3h:** FTIR (KBr) cm^{-1}: 3515 (O-H); 3005 (ArC-H); 2955 (C-H); 2875 (C-H thiazole); 1550 (-N=CH), 702 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.63 (s, 1H, CH); 7.83 (s, 1H, CH); 8.19 (s, 1H, N=CH); 7.18-7.34 (m, 8H, Ar-H); 5.20 (s, 2H, OH). MS spectrum, *m/z*: 356 $[M+1]^+$.

4-(2-{[(1*E*)-(4-hydroxyphenyl)methylene]amino}[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-5-yl)phenol **(3i).**

The following spectral data were recorded for compound **3i:** FTIR (KBr) cm^{-1}: 3530 (O-H); 3005 (ArC-H); 2955 (C-H); 2875 (C-H thiazole); 1535 (-N=CH), 780 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.63 (s, 1H, CH); 7.83 (s, 1H, CH); 8.19 (s, 1H, N=CH); 7.18-7.34 (m, 8H, Ar-H); 5.28 (s, 2H, OH). MS spectrum, *m/z*: 357 $[M+1]^+$.

5-[4-(dimethylamino)phenyl]-*N*-[(1*E*)-phenylmethylene] [1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3j).**

The following spectral data were recorded for compound **3j:** FTIR (KBr) cm^{-1}: 3110 (ArC-H); 2960 (C-H); 2880 (C-H thiazole); 1590 (-N=CH), 708 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.8 (s, 1H, CH); 9.3 (s, 1H, N=CH); 6.3 (s, 1H, CH); 7.17-7.72 (m, 9H, Ar-H); 1.42-1.47(s, 6H, CH_3). MS spectrum, *m/z*: 368 $[M+1]^+$.

5-[4-(dimethylamino)phenyl]-*N*-[(1*E*)-(2-methoxyphenyl) methylene][1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3k).**

The following spectral data were recorded for compound **3k:** FTIR (KBr) cm^{-1}: 3110 (ArC-H); 2960 (C-H); 2880 (C-H thiazole); 1570 (-N=CH), 710 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.8 (s, 1H, CH); 9.3 (s, 1H, N=CH); 6.3 (s, 1H, CH); 7.80-7.95 (m, 8H, Ar-H); 3.15 (s, 3H, $-OCH_3$); 1.42-1.47(s, 6H, CH_3). MS spectrum, *m/z*: 398 $[M+1]^+$.

5-[4-(dimethylamino)phenyl]-*N*-[(1*E*)-(4-methoxyphenyl) methylene][1,3]thiazolo[4,3-*b*][1,3,4]thiadiazol-2-amine **(3l).**

The following spectral data were recorded for compound **3l:** FTIR (KBr) cm^{-1}: 3110 (ArC-H); 2960 (C-H); 2880 (C-H thiazole); 1585 (-N=CH), 714 (C-S-C). ^1H NMR chemical shifts at (400 MHz, $CDCl_3$, δ ppm): 9.8 (s, 1H, CH); 9.3 (s, 1H, N=CH); 6.3 (s, 1H, CH); 7.80-7.95 (m, 8H, Ar-H); 3.52 (s, 3H, $-OCH_3$); .1.42-1.47(s, 6H, CH_3). MS spectrum, *m/z*: 397 $[M+1]^+$.

The results of antibacterial study for **96a-l** series revealed that the compounds **3b**, **3f, 3h, 3i** and **3j** showed good antibacterial activity against four organisms tested but exhibited less activity than the standard drugs. Compound **3e** and **3i** showed most activity against *S. aureus* and compound **3b** showed better activity against *E. coli*. The remaining compounds showed moderate activity. Results of antifungal activity showed that compounds **3f**, **3i** & **3l** showed better activity against *C. albicans* and A. *niger*. The remaining compounds exhibited less activity than the standard drug.

CONCLUSION

A new series of 5-(Substituted) phenyl-*N*-[(1*E*)-(substituted) phenylmethylene][1,3]thiazolo[4,3 *b*][1,3,4] thiadiazol-2-amine **3a-l** were synthesized in good yield and were evaluated for antibacterial, antifungal and antitubercular activities.

The results of antibacterial study for **3a-l** series revealed that the compounds **3b**, **3f, 3h, 3i** and **3j** showed good antibacterial activity against four organisms tested but exhibited less activity than the standard drugs. The remaining compounds showed moderate activity. Results of antifungal activity showed that compounds **3f**, **3i** & **3l** showed better activity against *C. albicans* and A. *niger*. The remaning compounds exhibited less activity than the standard drug.

In Schiff base series **3a-l,** compound **3f, 3h** and **3i** showed good antitubercular activity compared to standard streptomycin.

In conclusion, various bioactive molecules have been designed and synthesized which exhibited *in vitro* potent antitubercular activity. Further clinical studies may lead to exploration and introduction of new antimicrobial drugs into the market.

ACKNOWLEDGEMENT

The author is very much thankful to President and Principal Maratha Mandal's college of Pharmacy Belgaum for providing facilities to carry out the research work and Maratha mandal's Nathajirao G. Halgekar Institute of Dental sciences and Research Centre, Belgaum for antibacterial and antifungal activities. I also thank to director of SAIF, Chandigarh, Punjab and Karnataka University, Dharwad for providing necessary spectral data.

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Source of support: Nil, Conflict of interest: None Declared