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SYNTHESIS AND *IN-VITRO* ANTIMICROBIAL ACTIVITY OF SOME NOVEL SUBSTITUTED 1,3,4-THIADIAZOLES

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ABSTRACT

A series of new 2-[(substituted benzylidene) imino]-5-(4'-methoxyphenyl)-1,3,4-thiadiazoles have been synthesized by the reaction of the thiosemicarbazide with methoxybenzaldehyde to obtaine 2-amino-5-(4'-methoxyphenyl)-1,3,4-thiadiazole in the presence of aqueous $FeCl_3$, which was then treated with various substituted aromatic aldehydes to yield the corresponding Schiff bases. The synthesized compounds were characterized by physicochemical and spectral data. All the synthesized compounds were screened for their *in-vitro* antimicrobial activity by cup plate diffusion method. It can be inferred from the results that the newly synthesized compounds possessing electron withdrawing groups at the aldehydic phenyl ring exhibits better antimicrobial activity than the compounds with electron donating groups.

Key Words: 1,3,4-Thiadiazole, Schiff bases, Antimicrobial activity.

INTRODUCTION

Thiadiazoles is an important class of heterocyclic compounds, of which specifically 1,3,4-thiadiazole have been of great interest and possesses a wide spectrum of pharmacological properties *viz.* antiproliferative[1], carbonic anhydrase inhibition[2], antibacterial[3-4], anti-helicobacter pylori[5], anti-leishmanial[6], anti-inflammatory and analgesic[7], antimicrobial[7-12], anti-tubercular[12], anti-tumor[12], anti-diabetic[12], diuretic[12], anticonvulsant[12-13], anti-histamine[12], antioxidant[12],as pesticides[12] and antidepressant[12], [14]. Moreover, thiadiazole nucleus occupies a very important place in treatment regimes with antibiotics. The search for novel molecules continues due to the fast development of pathogen resistance towards the

existing molecules. In view of the above observations we have synthesized some new 2-amino-1,3,4- thiadiazole derivatives (Schiff bases) in presumption to produce a new and better antimicrobial agent.

EXPERIMENTAL

Melting points of all the synthesized compounds (**SCHEME1**) were uncorrected. Reactions were monitored by thin layer chromatography (TLC) on pre-coated plates using different solvent systems. The purity of the compounds was ascertained by TLC, using UV lamp as visualizing agent. IR (KBr pellet) was recorded on Perkin Elmer Infra Red Spectrophotometer. ^{1}H NMR spectral data were recorded on Brucker Aveance NMR Spectrometer and the reported chemical shift values were reported in δ (ppm). Maldi MS was used to record Mass Spectra (m/z, %) and the UV spectral data (λ_{max} in nm) were recorded on Shimadzu UV-1602 double beam spectrophotometer.

Synthesis of (E)-1-(4-methoxybenzylidene)-thiosemicarbazide (SD01): A solution of 4-methoxybenzaldehyde (24.20 mL; 0.2 M), in 300 mL warm 95% ethanol and a solution of thiosemicarbazide (18.23 g; 0.2 M), in 300 mL warm water were mixed. The product separated immediately. The reaction mixture was cooled to room temperature, and filtered. The product was recrystallized in glacial acetic acid and the melting point of the product was found to be 174 ºC. The purity of compound was ascertained by TLC using chloroform: ethylacetate mixture (7:3) as mobile phase.

Synthesis of 2-amino-5-(4'-methoxyphenyl)-1,3,4-thiadiazole (SD02) : A mixture of **SD01** (4 g; 0.02 M), water (900 mL), ferric chloride pentahydrate (15 g) was stirred and heated at 80-90ºC for $1^1/_2$ h. The filtrate residue was made alkaline with 10% aqueous ammonia[15-16]. The insoluble material was filtered and dried. Subsequently, it was thoroughly extracted with boiling 95% 20 mL ethanol. Then the extract was concentrated to dryness and the residue was recrystallized from boiling water to give the desired product. The purity of the compound was ascertained by TLC using chloroform: ethanol mixture (7:3) as mobile phase and melting point of the pure compound was found to be 160 ºC.

Synthesis of 2-[(substituted benzylidene) imino]-5-(4'-methoxyphenyl)-1,3,4-thiadiazole (Schiff bases) (SD02a-l): A mixture of **SD02** (0.005 M), the required substituted benzaldehydes (0.005 M) in ethanol (30 to 40 mL) and a catalytic amount of concentrated H_2SO_4 (2 mL) were added[15]. The resultant mixture in each case was then heated under

reflux for 5 h and cooled at room temperature. Then the above solution was added to cold water and stirred for few minutes. The solid separated was filtered and recrystallized from benzene. Melting point and R_f values of all the synthesized compounds are reported in Table 1.

All the synthesized Schiff bases (**SD02a-l)** were screened for their *in-vitro* antibacterial activity against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and *in-vitro* antifungal activity against *Aspergillus niger* and *Candida albicans* by cup plate method at a concentration of 50 µg/mL. The zone of inhibition was measured (in mm) and the average of three readings was calculated and these data are illustrated in Table 1. The activity was compared with Ampicillin and Miconazole Nitrate (50 µg/mL) as standard [4], [8-12], [17-20].

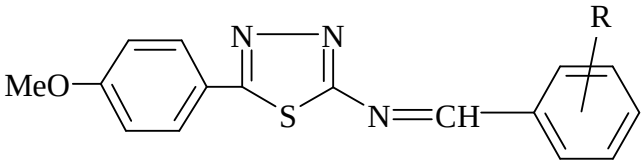
RESULTS AND DISCUSSION

The formation of (E)-1-(4-methoxybenzylidene)-thiosemicarbazide **(SD01)** was confirmed by the presence of specific IR peaks at 3336 cm^{-1} (NH_2), 1254 cm^{-1} (C-O methoxy) and 996 cm^{-1} (C=S), were the formation of 2-amino-5-(4'-methoxyphenyl)-1,3,4-thiadiazole **(SD02)** was confirmed by the presence of specific IR peaks at 3471 cm^{-1} (NH_2); 697 cm^{-1} (C=S), 1253 cm^{-1} (C-O methoxy), ^{1}H NMR spectral data δ = 3.85 (s, 3H, OCH_3); 4.89 (d, 2H, NH_2); 7.00 – 7.69(d, 4H, phenylring) and by the mass spectral data (m/z, %): 208 (100), 192 (15), 177 (14), 165 (10), 151 (13), 162 (11), 101 (12), 133 (22), 119 (16), 100 (9), 107 (8).

The formation and the purity of the Schiff bases 2-[(substituted benzylidene) imino]-5-(4'-methoxyphenyl)-1,3,4-thiadiazole **(SCHEME1: SD02a-l)** were confiormed by the difference in melting points, R_f values, specific IR peaks between 1690-1640 cm^{-1} and ^{1}H NMR spectral data. The physical and spectral data of the entire synthesized compound is illustrated in the Table 1 and Table 2 respectively.

The antimicrobial activity of all the synthesized Schiff bases was carried out by using cup plate diffusion method and the zone of inhibiton was mesaured. The result of the antimicrobial activity of the entire synthesized compound is illustrated in the Table 1. It can be concluded from the results that the antimicrobial potency of the novel compounds are based on the electron donating groups [methoxy (SD02-c, SD02-g, SD02-h), methyl (SD02-j), hydroxyl (SD02-d, SD02-i) dimethyl amino (SD02-b)] and the electron withdrawing

groups [chloro (SD02-a, SD02-k) and nitro (SD02-f, SDO2-l)] substituent attached at 'R'. Finally, it can be inferred from the results that the newly synthesized compounds possessing electron withdrawing groups at the aldehydic phenyl ring exhibits better antimicrobial activity than the compounds with electron donating groups.

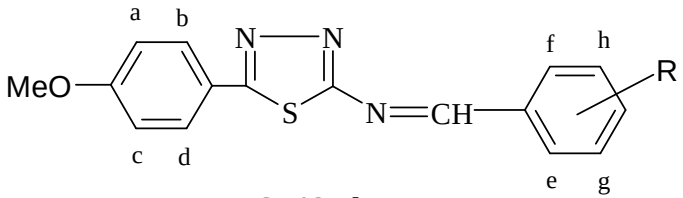


SD02a-l

TABLE 1: Antimicrobial and physical data of compounds SD02a-l:

Compd. Code	R	% yield	R_f	m.p. (°C)	Zone of inhibition (mm)					
					S.a.	***B.s.***	***E.c.***	***K.p.***	***A.n.***	***C.a.***
SD02-a	2-chloro	61.96	0.89	174	08	04	01	NA	09	07
SD02-b	4-dimethyl amino	51.66	0.82	172	03	04	02	03	02	NA
SD02-c	3,4-dimethoxy	64.03	0.81	171	05	04	05	06	08	01
SD02-d	4-hydroxy	66.16	0.87	181	06	05	06	04	05	02
SD02-e	H	45.36	0.77	183	05	03	02	NA	05	NA
SD02-f	3-nitro	61.18	0.86	175	11	09	08	11	10	07
SD02-g	3,4,5-trimethoxy	73.17	0.85	179	04	02	NA	NA	05	03
SD02-h	3-methoxy-4-hydroxy	65.75	0.81	188	05	04	02	06	02	NA
SD02-i	2-hydroxy	53.66	0.83	186	05	06	NA	NA	05	NA
SD02- j	4-methyl	69.84	0.79	197	04	02	04	03	04	NA
SD02- k	4-chloro	52.35	0.91	170	08	09	05	01	09	04
SD02-l	2-nitro	70.25	0.78	180	10	08	11	09	05	02
Ampicillin	----	----	----	----	11	12	19	12	----	----
Miconazole nitrate	----	----	----	----	----	----	----	----	20	15

S.a.= S.aureus, B.s.= B.subtilis, E.c.= E.coli, K.p.= Klebsiella Pneumoniae, A.n.= A.niger, C.a.= C.albicans. NA= No Activiy



SD02a-l

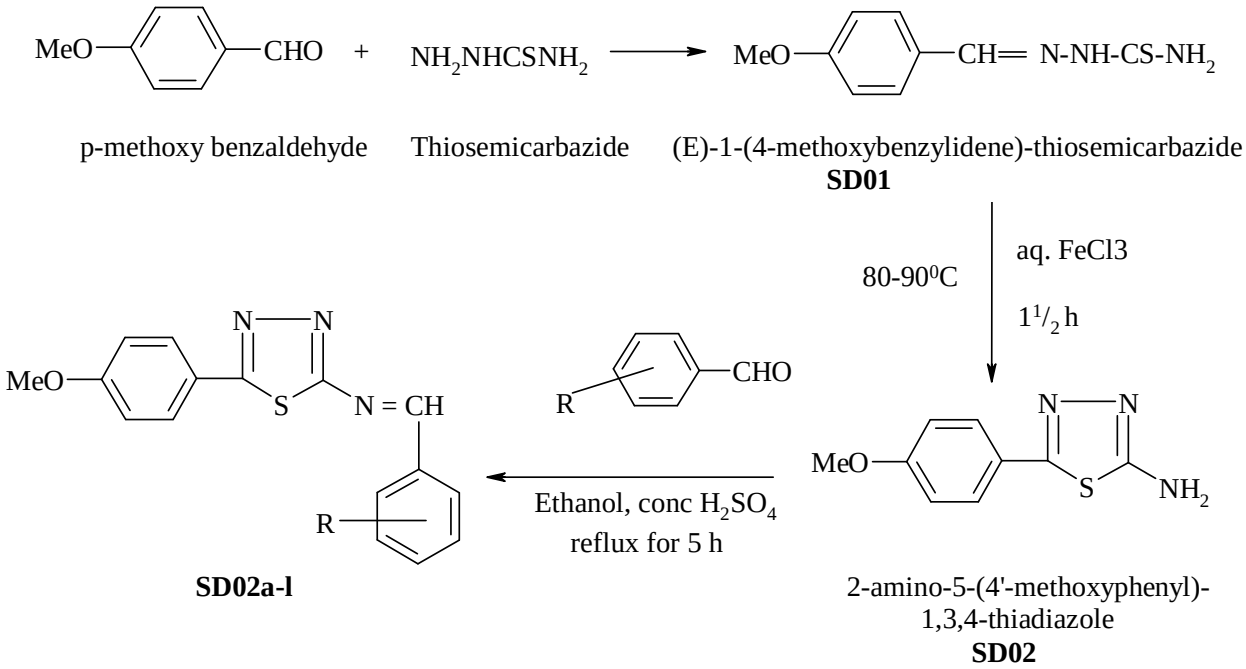
TABLE 2: Spectral data of compounds SD02a-l:

Compd. Code	**R**	**λ_{max} (nm)**	**IR (KBr) cm^{-1}**	**^1H NMR ($CdCl_3$) δ/ppm**
SD02-a	2-chloro	303	2945 (Ali-CH); 1165(C-O); 3083(Ar-H str); 1677 (Ar-C=C); 808 (Ar-H bend); 1689 (C=N); 723 (C-S); 825(C-N); 1618 (N=CH); 507 (C-Cl).	_____
SD02-b	4-dimethyl amino	385	2924 (Ali-CH); 1275(C-O); 3080(Ar-H str); 1556 (Ar-C=C); 825 (Ar-H bend); 1686 (C=N); 776 (C-S); 1650 (N=CH); 1350 (C-N of N-CH_3).	3.01 (s, 6H, N-$(CH_3)_2$); 3.82 (s, 3H, OCH_3); 6.62 (d, 2H, phenylring g,h); 6.87 (d, 2H, phenylring a,c); 7.36 (d, 2H, phenylring b,d); 7.44 (d, 2H, phenylring e,f); 9.13 (s,1H, N=CH).
SD02-c	3,4-dimethoxy	350	2920 (Ali-CH); 1273 (C-O); 3078(Ar-H str); 1540 (Ar-C=C); 798 (Ar-H bend); 1690 (C=N); 746 (C-S); 825 (C-N); 1653 (N=CH).	_____

SD02-d	4-hydroxy	369	2866 (Ali-CH); 1088 (C-O); 3087(Ar-H str); 1553 (Ar-C=C); 832 (Ar-H bend); 1675 (C=N); 834 (C-S); 820 (C-N); 1645 (N=CH); 3626 (O-H).	_____
SD02-e	H	378	2825 (Ali-CH); 1268 (C-O); 3067(Ar-H str); 1540 (Ar-C=C); 840 (Ar-H bend); 1693 (C=N); 43 (C-S); 818 (C-N); 1641 (N=CH).	_____
SD02-f	3-nitro	354	2924 (Ali-CH); 1342 (C-O); 3086(Ar-H str); 1541 (Ar-C=C); 826 (Ar-H bend); 1640 (C=N); 738 (C-S); 813 (C-N); 1657 (N=CH); 1518 (N=O).	_____
SD02-g	3,4,5-trimethoxy	347	2935 (Ali-CH); 1275 (C-O); 3087(Ar-H str); 1556 (Ar-C=C); 804 (Ar-H bend); 1687 (C=N); 746 (C-S); 833 (C-N); 1643 (N=CH).	_____
SD02-h	3-methoxy-4-hydroxy	373	2726 (Ali-CH); 1298 (C-O); 3087(Ar-H str); 1552 (Ar-C=C); 768 (Ar-H bend); 1665 (C=N); 749 (C-S); 825 (C-N); 1642 (N=CH); 3552 (O-H).	_____
SD02-i	2-hydroxy	403	2935 (Ali-CH); 1342 (C-O); 3067(Ar-H str); 1550 (Ar-C=C); 786 (Ar-H bend); 1663 (C=N); 752 (C-S); 825 (C-N); 1644 (N=CH); 3650 (O-H).	_____

SD02- j	4-methyl	383	2846 (Ali-CH); 1338 (C-O); 3051(Ar-H str); 1600 (Ar-C=N); 820 (Ar-H bend); 1678 (C=N); 813 (C-S); 828 (C-N); 1650 (N=CH).	_____
SD02- k	4-chloro	396	2924 (Ali-CH); 1298 (C-O); 3070(Ar-H str); 1553 (Ar-C=C); 809 (Ar-H bend); 1687 (C=N); 817 (C-S); 830 (C-N); 1651 (N=CH); 509 (C-Cl).	3.84(s, 3H, OCH_3); 6.91(d, 2H, phenylring a,c); 7.30(d, 2H, phenylring g,h); 7.38(d, 2H, phenylring b,d); 7.52(d, 2H, phenylring e,f); 9.15 (s, 1H, N=CH).
SD02-l	2-nitro	352	2914 (Ali-CH); 1272 (C-O); 3102(Ar-H str); 1544 (Ar-C=C); 821 (Ar-H bend); 1667 (C=N); 738 (C-S); 824 (C-N); 1646 (N=CH); 1534 (N=O).	_____

SCHEME 1



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