Iranian Journal of Pharmaceutical Sciences
Summer 2010: 6(3): 179-184
ijps.sums.ac.ir

Original Article

Synthesis, Characterization and Study of *In Vitro* Antimicrobial Activity of Some Substituted *N′*-[Arylidene]-2-(5-Phenyl-1H-Tetrazol-1-yl) Acetohydrazide

Popat B. Mohite[a,*], Vaidhun H. Bhaskar[b]

[a]*Department of Pharmaceutical Chemistry, MES College of Pharmacy, Sonai, Ahmednagar, Maharashtra, India*
[b]*MP Patel College of Pharmacy, Kapadwanj, Gujrat, India*

Abstract

Reaction of 5-phenyl tetrazole with ethyl chloroacetate to form ethyl (5-phenyl-1*H*-tetrazol-1-yl) acetate (1). Compound 1 react with hydrazine hydrate in ethanol yield 2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide (2). The condensation of (2) with various aldehydes yield the corresponding substituted *N′*-[-arylidene]-2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide (3a- j). The compounds obtained were identified by spectral data and have been screened for antimicrobial activity. The most promising compounds having good antibacterial activity were 3b, 3c and 3i, and the best for antifungal activity were 3b, 3c and 3e.

Keywords: Antimicrobial activity; Ciprofloxacin; Griseofulvin; Schiff's bases; Tetrazole.
Received: May 1, 2010; *Accepted:* July 20, 2010.

1. Introduction

Tetrazole derivatives possess very interesting pharmacological and biological properties and are reported to exhibit variety of biological activities like antibacterial [1], antifungal [2, 3], analgesic [4], anti-inflammatory [5, 6], and antitubercular effects [7]. Similarly 1,5 substituted tetrazoles have long been known for their pharmacological activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic, antithrombotic and antimicrobial properties. Compounds containing azomethine group (-CH=N-) are known as Schiff bases. Day by day, Schiff bases are more frequently applied for the betterment of human welfare. The importance of the Schiff base is due its versatile nature. Literature survey shows that many Schiff bases exhibit biological activities such as antibacterial and antifungal [8, 9], antitumor [10], anti-inflammatory [11, 12], and anticonvulsant effects [12]. Azole derivatives are well known for their antifungal and antibacterial effects. Various azole derivatives have also shown potent anticancer activity *in vitro* and *in vivo* in mice inoculated with human melanoma cells. Well known antifungal derivatives that are currently being clinically used are vorozole, letrozole, anastrazole,

*Corresponding author:
Popat B. Mohite, MES College of Pharmacy, Sonai, Tal-Newasa, Dist-Ahmednagar, Maharashtra-414105, India
Tel. 09890520854
E-mail- mohitepb@rediffmail.com

Table 1. Physicochemical characterization of *N'*-[-arylidene]-2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide.

Sr. No	R	Molecular Formula	MW	M.P. (°C)	Yield (%)
1	H	$C_{16}H_{14}N_6O$	306	189° C	70%
2	2-Cl	$C_{16}H_{13}ClN_6O$	340	220° C	34%
3	4-Cl	$C_{16}H_{13}ClN_6O$	340	222° C	88%
4	4-Br	$C_{16}H_{13}BrN_6O$	385	245° C	64%
5	4-CH3	$C_{17}H_{16}N_6O$	320	256° C	69%
6	3-OCH3	$C_{17}H_{16}N_6O_2$	336	258° C	78%
7	4-OCH3	$C_{17}H_{16}N_6O_2$	336	260° C	74%
8	3-NO2	$C_{16}H_{13}N_7O_3$	351	198° C	64%
9	4-NO2	$C_{16}H_{13}N_7O_3$	351	199° C	83%
10	(CH3)2-N-	$C_{18}H_{92}N_7O$	349	206° C	78%

ketokonazole and liarozole.

With the discovery of these application of tetrazoles as well as Schiff bases medicinal chemistry and in continuation of our interest in synthesis of tetrazole derivatives, different Schiff bases of 5-phenyl tetrazole in the same matrix have been synthesized to serve as new scaffold for the antimicrobial agents. The present work deals with the reaction of 2-(5-phenyl-^{1}*H*-tetrazol-1-yl) acetohydrazide (2) with different aromatic aldehydes to form Schiff's bases (3a-j). The reaction sequence for titled compounds is outlined in Scheme I. Finally, the structures of all the various synthesized compounds were assigned on the basis of IR and 1H NMR spectral data and these compounds were screened for their *in vitro* antimicrobial activity.

2. Material and methods

2.1. Materials

All chemicals and solvents were purchased from Qualigens. All reactions are carried out at laboratory condition. Melting points were determined with open capillary and uncorrected. FT-IR spectra were recorded on a Shimadzu FT-IR model 8010 spectrophotometer, ^{1}H NMR spectra were recorded in DMSO on a Varian mercury FT-NMR model YH- 300 instrument using TMS as internal standard.

2.2. General procedure for synthesis of ethyl (5-phenyl-1H-tetrazol-1-yl)acetate (1)

An equimolar mixture of 5-phenyl tetrazole (0.03 mol, 5 g), ethyl chloroacetate (0.03 mol, 3.67 ml) and anhydrous potassium carbonate (0.03 mol, 3.76 g) in methanol (40 ml) was refluxed on a water bath for 4 h and cooled to the room temperature. The product obtained was filtered, dried and recrystalized from ethanol. The compound was separated as white amorphous powder.

2.3. General procedure for synthesis of 2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide (2)

To a mixture of compound 1, (0.03 mol, 9 g) in methanol (40 ml), 99% hydrazine hydrate (0.03 mole, 1.95 ml) was added with continuous stirring to get clear solution. Reflux the reaction mixture on water bath for about 5 h. The solution was concentrated and allowed to cool overnight. The resulting solid obtained was filtered, washed with cold ethanol, dried and recrystalized from ethanol. The compound was separated as white powder.

2.4. General procedure for the synthesis of N'-[-arylidene]-2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide (3a- j)

Equimolar quantity of the hydrazide compound (2, 0.009 mol) and various aromatic aldehydes (0.009 mol) in ethanol and dioxane (50 ml) were heated on a water bath for 8 h. The resulting Schiff's bases (3a-j) were cooled and poured into crushed ice. The precipitate thus obtained was filtered

Table 2. Spectral characterization of N'-[-arylidene]-2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide.

Sl No.	R	IR (KBr) cm-1	^{1}H NMR (DMSO D6,) δ ppm
3a	H	3430(-NH), 3054(Ar-CH), 2376,2247(-NCH2), 1650 (-CO), 1625(-N=CH-),	9.27 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 10H, Ar), 5.56 (s, 2H, -CH2)
3b	2-Cl	3442(-NH), 3054(Ar-CH), 2378,2242(-NCH2), 1652 (-CO), 1623(-N=CH-),785(C-Cl)	9.20 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2).
3c	4-Cl	3445(-NH), 3054(Ar-CH), 2378,2243(-NCH2), 1652 (-CO), 1623(-N=CH-),785(C-Cl)	9.20 (s, 1H, NH) , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2).
3d	4-Br	3435(-NH), 3054(Ar-CH), 2380,2247(-NCH2), 1656 (-CO), 1620(-N=CH-),697(C-Br).	9.27 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2).
3e	4-CH_3	3428 (-NH), 3054(Ar-CH), 2370,2240(-NCH2), 1655 (-CO), 1625(-N=CH-).	9.24 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2),2.88(s,3H,CH3).
3f	3-OCH_3	3450(-NH), 3054(Ar-CH), 2372,2240(-NCH2), 1656 (-CO), 1627(-N=CH-),1165(-OCH3).	9.25 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.50 (s, 2H, -CH2),4.02(s,3H,-OCH3)
3g	4-OCH_3	3442(-NH), 3054(Ar-CH), 2371,2238(-NCH2), 1656 (-CO), 1627(-N=CH-), 1165(-OCH3).	9.23 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.50 (s, 2H, -CH2),4.02(s,3H,-OCH3
3h	3-NO_2	3430(-NH), 3054(Ar-CH), 2376,2249(-NCH2), 1653 (-CO), 1628(-N=CH-),1564 (-NO2).	9.22 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2).
3i	4-NO_2	3438(-NH), 3054(Ar-CH), 2376,2248(-NCH2), 1656 (-CO), 1628(-N=CH-),1564 (-NO2).	9.30 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2).
3j	(CH3)2-N	3425(-NH),3054(Ar-CH), 3155(-N(CH3)2). 2380,2250(-NCH2), 1651 (-CO),1626(-N=CH-).	9.20 (s, 1H, NH), , 7.95 (s, 1H, N=CH).7.91- 6.80 (m, 9H, Ar), 5.56 (s, 2H, -CH2),2.44 (s,6H,-N(CH3)2)

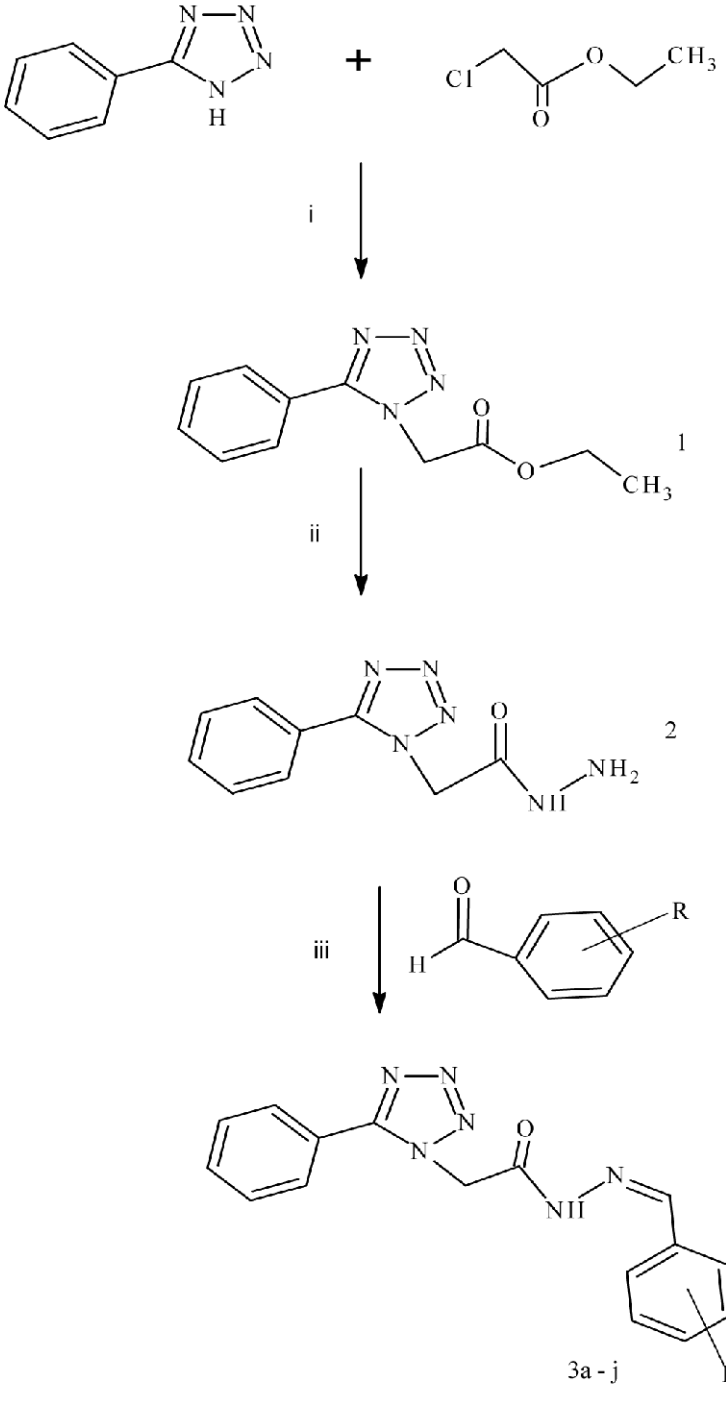
washed with cold water and purified by recrystallized from ethanol (Compound 3a,MS:(m/z):306(M+).

2.5. Antimicrobial activity

All of the newly synthesized compounds (3a-j) were screened for antimicrobial activity against both gram positive *S. aureus* and gram negative *E .coli* bacteria and antifungal activity against *C. albicans* and *A. niger* according to cup plate method at concentrations of 50 and 100 µg/ml. Ciprofloxacin and griseofulvin were used as standard for comparison of antibacterial and antifungal activity. Solvent dimethyl sulphoxide (DMSO) was used as control.

3. Results and discussion

In the course of our search for therapeutically useful antimicrobial agent, we have prepared new series of Schiff bases of 5-phenyl tetrazole by reaction of 2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide with aromatic aldehyde in ethanol: dioxane.



Scheme I. Synthesis of some substituted *N'*-[-arylidene]-2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide: *Where R:* H, , 2-Cl ,4-Cl, 4-Br, 4-CH3, 3-OCH_3,4-OCH_3,3-NO2, 4-NO_2, 4-N $(CH_3)_2$, ***Reagents and conditions:*** i. methanol, K_2CO_3 reflux 2 h; ii. NH_2NH_2, methanol, reflux 5 h; iii. aryl Aldehydes, abs. EtOH:Dioxane, reflux 8h.

Table 3. Antibacterial and antifungal activity data of the syntesyzed Schiff bases.

Comp.	Zone of inhibition in mm							
	S. aureus		*E. coli*		*C. albicans*		*A. niger*	
	50 µg/ml	100µg/ml	50 µg/ml	100µg/ml	50 µg/ml	100µg/ml	50 µg/ml	100µg/ml
3a	13	15	10	12	12	15	10	12
3b	15	16	15	17	18	20	15	17
3c	15	16	14	15	18	20	13	15
3d	11	14	10	12	16	18	11	13
3e	12	17	08	10	19	22	20	22
3f	12	15	08	11	12	15	08	11
3g	13	15	10	11	13	15	10	12
3h	12	13	10	12	15	17	09	11
3i	13	17	09	13	12	14	11	12
3j	13	14	12	15	11	13	11	14
Ciprofloxacin	20	24	20	24	-	-	-	-
Griseofulvin	-	-	-	-	20	24	20	24

5-Phenyl tetrazole reacted with ethyl chloroacetate to form ethyl (5-phenyl-1*H*-tetrazol-1-yl) acetate which on further reaction with hydrazine hydrate in ethanol yield 2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide. The series contain ten analogues. All of the compounds were prepared in good yields. The results of physicochemical properties are depicted in Table 1. The structure confirmation of synthesized compounds was done by IR, NMR spectroscopy and results of analysis were in good agreement with the structure of synthesized compounds. Results of spectral data are summarized in Table 2.

From the results of antibacterial screening in Table 3, it is evident that most of the compounds are very weakly active and few are moderately active against *Staphylococcus aureus* and *Escherichia coli* but compounds 3b, 3c, 3e and 3i possess very good activity against *Staphylococcus aureus* and Escherichia coli at concentration of 100 µg/ml. Similarly from the results of antifungal screening, it is evident that the compounds 3b, 3c and 3e possess very good activity against fungi *Candida albicans* and *Aspergillus niger*, and compound 3d showed moderate activity all bacteria and fungi tested.

4. Conclusion

We prepared a series of tetrazole Schiff bases and demonstrated that these compounds possessed good antibacterial and antifungal activities tested by cup plate method. The most promising compounds having good antibacterial and antifungal activity were 3b, 3c and 3i and 3b, 3c and 3e, respectively.

Acknowledgments

We are highly thankful to Professor V.K. Deshmukh, Principal of M.E.S. College of Pharmacy, Sonai and Prashant Patil Gadakh, Secreatary, Mula Education Society for providing excellent research facilities.

References

[1] Mulwad VV, Pawar Rupesh B, Chaskar Atul C. Synthesis and antibacterial activity of new tetrazole derivatives. *J Korean Chem Soc* 2008; 52: 249-56.

[2] Rajasekaran A, Sankaranarayanan M, Rajagopal KA. Antibacterial, antifungal and anticonvulsant evaluation of novel newly synthesized 1-[2-(1 H -tetrazol-5-yl)ethyl]-1 H-benzo[d][1,2,3]triazoles. *Arch Pharm Res* 2006; 29: 535-40.

[3] Upadhayaya RS, Jain S, Sinha N, Kishore N, Chandra R, Arora SK. Synthesis of novel substituted tetrazoles having antifungal activity. *Eur J Med Chem* 2004; 39: 579-92.

[4] Bachar SC, Lahiri SC. Synthesis of chloro and bromo substituted 5-(indan-1′-yl) tetrazoles and 5-(indan-1′-yl) methyltetrazoles as possible analgesic agents. *Pharmazie* 2004; 59: 435-8.

[5] Ray SM, Lahiri SC. Studies on 5-(indan-1′-yl)tetrazoles as potential non-steroidal anti-inflammatory agents. *J Indian Chem Soc* 1990; 67: 324-6.

[6] Aliasghar J, Khalili D, Clercq ED, Salmi C,

Brunel JM. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. *Molecules* 2007; 12:1720-30.

[7] Adamec J, Waisser K, Kunes J, Kaustova J. A note on the antitubercular activities of 1 aryl-5-benzyl-sulfanyltetrazoles. *Arch Pharm* 2005; 338: 385-9.

[8] Mohammed IA, Subrahmanyam EVS. Synthesis, characterization and antimicrobial activity of some substituted N'-arylidene-2-(quinolin-8-yloxy) aceto hydrazides. *Acta Pharm Sinic* 2009; 51: 163-8.

[9] Sari N, Arslan S, Loğoğlu E, Sakiyan İ. Antibacterial activites of some new amino acid-Schiff bases. *Gazi Univ J Sci* 2003; 16: 283-8.

[10] Saidul İM, Akhter FM, Bodruddoza MAK. Antineoplastic activity of 2-oxo benzylidine (3-oxo aniline) Cu (II) ethylene diamine. *Pakistan J Biol Sci* 2002; 5: 335-7.

[11] Iana V, Emanuela T, Francesca M, Fabio S. Aromatic Schiff bases and 2,3-disubstituted-1,3-thiazolidin-4-one derivatives as anti-inflammatory agents. *Arkivoc* 2004; 5: 364-74.

[12] Manjusha V, Surendra NP, Krishna NS, James SP. Anticonvulsant activity of Schiff bases of isatin derivatives. *Acta Pharm* 2004; 54: 49-56.

[13] Mohite PB, Pandhare RB, Khanage SG, Bhaskar VH. Synthesis and anti-inflammatory activity of some 5-phenyl-1-(acyl)-1,2,3,4-tetrazole. *J Pharm Res* 2010; 3: 43-6.

[14] Mohite PB, Pandhare RB, Khanage SG, Bhaskar VH, A novel approach for synthesis of substituted tetrazoles. *Digest J Nanomat Biostruct* 2009; 4: 803-7.

[15] Menozzi G, Mosti L, Fossa P, Musiu C, Murgioni C, La Colla P. Synthesis and biological evaluation of azole derivatives, analogues of bifonazole, with phenylisoxazolyl or phenylpyrimidinyl moiety. *Farmaco* 2001; 56: 633-40.

[16] Menozzi G, Mosti L, Bruno O, Lo Presti E, Musiu C, Longu S, La Colla P, Filippelli W, Falcone G, Piucci B. Synthesis and biological evaluation of [a-(1,5-disubstituted 1H-pyrazolyl-4-yl)benzyl]azoles, analogues of bifonazole. *Farmaco* 1999; 54: 416.

[17] Liu LT, Lin YCh, Wang CLJ, Lin MS, Yen SCh, Chen HJ. Synthesis and biological activities of novel class of azole containing antifungal agents. *Bioorg Med Chem Lett* 1996; 6: 1335-8.

[18] Fringuelli R, Schiaffella F, Bistoni F, Pitzurra L, Vecchiarelli A. Azole derivatives of 1,4-benzothiazine as antifungal agents. *Bioorg Med Chem* 1998; 6: 103-8.