J. Serb. Chem. Soc. 69 (12) 991-998 (2004)
JSCS-3227

UDC 542.913+54.02:547.576+547.77
Original scientific paper

Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine

YOGESH KUMAR VAGHASIYA[a], RATHISH NAIR[a], MAYUR SONI[b*], SHIPRA BALUJA[b] and SUMITRA CHANDA[a*]

[a]*Department of Biosciences and* [b]*Department of Chemistey, Saurashtra University, Rajkot 360005, India (e-mail: sumitrachanda@yahoo.com)*

(Received 11 February 2004)

Abstract: Schiff bases derived from 4-aminoantipyrine and vanillin were evaluated for their potential as antibacterial agents against some Gram positive and Gram negative bacterial strains. The antibacterial activity was studied against *P. pseudoalcaligenes* ATCC 17440, *P. vulgaris* NCTC 8313, *C. freundii* ATCC 10787, *E. aerogenes* ATCC 13048, *S. subfava* NCIM 2178 and *B. megaterium* ATCC 9885. The determination of the antibacterial activity was done using the Agar Ditsh method. The Schiff bases produced were: (**1**) 4-(4-hydroxy-3-methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one [VV1]; (**2**) 4-(benzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one [VY2]); (**3**) 4-[(furan-3-ylmethylene)amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one [VY3]; (**4**) 4-(4-methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one [VY4]; (**5**) 2-methoxy-4-[(4-methoxyphenylimino)methyl]phenol [VY5]; (**6**) 4-[(2,4-dimethylphenylimino)methyl]-2-methoxyphenol [VY6]); (**7**) 2-methoxy-4-(naphthalene-1-yliminomethyl)phenol [VY7] and (**8**) 4-[(4-hydroxy-3-methoxybenzylidene)amino]-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide [VY8]. The antibacterial activity was evaluated in two polar solvents, DMSO and DMF. The Schiff bases derived from vanillin as the central molecule with 2,4-dimethylaniline and sulphamethoxazole as the side chain in DMSO effectively inhibited the investigated bacteria and appear to be promising antimicrobial agents.

Keywords: Schiff bases, antibacterial activity, DMSO, DMF.

INTRODUCTION

Schiff bases are characterized by the –N=CH– (imine) group which is important in elucidating the mechanism of transamination and racemisation reactions in biological systems.[1,2] Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviour studied.[3] They have been synthesized from a variety of compounds, such as amino thiazoles, 2-hydroxy-1-napthalaniline, amino sugars, aromatic aldehydes,

* Author for correspondence

isatin, the triazole ring, thiosemicarbazides, amino acids, pyrazolone, *etc.*[4–8] Literature survey shows that Schiff bases show bacteriostatic and bactericidal activity.[9] Antibacterial, antifungal, antitumor, anticancer activity has been reported and they are also active against a wide range of organisms, *e.g. C. albicans, E. coli, S. aureus, B. polymyxa, P. viticola*, *etc.*[10-12] Many Schiff bases are known to be medicinally important and are used to design medicinal compounds.[13–14]

The present paper describes the synthesis of some new Schiff bases from 4-aminoantipyrine and vanillin and reports on their antibacterial activity. The synthesized compounds were tested for antibacterial activity against *Pseudomonas pseudoalcaligenes* ATCC 17440, *Proteus vulgaris* NCTC 8313, *Citrobacter freundii* ATCC 10787, *Enterobacter aerogenes* ATCC 13048, *Staphylococcus subfava* NCIM 2871 and *Bacillus megaterium* ATCC 9885. The determination of antibacterial activity was done using the Agar Ditsh method. The antibacterial activity was evaluated in two solvents, dimethyl sulphoxide and *N,N*-dimethylformamide. Dimethyl sulphoxide (DMSO) is a versatile non-aqueous dipolar aprotic solvent having a dielectric constant of 46.6 (25 °C) and a dipole moment of 3.9 D (25 °C). It is a highly polar but aprotic solvent, which can mix very well with any liquid. It is also called a super solvent and exhibits quite interesting properties. *N,N*-Dimethylformamide (DMF) is a very good aprotic protophilic medium for organic and inorganic substances.[15] It is one of the most important solvents in analytical chemistry and for practical purposes. Its dielectric constant and dipole moment are 36.71 (25 °C) and 3.86 D (25 °C), respectively.

Considering the aforesaid, in the present paper, the synthesis of some Schiff bases, their structural determination by spectral analysis and their antimicrobial analysis are reported.

EXPERIMENTAL

The following Schiff bases were synthesized:

1. 4-(4-Hydroxy-3-methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one [VY1]
2. 4-(Benzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one [VY2]
3. 4-[(Furan-3-yl-methylene)amino]-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one [VY3]
4. 4-(4-Methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one [VY4]
5. 2-Methoxy-4-[(4-methoxyphenylimino)methyl]phenol [VY5]
6. 4-[(2,4-Dimethylphenylimino)methyl]-2-methoxyphenol [VY6]
7. 2-Methoxy-4-(naphthalene-1-yl-iminomethyl)phenol [YV7]
8. 4-[(4-Hydroxy-3-methoxybenzylidene)amino]-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide [VY8].

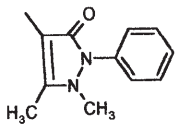
Synthesis of Schiff bases derived from 4-aminoantipyrine

To the required amount of aldehyde dissolved in 200 ml methanol was added 0.1 mol of amine and few drops of glacial acetic acid, which acts as a catalyst. The mixture was refluxed for 10–12 h at 70–80 °C in a water bath. The resulting solution was cooled to room temperature, and then poured onto crushed ice with constant stirring. The precipitate was filtered off and washed with sodium bisulfite solution to remove the excess aldehyde. The product was crystallized from hot methanol and dried.

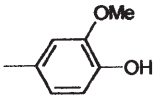
$$R\text{-}CHO + R'\text{-}NH_2 \rightarrow R\text{-}CH{=}N\text{-}R'$$

In this reaction, R'-NH_2 is 4-aminoantipyrine, and R is a given.

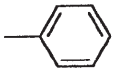
R'=



VY1, R=

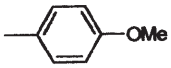


VY2, R=



VY3, R=

VY4, R=



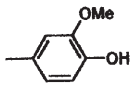
Synthesis of Schiff bases derived from vanillin

To 15.2 g of vanillin dissolved in 200 ml methanol were added 0.1 mol of the required aniline derivative and a few drops of glacial acetic acid. The mixture was refluxed for 10–12 h at 70–80 °C. The mixture was then poured onto crushed ice with constant stirring, filtered and dried.

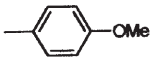
$$R\text{-}CHO + R'NH_2 \rightarrow R\text{-}CH{=}N\text{-}R'$$

In this reaction R is vanillin and R' is as indicated:

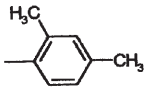
R=



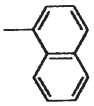
VY5, R'=



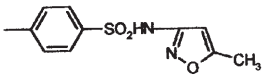
VY6,R'=



VY7,R'=



VY8,R'=



Test microorganisms

The bacterial strains studied are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The investigated microorganisms were *P. pseudoalcaligenes* ATCC 17440, *P. vulgaris* NCTC 8313, *C. freundii* ATCC 10787, *E. aerogenes* ATCC 13048, *S. subfava* NCIM 2871 and *B. megaterium* ATCC 9885.

Preparation of the test compound

The compounds were dissolved at a concentration of 10 mg/ml in either of the two solvents (DMSO or DMF) in order to obtain a final concentration of 1 mg/0.1ml, 0.01mg. The synthesized Schiff bases are soluble only in DMF, 1,4-dioxane and DMSO.

From these solvents, two, *i.e.*, DMSO and DMF, were selected for the present study.

Preparation of the plates and microbiological assays

A loop full of the given test strain was inoculated into 25ml of N-broth (Nutrient Broth) and incubated for 24 h in an incubator at 37 °C in order to activate the bacterial strain. A petri-dish of 100 mm diameter was filled with 28–30 ml of Mueller Hinton Agar No.2 media. Inoculation was performed by the Pour-plate technique. 0.2 ml of the activated strain was inoculated into the media when it had reached a temperature of 40–45 °C. The complete procedure of the ditch preparation was done in a laminar airflow to maintain strict sterile and aseptic condition. The media was allowed to solidify. After solidification of the media, a well was made in the media with the help of a cup-borer (0.85 cm) and then 0.1 ml of the synthetic compound (dissolved in DMSO/DMF) was inoculated into the well. Controls were performed (for each bacterial strain and each solvent), where 0.1 ml of the pure solvent was inoculated into the well. The plates were incubated for 24 h at 37 °C. The inhibition zone formed by the compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds. The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample.

DISCUSSION

In total 8 compounds were synthesized. Analysis of their IR and NMR spectral data confirmed their molecular sructure. Their data are given below.

VY1: IR (KBr, cm^{-1}): –OH (str.): 3412,–OCH_3:2852, C=O (str.): 1640,–C=N: 1581, –OH (bend.): 1381, –C-H (def) asym: 1377.
^{1}H-NMR(δ, ppm): 2.46 (3H, –CH_3), 3.10 (3H, N–CH_3), 6.91–7.31 (8H, Ar–H), 9.6 (1H, N=CH).

VY2: IR (KBr, cm^{-1}): N–CH_3 (str.): 3160, C=O (str.): 1644, –C=N: 1595, C=C: 1580, –C–H (def) asym: 1414, –C–H (def.) sym: 1362.
^{1}H-NMR(δ, ppm): 2.49 (3H,–CH_3), 3.15 (3H,–OCH_3), 7.39–7.66 (10H, Ar–H), 9.76 (1H, N=CH).

VY3: IR (KBr, cm^{-1}): –C-H (str.) asym: 2923, –C–H (str.) sym: 2854, C=O (str.): 1649, –N=C (str.)1593.
^{1}H-NMR (δ, ppm): 2.47 (3H,–CH_3), 3.45 (3H, N–CH_3), 6.46–6.56: (8H, Ar–H), 7.45: (1H, N=CH).

VY4: IR (KBr, cm^{-1}): –O–CH_3 : 2832, N–CH_3: 3049, N=C: 1647, C=C (Ar.): 1593.
^{1}H-NMR (δ, ppm): 2.46 (3H,–CH_3), 3.10 (3H, N–CH_3), 3.65 (3H, N–CH_3), 6.44–7.69 (9H, Ar–H), 9.66(1H, N=CH).

VY5: IR (KBr, cm^{-1}): –OH (str.): 3470, –C=N: 1622, –C=C: 1589, –OH (bend.): 1379, C–C (o.o.p.d.): 710.
^{1}H-NMR (δ, ppm): 3.66 (3H,–OCH_3), 3.93 (3H,–OCH_3), 6.35 (1H, = CH), 6.92–7.23 (7H, Ar–H), 9.76 (1H, –OH).

VY6: IR (KBr, cm^{-1}): –OH (str.): 3400,–C=N: 1620, –OH (bend): 1433, –C–H (def) sym: 1369, C–C (o.o.p.d.): 702.

^{1}H-NMR (δ, ppm): 2.46 (3H, –CH_3), 2.49 (3H, –CH_3), 3.93 (3H, –OCH_3), 6.36 (1H, = CH), 6.70–7.11 (6H, Ar–H), 9.70 (1H, –OH).

VY7: IR (KBr, cm^{-1}): –OH (str.): 3430,–OCH_3(str.): 2852, –C=N(str.): 1624,–C=C(str): 1595, –OH (bend.): 1398.
^{1}H-NMR (δ, ppm): 2.30 (3H,–OCH_3), 6.61–7.59 (12H, Ar–H), 9.76 (1H, =CH), 10.42 (1H, –OH).

VY8: IR (KBr, cm^{-1}): –OH(str.): 3440,–C=N: 1715, –OH (bend): 1390, S=O (str) asym: 1170, –N-H(def) 1635.
^{1}H-NMR (δ, ppm): 3.54 (3H, –CH_3), 3.63 (1H,–NH), 3.97 (3H, –OCH_3), 6.36–7.74 (9H, Ar–H), 9.60 (1H, =CH), 10.42 (1H,–OH).

The molecular formula, molecular weights, melting points, % yields and R_f values along with the solvent systems of the 8 Schiff bases are given in Table I.

TABLE I. Analytical and physical data of the investigated compounds

Compound code	Molecular formula	Molecular weight/g mol^{-1}	M.p. °C	Yield %	R_f value	Solvent system
VY1	$C_{15}H_{15}NO_3$	257.28	138	58	0.61	EA:H 4:6
VY2	$C_{16}H_{17}NO_2$	255.31	142	61	0.50	EA:H 4:6
VY3	$C_{18}H_{15}NO_2$	277.32	108	58	0.53	EA:H 4:6
VY4	$C_{18}H_{17}N_3O_5S$	387.41		63	0.48	EA:H 4:6
VY5	$C_{19}H_{19}N_3O_3$	337.37	194	67	0.30	EA:H 3:7
VY6	$C_{18}H_{17}N_3O$	291.35	163	56	0.60	EA:H 3:7
VY7	$C_{16}H_{15}N_3O_2$	281.31	216	45	0.38	EA:H 3:7
VY8	$C_{19}H_{19}N_3O_2$	321.37	159	59	0.48	EA:H 2:8

EA = Ethyl acetate; H = Hexane

All the compounds had different antibacterial activity *in vitro* against the tested bacterial strains in different solvents. The controls were deducted from the tested compounds; their effect was noticeably different depending on the type of the solvent used. The antibacterial activity of the Schiff bases synthesized from 4-aminoantipyrine and vanillin against the Gram positive bacteria *S. subfava* and *B. megaterium* are shown in Fig. 1. The inhibitory activity was greater in DMSO than in DMF. Of these Gram positive bacteria, *S. subfava* was more resistant to the synthesized compounds than *B. megaterium*. The compounds synthesized from 4-aminoantipyrine did not produce any inhibitory zone against *S. subfava* in either of the used solvents; while of the compounds synthesized from vanillin, only VY6 in both the solvents and VY7 in DMSO showed inhibitory activity. Considering the Gram positive *B. megaterium*, DMF was ineffective while almost all the compounds in DMSO produced inhibitory zones. The most active compounds were VY1, VY2 and VY6 followed by VY7 and VY3; the compounds VY4, VY5 and

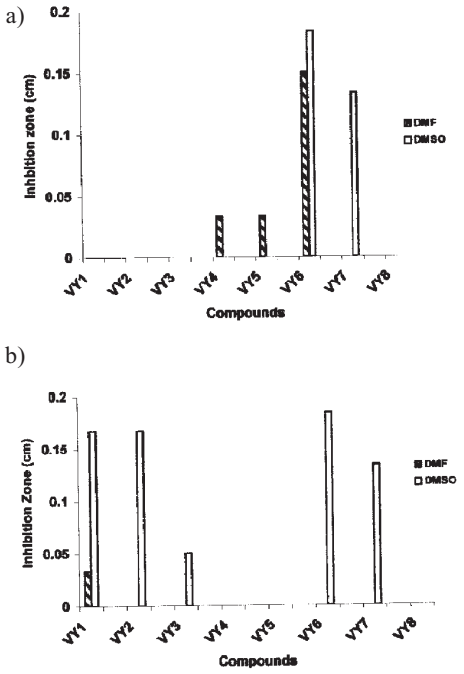


Fig. 1. Antibacterial activity of some synthetic compounds against: a) *S. subfava*, b) *B. megaterium*.

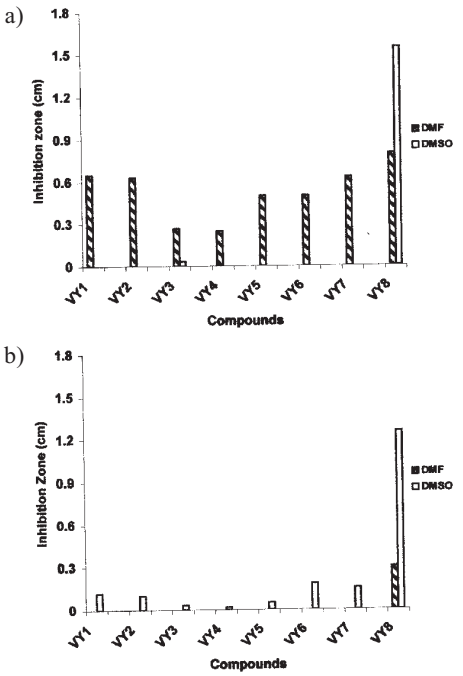


Fig. 2. Antibacterial activity of some synthetic compounds against: a) *P. pseudoalcaligenes*, b) *P. vulgaris*.

VY8 completely failed to inhibit this microorganism. These differences in the inhibitory activity are because of molecular diversity. In the present investigation, two central ligands were used. In VY1-VY4, the central ligand was 4-aminoantipyrine while in VY5-VY8 it was vanillin. All eight compounds had different side chains *viz* in VY1 it was vanillin, in VY2 it was benzaldehyde, in VY3 it was furfuraldehyde, in VY4 it was *p*-anisaldehyde, in VY5 it was *p*-anisidine, in VY6 it was 2,4-dimethylaniline, in VY7 it was α-naphthylamine and in VY8 it was sulphamethoxazole. From the above it can be concluded, that these two Gram positive bacteria were more inhibited when the central ligand was vanillin than when it was 4-aminoantipyrine. The side chains 2,4-dimethylaniline and alpha–naphthylamine are the best and the solvent DMSO is better than DMF. The interaction between the solvent and the Schiff base plays an important role in inhibiting these bacterial strains.

In vitro antibacterial evaluation of the eight synthesized compounds against *P. pseudoalcaligenes* and *P. vulgaris* are shown in Fig. 2. All the compounds dissolved in DMF showed considerable inhibitory zones against *P. pseudoalcaligenes*. The compounds VY5-VY8 were more active than the compounds VY1-VY4. The compound VY8 was the only compound in DMSO which showed a high inhibition zone while all the other 7 compounds were ineffective in DMSO. An entirely different trend was observed with *P. vulgaris*. The compounds extracted in DMF did

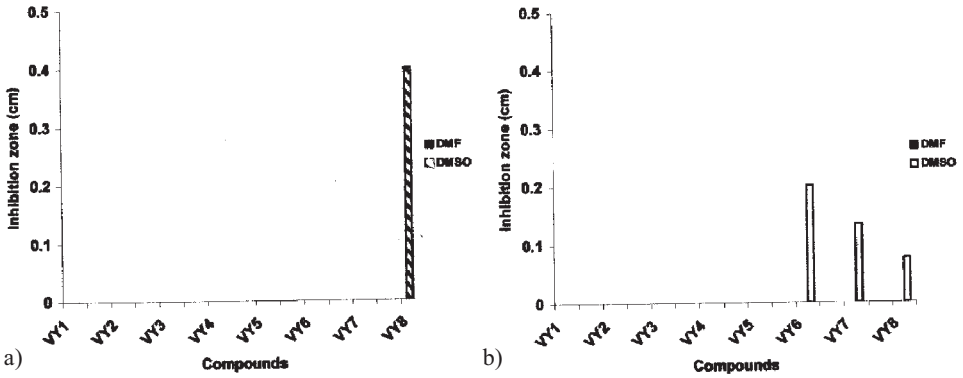


Fig. 3. Antibacterial activity of some synthetic compounds against: a) *C. freundii*, b) *E. aerogenes*.

not inhibit this Gram negative bacteria. Also when dissolved in DMSO only VY8 showed a great inhibitory activity. Neither central ligands nor the attached side chains had a differential effect on *P. pseudoalcaligenes, i.e.*, all produced almost same inhibitory activity, except VY8 in DMF, while only VY8 in DMSO could inhibit *P. vulgaris*, *i.e.*, the sulphamethoxazole side chain with vanillin as the central ligand proved to be best for inhibiting this bacteria.

The differential effect of the compounds dissolved in the employed polar solvents in inhibiting the Gram negative bacteria *E. aerogenes* and *C. freundii* are shown in Fig. 3. The compounds VY1-VY4 irrespective of whether they were dissolved in DMF or DMSO did not produce any inhibitory zones against either of the bacteria. VY8 in DMF was the only Schiff base that showed inhibitory activity against *C. freundii* while compounds VY6-VY8 showed inhibitory activity against *E. aerogenes* when dissolved in DMSO. The other compounds did not inhibit either of the studied bacteria.

CONCLUSIONS

From the results given above, it can be concluded that vanillin is more effective than 4-aminoantipyrine and DMSO proved to be a better solvent than DMF. Of the side chains, 2,4-dimethylaniline and sulphamethoxazole are the best and can be used as leading molecules in drug design *i.e.* in inhibiting these medically important bacterial strains. *p*-Anisidine, *p*-anisaldehyde and furfuraldehyde as side chains did not produce any inhibitory activity. This once again proves the earlier conclusion that antibacterial activity is dependent on the molecular structure of the compound, the employed solvent and the bacterial strain under consideration. Such screening of various organic compounds and identifying the active agents is essential because the successful prediction of a lead molecule and drug-like properties at the onset of drug design will pay off later in drug development.

ИЗВОД

СИНТЕЗА ДЕРИВАТА ВАНИЛИНА И 4-АМИНОАНТИПИРИНА, ЊИХОВЕ СТРУКТУРЕ И АНТИБАКТЕРИЈСКА АКТИВНОСТ

YOGESH KUMAR VAGHASIYA[1], RATHISH NAIR[1], MAYUR SONI[2], SHIPRA BALUJA[2] и SUMITRA CHANDA[1]

[1]Department of Biosciences and [2]Department of Chemistry, Saurashtra University, Rajkot 360005, India

Испитана су антибактеријска деловања Шифових база изведених од ванилина и 4-аминоантипирина на неке грам-позитивне и грам-негативне бактерије, и то: *P. pseudoalcaligenes ATCC 17440, P. vulgaris NCTC 8313, C. freundii ATCC 10787, E. aerogenes ATCC 13048, S. subfava NCIM 2178* и *B. megaterium ATCC 9885*. Добијене су следеће Шифове базе: (**1**) 4-(4-хидрокси-3-метокси-бензилиденамино)-1,5-диметил-2-фенил-1,2-дихидро-пиразол-3-он [VY1]; (**2**) 4-(бензилиден-амино)-1,5-диметил-2-фенил-1,2-дихидро-пиразол-3-он [VY2]; (**3**) 4-[(фуран-3-илметилен)-амино]-1,5-диметил-2-фенил-1,2-дихидро-пиразол-он [VY3], (**4**) 4-(4-метокси-бензилиденамино)-1,5-диметил-1,2-фенил-1,2-дихидро-пиразол-3-он [VY4]; (**5**) 2-метокси-4-[(4-метокси-фенилимино)-метил]-фенол [VY5]; (**6**) 4-[(2,4-диметил-фенилимино)-метил]-2-метокси-фенол [VY6]; (**7**) 2-метокси-4-(нафтален-1-илиминометил)-фенол [VY7] и (**8**) 4-[(4-хидрокси-3-метокси-бензилиден)-амино]-*N*-(5-метил-изоксазол-3-ил)-бензенсулфонамид [VY8]. Антибактеријска активност испитивана је у два паралелна растварача DMSO и DMF. Шифове базе са ванилином као централним молекулом и 2,4-диметиланилином и сулфаметоксазолом као бочним гранама у DMSO ефикасно инхибирају испитиване бактерије и представљају обећавајуће антибактеријске агенсе.

(Примљено 11. фебруара 2004)

REFERENCES

1. K. Y. Lau, A. Mayr, K. K. Cheung, *Inorg. Chim. Acta* **285** (1999) 223
2. A. S. Shawali, N. M. S. Harb, K. O. Badahdah, *J. Heterocyclic Chem.* **22** (1985) 1397
3. N. Raman, V. Muthuraj, S. Ravichandran, A. Kulandaisamy, *Proc. Ind. Acad. Sci*. **115** (2003) 161
4. S. K. Sridhar, A. Ramesh, *Ind. J. Chem. Soc.* **41** (2002) 668
5. Z. Wei, C. Qiong, H. Chao-gang, *Huazhong Shifan Daxue. Xuebao Zirankexue ban* **36** (2002) 478
6. Y. Dong-Dong, J. Yan Lan, S. Lu, *Chinese J. Chem.* **19** (2001) 1136
7. P. Piotr, B. Bogumil, *Biopolymers* **67** (2002) 61
8. Rh. Miao, Li. Shuoliong, Y. Rudong, V. L. Y. Welbing, *Ind. J. Chem.* **42** (2003) 318
9. Z. Yuxia, Z. Tao, M. Wanshan, Z. Haibin, C. Suifeng, *Hauxue Shiji* **24** (2002) 117
10. M. A. Gawad, Y. M. Issa, S. M. Abd-Alhamid, *Egypt J. Pharm. Sci* **34** (1993) 219
11. V. V. Mulwad, J. M. Shirodkar, *Ind. J. Hetrocyclic Chem.* **11** (2002) 199
12. N. Sari, S. Arslan, E. Logoglu, I. Sariyan, *G. U. J. Sci **16*** (2003) 283
13. S. Rao, A. S. Mitra, *J. Ind. Chem. Soc.* **55** (1978) 420
14. S. A. Khan, A. A. Siddiqui, B. Shibeer, *Asian J. Chem.* **14** (2002) 117
15. A. Marchetti, C. Preti, M. Tagliazucchi, L Tassil, G. Tosi, *J. Chem. Eng. Data* **36** (1991) 360.