Journal of Enzyme Inhibition and Medicinal Chemistry, April 2005; 20(2): 183–188

In-vitro antibacterial, antifungal and cytotoxic properties of sulfonamide—derived Schiff's bases and their metal complexes

ZAHID H. CHOHAN[1,†], MAHMOOD-UL-HASSAN[2], KHALID M. KHAN[3] & CLAUDIU T. SUPURAN[4]

[1]*Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan,* [2]*Department of Chemistry, Islamia University, Bahawalpur, Pakistan,* [3]*HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan, and* [4]*University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia 3, Rm. 188, Polo Scientifico, 50019, Sesto Fiorentino, Firenze, Italy*

(Received 25 August 2004; accepted 1 December 2004)

Abstract

A series of new antibacterial and antifungal Schiff's bases derived from sulfonamides, as well as their transition metal complexes incorporating cobalt (II), copper (II), nickel (II) and zinc (II) were synthesized, characterized and screened for their *in-vitro* antibacterial activity against six Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysentriae*) and four Gram-positive (*Bacillus cereus*, *Corynebacterium diphtheriae*, *Staphylococcus aureous* and *Streptococcus pyogenes*) bacterial strains and for *in-vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, *Candida glaberata*. The results of these studies show the metal complexes to be more antibacterial and antifungal as compared to the uncomplexed Schiffs' bases. The brine shrimp bioassay was also carried out to study the *in-vitro* cytotoxic properties of these synthesized ligands and their complexes.

Keywords: *Sulfonamides, metal complexes, antibacterial, antifungal, cytotoxicity*

Introduction

Sulfonamides constitute an important class of drugs [1] with several types of pharmacological actions. Their significance appeared when sulfanilamide, an important analogue of sulfonamide, was reported [2] to be the first metabolite of an antibacterial drug. Later on, a large number of sulfanilamide derivatives were synthesized, characterised and tested for antibacterial [3], antitumor [4], anti-carbonic anhydrase [5,6], diuretic [7,8], hypoglycaemic [9], antithyroid [10] or protease inhibitory activity [11,12] among others. Sulfanilamide thus became the basis for the development of all other types of medicinally important compounds with a varied spectrum of biological action. Further extension to this area highlighted their significant role in enhancing the biological activity by the formation of first silver (I) complex of sulfanilamide metal from the sodium salt of sulfanilamide and silver nitrate [13]. Then other metal complexes of substituted sulfanilamides were subsequently investigated for biological activity in detail [14]. These studies attracted the attention of researchers and thus, several heterocyclic/aromatic sulfonamides such as acetazolamide, methazolamide, ethoxzolamide, dichlorophenamide, dorzolamide, or brinzolamide [6,15] were used as ligands for complexation [16–22] and their further use as potential biologically active compounds. Taking the above into account, we have also commenced a program to explore further into this potential area of research by designing and investigating

Correspondence: C. T. Supuran, University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia 3, Rm. 188, Polo Scientifico, 50019, Sesto Fiorentino, Firenze, Italy. Tel. 39 055 4573005. Fax. 39 055 4573385. E-mail: claudiu.supuran@unifi.it

† E-mail: zchohan@mul.paknet.com.pk

ISSN 1475-6366 print/ISSN 1475-6374 online © 2005 Taylor & Francis Ltd
DOI: 10.1080/14756360500043257

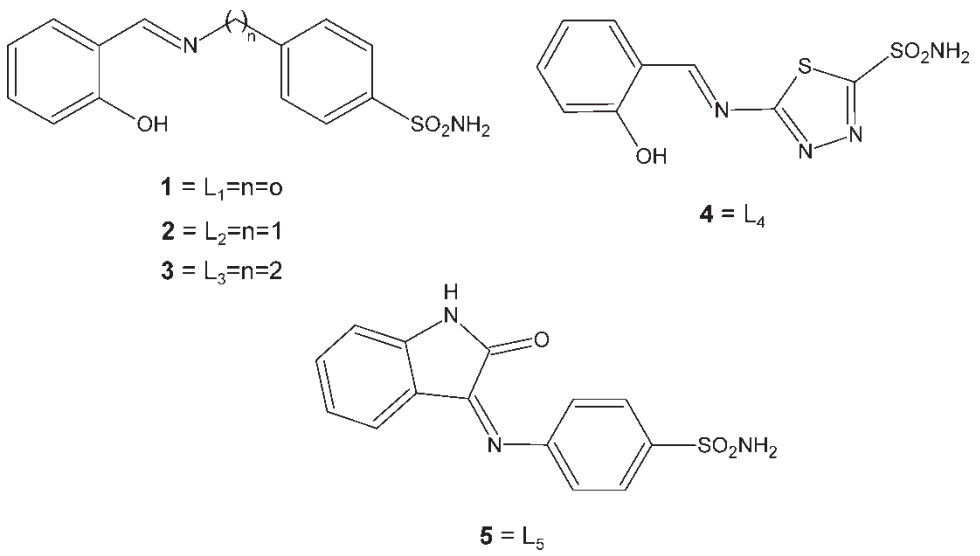

Figure 1. Structure of Schiff's Bases

[23] metal complexes of newly synthesized compounds. In this respect, we have recently reported [24] the synthesis and evaluation as CA inhibitors of Schiff's base transition metal [Co(II), Cu(II), Ni(II) and Zn(II)] complexes of sulfanamide-derived salicylaldehyde compounds that were obtained from sulfonilamide analogues i.e. homosulfanilamide, 4-aminoethyl-benzene-sulfonamide, and 1,3,4-thiadiazole-2-sulfonamide (**1**–**4**) and sulfonamide-derived isatin (**5**) Figure (1). These were characterized by standard procedures and assayed as inhibitors of the physiologically relevant CA isozymes: hCA I, and hCA II. We have also studied antibacterial/antifungal activity and cytotoxic properties of these compounds and their Co(II), Cu(II), Ni(II) and Zn(II) complexes (**6-25**) which had not been investigated before for such activities. The results of these studies show the metal complexes to be more antibacterial and antifungal as compared to their uncomplexed Schiffs' bases.

Materials and methods

Solvents used were analytical grades; all metal (II) were used as chloride salts. IR, NMR,UV-Visible spectra, C, H and N analyses, Conductance and Magnetic measurements were carried out on solid compounds using the respective instruments and reported [24] by us elsewhere. In-vitro antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of Schiff's Base (5)

To an ethanolic (25 mL) solution of sulfonilamide (1.2 g, 0.007 moles) an ethanol solution of isatin (1 g, 0.007 moles) was added with stirring. Then 1 2 drops of conc. H_2SO_4 were added and the mixture refluxed for 2 h. After cooling to room temperature, the solution was filtered and left overnight at room temperature, which led to the formation of a crystallized product. The crystallized product thus obtained was filtered and recrystallized from ethanol: chloroform (50%) to give the orange-red desired product (68%). Purification was checked by TLC, which indicated a single spot. All other Schiff's bases (**1**–**4**) were prepared following the same method as reported elsewhere [23,24].

Preparation of coordination compounds (6–25)

To a hot ethanol (25 mL) solution of sulfonamide **1**–**5** (0.02 moles) an aqueous solution of the corresponding metal(II) salt (0.01 M) was added. The mixture was refluxed for 3 h and the obtained solution filtered and reduced to half of its volume by evaporation of the solvent *in vacuo*. The concentrated solution was left overnight at room temperature, which led to the formation of a solid product. This mixture was filtered and the solid washed with ethanol (2 × 15 mL) and dried. Recrystallization from 50% aqueous ethanol gave the desired product. Unfortunately only microcrystalline powders could be obtained, which could not be used for X-ray structural determinations. In fact this is the usual technical problem related to the thorough characterization of this type of metal complex [19].

Antibacterial bioassay (in-vitro)

The synthesized compounds were screened for antibacterial activity against six Gram-negative bacterial strains *i.e. Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae* and four Gram-positive

bacterial strains *i.e. Bacillus cereus*, *Corynebacterium diphtheriae*, *Staphylococcus aureous* and *Streptococcus pyogenes* using the agar well diffusion method [25,26]. Two to eight hours old bacterial inoculums containing approximately $10^4 - 10^6$ colony forming units (CFU)/ml were used in these assays. The wells were dug into the media with the help of a sterile metallic borer with centres at least 24 mm. Recommended concentration (100 μl) of the test sample (1 mg/ml in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and the reference antibacterial drug, imipenum, served as negative and positive controls respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO or DMF in the biological screening, separate studies were carried out with the solutions alone of DMSO and DMF and they showed no activity against any bacterial strains.

Antifungal activity (in-vitro)

Antifungal activities of all compounds were studied against six fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspension and transferred to petri plates. Discs soaked in 20 ml (10 μg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for seven days. The results were recorded [27] as zones of inhibition (mm) and compared with those for the standard drugs miconazole and amphotericin B.

Cytotoxicity (in-vitro)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22x32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMF. From this stock solutions 500, 50 and 5 μg/ml were transferred to 9 vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when the shrimp larvae were ready, 1 mL of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 mL per vial. After 24 h the number of survivors was counted [28,29]. Data were analyzed by Finney computer program to determine the LD_{50} values [30].

Results and discussion

The Schiff's bases **1**–**5**, prepared from aromatic/heterocyclic sulfonamides and salicylaldehyde, [22] were shown to behave as a moderate CAIs against isozyme I, and as much more effective CA II inhibitors [24]. Since metal complexes of heterocyclic/aromatic sulfonamides have recently been shown [10–25] to possess even stronger inhibitory properties, it appeared of interest to synthesize some metal complexes of these Schiff's bases, mainly due to their interesting donor system, comprising the nitrogen incorporated in the Schiff's base moiety as well as the phenolic OH group. Thus, the Co(II), Cu(II), Ni(II) and Zn(II) complexes (Table I) of ligands **1**–**5** have been obtained. It should be noted that similarly to other ligands investigated in previous studies as metal complexing sulfonamides with CA inhibitory properties [19], the compound investigated here acts as a neutral ligands, and not in the deprotonated state. These ligands and their complexes were thoroughly characterized and reported earlier [24] but were studied only for their CA inhibitory properties.

Antibacterial bioassay

All compounds were tested against six Gram-negative (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi* and *S. dysenteriae*) and four Gram-positive (*B. cereus*, *C. diphtheriae*, *S. aureous* and *S. pyogenes*) bacterial strains (Table I) according to literature protocol. [25,26] The results were compared with those of the standard drug imipenum. Compound **1** exhibited significant activity against *K. pneumoniae*, *E. coli*, *P. aeruginosa,* and *S. dysentriae*, a moderate activity against *P. mirabilis*, *S. pyogenes*, *S. typhi* and *S. aureous* and, a weak activity against *B. cereus* and *C. diphtheriae*. Compounds **2** and **3** showed enhanced activity against the same strains as compared to their analogue compound **1**. It was interesting to note that a methylene and ethylylene carbon chain in these compounds (**1**–**3**) had an impact on the bactericidal activity. As the carbon chain increased bactericidal activity was also increased. Compound **4** showed a significant activity against both Gram-positive and Gram-negative strains. Compound **5** showed a significant activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae* and *S. aureous;* a moderate activity against *P. mirabilis*, *C. diphtheriae* and *S. pyogenes* and, a weak activity against *B. cereus*. The structure-activity relationship studies (SAR) suggested that compound **4** with thiadiazole moiety was generally found to be more active against a wide

Table I. *In-vitro* antibacterial activity data for Schiff's bases (**1**–**5**) and their metal (II) complexes (**6**–**25**).

		Diameter of zones showing complete inhibition of growth (mm)									
Compound		*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*	*(g)*	*(h)*	*(j)*	*(k)*
1	L_1	16	17	11	16	12	17	06	05	10	11
2	L_2	18	18	14	17	16	18	08	10	12	13
3	L_3	20	20	16	18	18	19	12	13	14	15
4	L_4	22	22	18	19	19	20	15	14	15	17
5	L_5	16	14	10	15	20	14	06	10	11	15
6	$Co(L_1)_2Cl_2$	20	24	21	20	21	22	18	18	18	19
7	$Co(L_2)_2Cl_2$	22	23	20	22	22	21	20	16	17	20
8	$Co(L_3)_2Cl_2$	22	22	21	20	22	21	19	17	20	20
9	$Co(L_4)_2Cl_2$	13	23	19	20	21	22	18	18	21	19
10	$Co(L_5)_2Cl_2$	22	24	21	20	22	20	20	19	20	20
11	$Cu(L_1)_2Cl_2$	23	24	22	24	23	22	20	20	18	21
12	$Cu(L_2)_2Cl_2$	23	25	20	22	23	22	20	18	19	21
13	$Cu(L_3)_2Cl_2$	24	23	23	23	22	20	21	20	20	20
14	$Cu(L_4)_2Cl_2$	22	22	24	24	21	21	20	18	18	20
15	$Cu(L_5)_2Cl_2$	22	24	24	22	21	23	22	16	18	19
16	$Ni(L_1)_2Cl_2$	23	25	22	25	21	22	23	15	18	19
17	$Ni(L_2)_2Cl_2$	20	24	22	23	23	22	21	16	19	18
18	$Ni(L_3)_2Cl_2$	22	23	22	24	22	22	23	15	20	18
19	$Ni(L_4)_2Cl_2$	24	24	20	23	22	21	20	16	18	19
20	$Ni(L_5)_2Cl_2$	23	25	24	22	22	21	19	17	18	18
21	$Zn(L_1)_2Cl_2$	23	24	22	22	21	24	20	15	20	20
22	$Zn(L_2)_2Cl_2$	24	25	21	22	23	22	21	17	17	19
23	$Zn(L_3)_2Cl_2$	22	23	19	22	21	24	20	15	18	20
24	$Zn(L_4)_2Cl_2$	24	23	19	22	22	23	21	15	17	20
25	$Zn(L_5)_2Cl_2$	23	24	20	22	21	23	20	18	20	19
Imipenum		30	25	30	32	30	34	32	30	35	35

Ligand: $>$ 15 mm = significant activity; 7–14 mm = moderate activity; $<$ 7 mm = weak activity.
(a) = *Escherichia coli*, *(b)* = *Klebsiella pneumoniae*, *(c)* = *Proteus mirabilis*, *(d)* = *Pseudomonas aeruginosa*, *(e)* = *Salmonella typhi*, *(f)* = *Shigella dysentriae*, *(g)* = *Bacillus cereus*, *(h)* = *Corynebacterium diphtheriae*, *(j)* = *Streptococcus pyogenes*, *(k)* = *Staphylococcus aureous*. Imipenum = standard drug.

range of Gram-positive and Gram-negative bacteria as compared to other compounds. Compounds (**1**–**3**) were found less active than compound 4 and slightly more active as compared to compound **5**.

Cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes (**6**–**25**) of these synthesized ligands/compounds (**1**–**5**) were also screened against the same bacterial Gram-negative and Gram-positive strains. It was evident that overall potency of the uncoordinated compounds was enhanced on coordination with the metal ions.

Antifungal bioassay

The antifungal screening of all compounds was carried out against *Trichophyton longifusus*, *Candida albican*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberate* fungal strains according to the literature protocol [27]. The results were compared with those from the standard drugs miconazole and amphotericin B. These results illustrated in Table II indicate that compound **1** exhibited a significant activity against *T. longifusus*, *F. solani*, *C. albican*, *M. canis* and *A. flavus;* and a moderate activity against *C. glaberata*. Compound **2** showed a significant activity against all fungal species except a moderate activity against *C. albicans*. Compound **3** showed a significant activity against all species. Compound **4** showed a significant activity against *T.longifusus*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata* and, a moderate activity against *C. albican*. Compound **5** showed a significant activity against *T. longifusus*, *A. flavus M. canis,* and *C. glaberata*; a moderate activity against *C. albicans* and a weak activity against *F. solani*. The metal (II) complexes (**6**–**25**) of these compounds relatively showed much enhanced activity as compared to the uncoordinated compounds.

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [29]. From the data recorded in Table III, it is evident that only two compounds, **4** and **5** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive in this assay. Compound **4** showed maximum activity (LD_{50} = 110.2 μg/ml) in the present series of compounds, whereas the other active compound **5** of the series demonstrated slightly lesser activity (LD_{50} = 125.1 μg/ml) than compound **4**. Structure-activity relationships (SAR) in compounds (**1**–**3**) revealed that cytotoxicity increased with the increase in the number

Table II. *In-vitro* antifungal activity data for Schiff's bases (**1**–**5**) and their metal (II) complexes (**6**–**25**).

	Diameter of zones showing complete inhibition of growth (mm)					
Compound	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*
1	26	15	29	24	22	10
2	28	12	30	23	20	25
3	26	24	30	23	18	20
4	27	12	27	25	20	22
5	27	11	28	23	06	20
6	27	18	31	24	24	25
7	28	18	30	22	23	24
8	26	16	31	23	22	26
9	28	15	29	24	25	27
10	26	16	27	27	24	26
11	25	15	29	24	25	25
12	27	14	29	22	27	25
13	26	16	30	23	25	27
14	27	17	29	24	24	25
15	26	18	27	22	25	27
16	27	20	31	25	26	26
17	28	18	32	23	24	26
18	28	14	32	24	26	27
19	28	13	30	25	27	28
20	26	15	34	22	26	27
21	26	11	34	22	25	28
22	26	16	34	22	26	27
23	26	14	34	22	27	29
24	26	11	34	22	25	27
25	26	11	34	22	27	28
Miconazole	30	20	36	20	30	30
Amphotericin B	32	25	32	27	30	35

Ligand: >14 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity [15].
(a) = *Trichophyton longifusus*, *(b)* = *Candida albicans*, *(c)* = *Aspergillus flavus*, *(d)* = *Microsporum canis*, *(e)* = *Fusarium solani*, *(f)* = *Candida glaberata*. Miconazole and Amphotericin B = standard drugs.

of methylene groups. It was also observed that the cytotoxic properties of these compounds decreased on coordination with the metal ions.

The enhancement in antibacterial and antifungal activity on coordination with the metal ions is probably due to the presence of donor systems in the uncoordinated compounds and may inhibit enzyme production, since the enzymes, which require these groups for their activity, appear to be especially more susceptible to deactivation upon coordination/chelation. Chelation reduces the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalisation within the whole chelate ring system thus formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn favours its permeation through the lipoid layer of the membrane [31–35]. It has also been observed that some moieties such as the azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit [36–40] extensive biological activities that may be responsible for the increase in hydrophobic character

Table III. Brine shrimp bioassay data for Schiff's bases (**1**–**5**) and their metal(II) complexes (**6**–**25**).

Compound	LD_{50} (μg/ml)
1	630.5
2	585.2
3	552.6
4	110.2
5	125.1
6	945,1
7	988.4
8	967.2
9	987.1
10	958.6
11	955.8
12	978.3
13	1000.2
14	987.6
15	945.7
16	955.9
17	923.4
18	982.1
19	990.3
20	965.3
21	961.9
22	988.1
23	1000.3
24	1000.1
25	989.7

and liposolubility of the molecules in crossing the cell membrane of the micro-organism and hence enhance the biological utilization ratio and activity of the compounds.

References

[1] Domagk G. Deut Med Wochensch 1935;61:250–253.
[2] Mandell GL, Petri WA. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman's and Gilman's the Pharmacological basis of therapeutics. 9th ed. New York: McGraw-Hill; 1996. p 1057–1072.
[3] Drew J. Science 2000;287.
[4] Owa T, Nagasu T. Exp Opin Ther Patents 2000;10:1725–1740.
[5] Supuran CT, Scozzafava A. Exp Opin Ther Patents 2000; 10:575–600.
[6] Supuran CT, Scozzafava A. Curr Med Chem-Imm., Endoc. Metab. Agents 2001;1:61–97.
[7] Maren TH. Annu Rev Pharmacol Toxicol 1976;16:309–327.
[8] Boyd AE. Diabetes 1988;37:847–850.
[9] Thornber CW. Chem Soc Rev 1979;8:563–580.
[10] Ogden RC, Flexner CW. Protease Inhibitors in AIDS Therapy. New York, U.S.A: Marcel Dekker; 2001.
[11] Supuran CT, Scozzafava A, Mastrolorenzo A. Exp Opin Therap Patents 2001;11:221–259.
[12] Scozzafava A, Supuran CT. J Med Chem 2000;43:3677–3687.
[13] Brown CE, Towle JL. J Am Chem Soc 1941;63:3523.
[14] Bult A. In: Sigel H, Sigel A, editors. Metal ions in biological systems. New York: M. Dekker; 1983. p 261–268, and references cited therein.
[15] Supuran CT, Scozzafava A, Casini A. Med Res Rev 2003;23:146–189.
[16] Alzuet G, Ferrer S, Borras J, Supuran CT. Roum Chem Quart Rev 1994;2:283–300.
[17] Supuran CT, Manole G, Andruh M. J Inorg Biochem 1993;49:97–103.

[18] Supuran CT. Rev Roum Chim 1992;37:849–855.
[19] Sumalan SL, Casanova J, Alzuet G, Borras J, Castiñeiras A, Supuran CT. J Inorg Biochem 1996;62:31–39.
[20] Supuran CT, Almajan GL. Main Group Met Chem 1995;18:347–351.
[21] Supuran CT. Metal Based Drugs 1995;2:327–330.
[22] Supuran CT, Scozzafava A. J Enz Inhib 1997;12:37–51.
[23] Ul-Hassan M, Scozzafava A, Chohan ZH, Supuran CT. J Enz Inhib Med Chem 2001;16:499–505.
[24] Ul-Hassan M, Scozzafava A, Chohan ZH, Supuran CT. J Enz Inhib Med Chem 2004;19:263.
[25] Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay Techniques for Drug Development. The Netherlands: Harwood Academic Publishers; 2001. p 16.
[26] Khan KM, Rahat S, Choudhary MI, Atta-ur-Rahman, Ghani US, Perveen S, Khatoon A, Dar Malik A. Helv Chim Acta 2002;85:559.
[27] Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay techniques for drug development. The Netherlands: Harwood Academic Publishers; 2001. p 22.
[28] McLaughlin JL, Chang C-J, Smith DL. In: Atta-ur-Rahman, editor. Studies in natural products chemistry, "BenchTop" bioassays for the discovery of bioactive natural products: An update, structure and chemistry (part-B). Vol. 9. The Netherlands: Elsevier Science Publishers B.V.; 1991. p 383.
[29] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Planta Medica 1982;45:31.
[30] Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
[31] Chohan ZH, Pervez H, Rauf A, Supuran CT. Metal-Based Drugs 2002;8:42.
[32] Hassan MU, Chohan ZH, Supuran CT. Main Group Metal chemistry 2002;25:291.
[33] Chohan ZH, Scozzafava A, Supuran CT. J Enz Inhib Med Chem 2003;18:259.
[34] Chohan ZH, Scozzafava A, Supuran CT. J Enz Inhib Med Chem 2002;17(4):261.
[35] Chohan ZH, Supuran CT, Scozzafava A. J Enz Inhib Med Chem 2003;18:1.
[36] Chohan ZH. Synth React Inorg Met-Org Chem 2004;34:833.
[37] Chohan ZH, Supuran CT, Scozzafava A. J Enz Inhib Med Chem 2004;19:79.
[38] Chohan ZH, Khan KM, Supuran CT. Appl Organomet Chem 2004;18:305.
[39] Chohan ZH, Scozzafava A, Supuran CT. Synth React Inorg Met-Org Chem 2003;33:241.
[40] Chohan ZH, Pervez H, Scozzafava A, Supuran CT. J Chem Soc Pak 2003;25:308.