European Journal of Medicinal Chemistry 44 (2009) 3330–3339

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Mono and bis-6-arylbenzimidazo[1,2-*c*]quinazolines: A new class of antimicrobial agents

Rondla Rohini [a], Kanne Shanker [a], Puchakayala Muralidhar Reddy [a,b], Yen-Peng Ho [b,*], Vadde Ravinder [a,*]

[a] Department of Chemistry, Kakatiya University, Warangal 506 009, Andra Pradesh, India
[b] Department of Chemistry, National Dong Hwa University, Hualien 974, Taiwan

ARTICLE INFO

Article history:
Received 5 February 2009
Received in revised form
15 March 2009
Accepted 19 March 2009
Available online 27 March 2009

Keywords:
Mono, bis-6-arylbenzimidazo[1,2-*c*]quinazolines
Synthesis
Antibacterial activity
Antifungal activity

ABSTRACT

With the aim of obtaining novel biologically active compounds, we have synthesized a series of mono, bis-2-*o*-arylideneaminophenylbenzimidazoles and a second series of corresponding mono, bis-6-arylbenzimidazo[1,2-*c*]quinazolines respectively. The target benzimidazo[1,2-*c*]quinazoline compounds were obtained by the condensation of 2-(*o*-aminophenyl)benzimidazole with mono and di carbonyl compounds, followed by oxidative cyclisation of the resulting mono and bis-2-*o*-arylideneaminophenylbenzimidazoles.All the products were characterized via IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The antimicrobial activities of all quinazolines against various bacteria and fungi were evaluated. Among the compounds tested **IVd**, **IVe** exhibited good antibacterial and antifungal activities while **IIIb**, **IIIc** also showed notable antimicrobial activity with reference to standard drugs Ampicillin and Ketoconazole respectively.

1. Introduction

In the broad class of heterocyclic compounds, the nitrogen heterocycles play an important role. Among them, quinazolines are most important class of compounds and have received much attention from synthetic as well as medicinal chemists, because of the diverse range of their pharmacological properties [1–8] and also in several areas as materials in electronics, in electrochemistry as anticorrosive agents, as polymers or optical materials and fluorescent tags in DNA sequencing [9–12]. Previous reports described benzimidazo and benzothiazoloquinazoline derivatives as cytotoxic compounds with potential antitumoral [13–17] and particularly, there is one report that describes the antitumor activity of benzimidazo[1,2-*c*]quinazolines [17]. It was reported that benzimidazo[1,2-*c*]quinazoline derivatives show various therapeutic activities, such as anticancer [14,16,17], antiviral [18,19], antimicrobial [20], anti-inflammatory [13,18] and anticonvulsants [21]. Based on the importance of these molecules, our attention was attracted towards synthesis of novel quinazoline derivatives in order to find more potent biologically active molecules. In this paper, we describe the synthesis and characterization of both mono, bis-2-*o*-arylideneaminophenylbenzimidazoles (**Ia–e** & **IIa–e**) and mono, bis-6-substituted benzimidazo[1,2-*c*]quinazolines (**IIIa–e** & **IVa–e**). In addition, the antimicrobial activities of all synthesized quinazolines against different bacteria and fungi were evaluated. Among the compounds tested some of quinazolines were found to be superior in inhibiting all the bacterial and fungal strains.

2. Chemistry

In the present investigation, the starting compound, 2-(*o*-aminophenyl)benzimidazole (**A**) was prepared according to the procedure described by Heins et al. [22]. The target compounds **IIIa–e**, **IVa–e** were prepared by using the reaction sequence in Scheme 1. Initially, we synthesized ten 2-*o*-arylideneaminophenylbenzimidazoles (**Ia–e** & **IIa–e**) via the condensation between 2-(*o*-aminophenyl)benzimidazole (**A**) and various mono (Ar–CHO), di carbonyl compounds (CHO–Ar–CHO). At last, we prepared mono, bis-6-arylbenzimidazo[1,2-*c*]quinazolines through oxidative cyclisation [23] of corresponding 2-*o*-arylideneaminophenylbenzimidazoles.

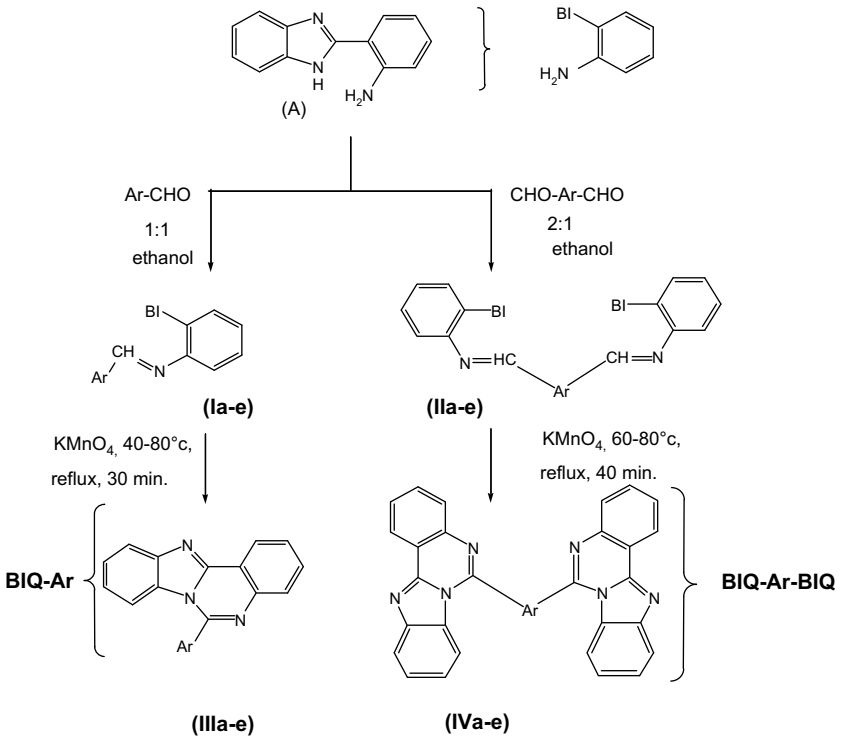
3. Pharmacology

3.1. Antibacterial activity

All the quinazolines prepared herein were screened for their potential biological activities such as, antibacterial activity against

* Corresponding authors. Tel.: +91 9390100594.
E-mail addresses: ypho@mail.ndhu.edu (Y.-P. Ho), ravichemku@rediffmail.com (V. Ravinder).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.03.022



Ia, IIIa. Ar = phenyl
Ib, IIIb. Ar = 2-pyridyl
Ic, IIIc. Ar = 2-thienyl
Id, IIId. Ar = 2-furyl
Ie, IIIe. Ar = 2-pyrrolyl

IIa, IVa. Ar = 1,2 phenyl
IIb, IVb. Ar = 1,3 phenyl
IIc, IVc. Ar = 1,4 phenyl
IId, IVd. Ar = 2,6 pyridyl
IIe, IVe. Ar = 2,5thienyl

Scheme 1. Synthetic route of mono, bis-benzimidazo[1,2-*c*]quinazolines.

Staphylococcus aureus, *Bacillus subtilis*, *Streptococcus pyogenes* (Gram positive) and *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumonia* (Gram negative) bacterial strains by agar diffusion method [29,30]. Ampicillin was used as a reference standard. Preliminary screening for ten quinazolines was performed at fixed concentrations of 1000 μg/ml. Screening results are summarized in Table 1. The antibacterial screening revealed that all the tested compounds **IIIa**–**e** and **IVa**–**e** showed moderate to good inhibition towards all bacterial strains. Out of ten quinazolines, the four compounds **IIIb**, **IIIc** and **IVd**, **IVe** were found to be very effective based on the obtained values of relative zone of inhibition. The maximum inhibition was observed in **IIIb** and **IIIc** against all the tested organisms except *S. typhimurium* and *K. pneumonia*. In addition, the most potent activity was observed in bis-benzimidazo[1,2-*c*]quinazolines (**IVd** and **IVe**) against all bacterial strains when compared them with standard drug Ampicillin. The minimum inhibitory concentration [31,32] of these quinazolines (**IIIb**, **IIIc** and **IVd**, **IVe**) was also verified by the liquid dilution

Table 1
Zone of inhibition of newly synthesized mono, bis-benzimidazo[1,2-*c*]quinazolines against different bacteria and fungi.

Compound (1000 μg/ml)	Zone of inhibition (mm)								
	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	S. aureus	*B. subtilis*	*S. pyogenes*	*S. typhimurium*	*E. coli*	*K. pneumonia*	*A. niger*	*C.albicans*	*T. viridae*
IIIa	18	14	13	12	18	16	10	10	9
IIIb	50	42	49	38	51	35	49	48	45
IIIc	51	45	50	40	50	36	51	48	46
IIId	25	21	18	15	20	12	13	11	6
IIIe	22	18	13	14	18	12	11	9	9
IVa	18	14	15	12	20	15	16	13	13
IVb	21	18	15	15	19	18	18	15	12
IVc	25	19	18	14	21	18	21	16	13
IVd	52	45	50	46	50	49	50	50	45
IVe	55	50	52	46	51	50	55	51	48
Std	48[a]	39[a]	35[a]	45[a]	40[a]	45[a]	45[b]	40[b]	41[b]

[a] Ampicillin.
[b] Ketoconazole.

Table 2
MIC values potent mono, bis-benzimidazo[1,2-*c*]quinazolines and standard drugs.

Compound	Range of concentration (μg/ml)								
	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	S. aureus	*B. subtilis*	*S. pyogenes*	*S. typhimurium*	*E. coli*	*K. pneumonia*	*A. niger*	*C.albicans*	*T. viridae*
IIIb	10	20	15	20	15	20	10	15	15
IIIc	5	15	10	15	10	15	5	15	10
IVd	2.5	15	10	5	5	5	2.5	5	10
IVe	2.5	10	5	5	2.5	5	2.5	2.5	5
Std	10[a]	20[a]	25[a]	10[a]	15[a]	10[a]	15[b]	25[b]	20[b]

[a] Ampicillin.
[b] Ketoconazole.

method in which the effectiveness was observed at lower concentrations (Table 2).

3.2. Antifungal activity

All the above-mentioned quinazoline compounds were also examined for antifungal activity against *Aspergillus niger*, *Candida albicans*, *Trichoderma viridae* fungal strains. Ketoconazole was used as standard drug for the comparison of antifungal results. The fungal strains were grown and maintained on Sabouraud glucose agar plates. Preliminary screening for ten quinazolines was performed at fixed concentrations of 1000 μg/ml. The plates were incubated at 26 °C for 72 h and zones of inhibition formed were measured [33]. The antifungal screening revealed that all the tested compounds **IIIa–e** and **IVa–e** showed moderate to good inhibition. The pyridyl and thienyl substituted quinazolines **IIIb**, **IIIc** and **IVd**, **IVe** showed same trend in the case of fungal strains (Tables 1 and 2).

In general, the order of both antibacterial and antifungal activity of the quinazolines is **IVe** ≥ **IVd** ≥ **IIIc** ≥ **IIIb** ≥ **IVc** ≥ **IIId** ≥ **IVb** ≥ **IIIe** ≥ **IVa** ≥ **IIIa**. Furthermore, the MIC values of potent compounds **IIIb**, **IIIc**, **IVd** and **IVe** (Table 2 and Fig. 5) showing their superior activity against bacterial and fungal strains than respective standard drugs Ampicillin and Ketoconazole.

4. Results and discussion

Formation of 2-*o*-arylideneaminophenylbenzimidazoles (**Ia–e**, **IIa–e**) and 6-arylbenzimidazo[1,2-*c*]quinazolines (**IIIa–e**), (**IVa–e**) was confirmed on the basis of elemental analysis, IR, NMR and mass spectral analysis.

4.1. Infrared spectral analysis

IR spectra of compound **A** showed absorption band at 3350 cm^{-1} assigned to aminophenyl ring and in the case of compounds **A**, **Ia–e** and **IIa–e**, two absorption bands in the region 3150–3300 cm^{-1} and 1420 cm^{-1} were assigned to –NH stretching and bending vibrations of imidazolyl ring, respectively [24,25]. The absence of aminophenyl ring absorption at 3350 cm^{-1} and appearance of a strong intensity band in the IR spectra of compounds (**Ia–e**, **IIa–e**) in the range of 1610–1655 cm^{-1} attributable to C═N provides a strong evidence for the condensation and also confirms the formation of the azomethines **Ia–e** and **IIa–e** [26]. The oxidative cyclisation of compounds **Ia–e**, **IIa–e** to **IIIa–e**, **IVa–e** was accompanied by the disappearance of the absorption maxima at 3150–3300 cm^{-1}, which are ascribed to the vibrations of NH group of the benzimidazole ring; at the same time the appearance of a new maximum at 1360–1388 cm^{-1}, which is characteristic for benzimidazoquinazoline ring with a tertiary nitrogen atom, appears [21,27].

4.2. ^{1}H & ^{13}C NMR spectral analysis

The ^{1}H NMR spectra of the compound **A** as well as its derivatives have been recorded in $CDCl_3$/DMSO-d_6 using TMS as internal standard. In the spectra of 2-(*o*-aminophenyl) benzimidazole (**A**), signals at 6.4 δ and 8.25 δ were appeared

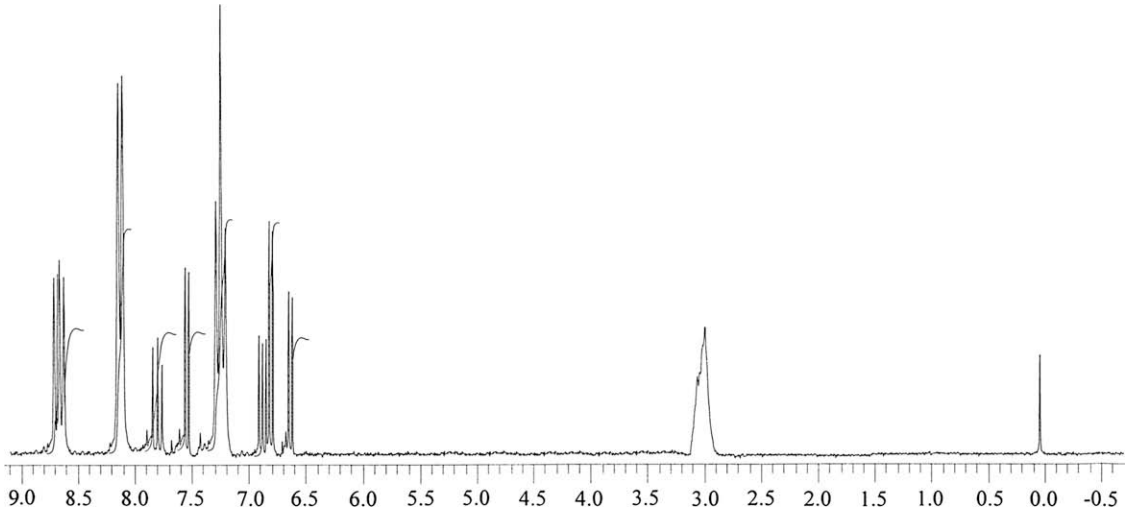


Fig. 1. ^{1}H NMR spectrum of compound **IVb**.

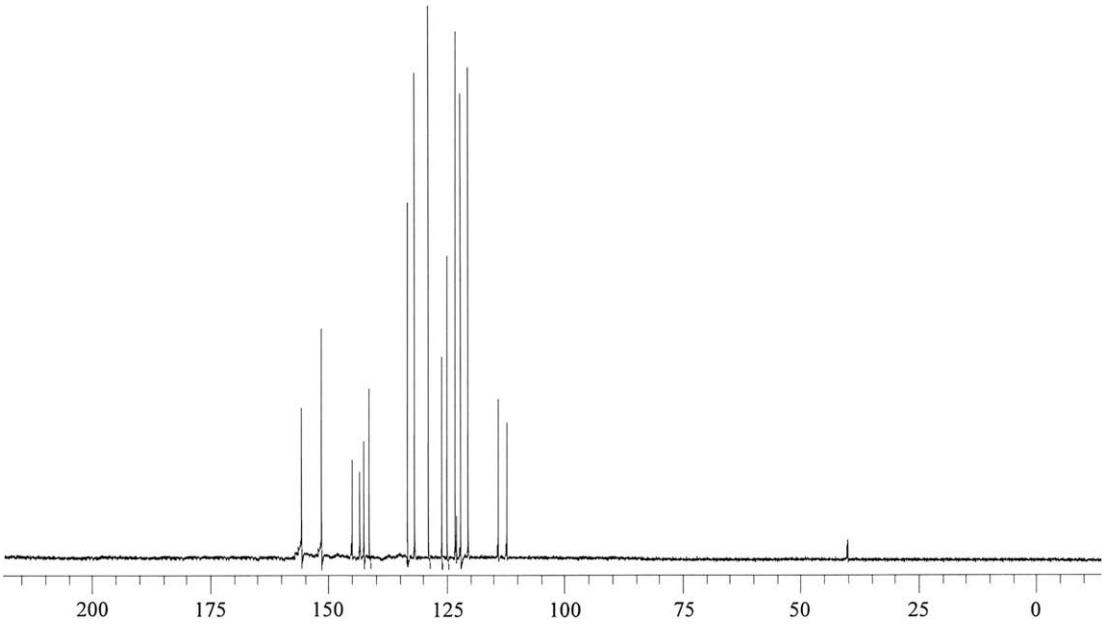


Fig. 2. ^{13}C NMR spectrum of compound **IVb**.

corresponding to free amino and imidazolyl protons respectively [24,26]. The aromatic protons of various environments present in all compounds appeared as multiplets in the range of 6.50–8.78 ppm [26,28]. ^{1}H NMR spectra of compounds **Ia–e**, **IIa–e** contain signals corresponding to CH═N protons in the range of 7.90–8.31 ppm suggesting the condensation of aromatic aldehydes with compound **A**. However, in the spectra of **Ia–e** and **IIa–e** compounds the disappearance of signals at 6.4 δ which is due to NH_2 protons of compound **A** supports the involvement of amino group in condensation and confirms the formation of azomethines. Finally, in the spectra of **IIIa–e**, **IVa–e** compounds disappearance of signals corresponding to –NH of imidazolyl ring and CH═N protons supports the benzimidazoquinazoline ring structure [21,27].

^{13}C NMR spectra of all the compounds contain signals in the range of 141.1–168.3 ppm confirming the presence of carbon, which is doubly bonded to nitrogen. The aromatic carbons of various environments present in all the compounds appeared as signals in the range of 110.3–156.2 ppm [28]. The ^{1}H & ^{13}C NMR spectra of compound **IVb** are presented in Figs. 1 and 2 respectively.

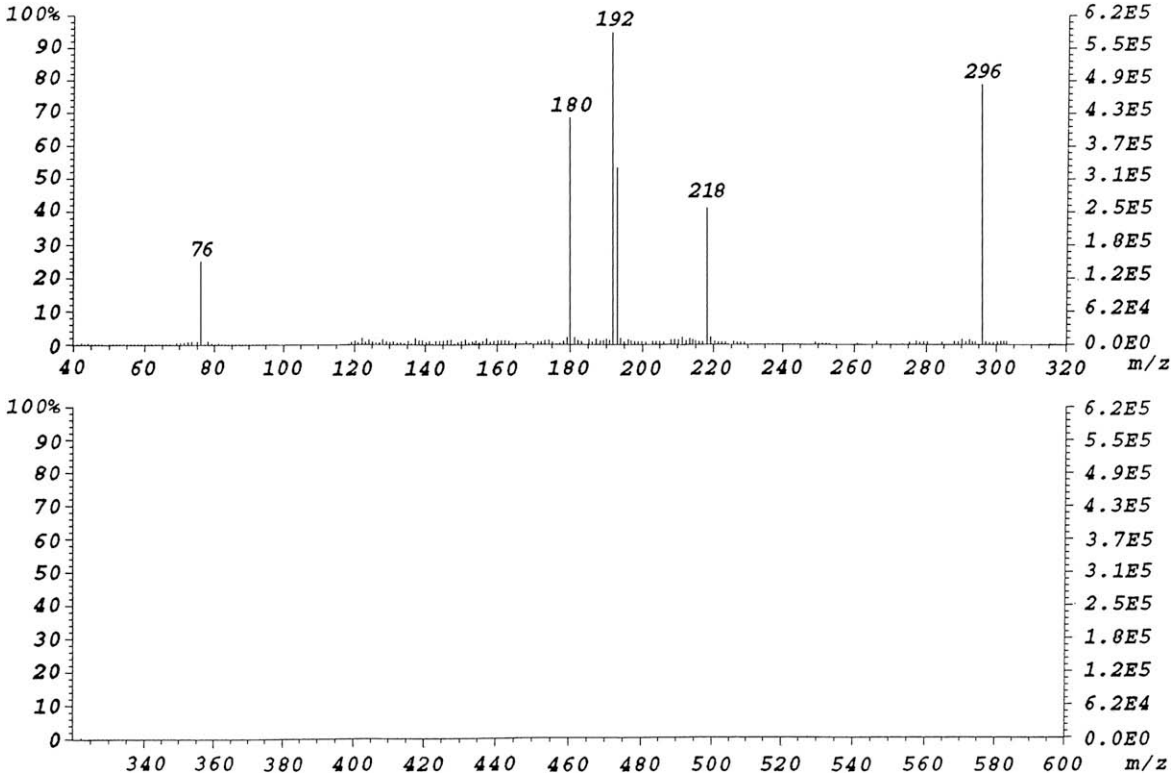


Fig. 3. FAB mass spectrum of compound **IIIb**.

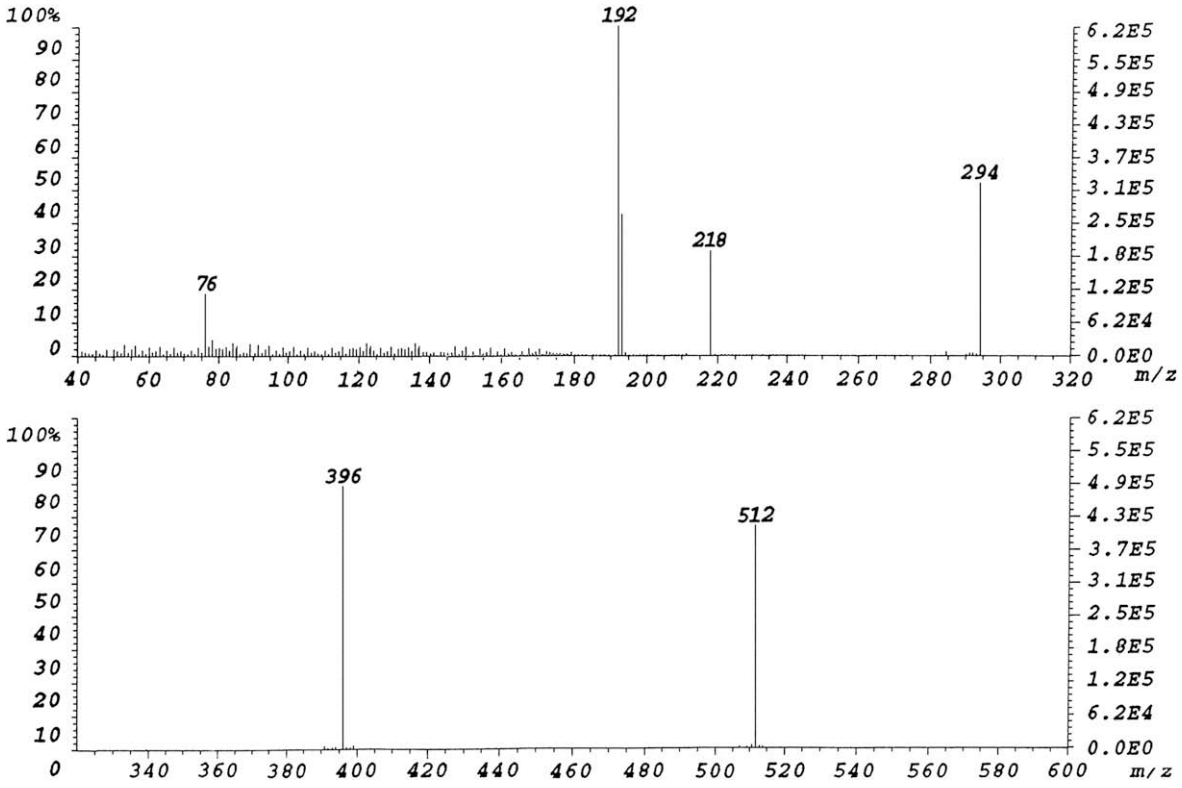


Fig. 4. FAB mass spectrum of compound **IVb**.

4.3. FAB mass analysis

All the compounds showed a single peak in ESI-MS suggesting the purity of the azomethines and quinazolines. The FAB mass spectrum of compound (**IIIb**) shows a parent peak at *m*/*z* (M^+) 296 (80.5%) corresponding to the molecular formula $C_{19}H_{12}N_4$. The mass spectrum shows major fragments at *m*/*z* values of 218 (40.2%), 193 (52.1%), 192 (100%), 180 (74.6%) and 76 (24.2%). The spectrum of (**IVb**) shows a molecular ion peak (M^+) at *m*/*z* 512 (65.4%), which confirms the proposed formula ($C_{34}H_{20}N_6$). The peaks have been observed at *m*/*z* values 396 (80.2%), 294 (51.5%), 218 (30.8%), 193 (42.2%), 192 (100%), and 76 (18.6%), which indicate the fragmentation pattern and their intensity gives an idea about the abundance and stability of the fragments. The mass fragmentation pattern of compounds **IIIb** and **IVb** as assigned on the basis of mass spectra (Figs. 3 and 4) is presented in Schemes 2 and 3.

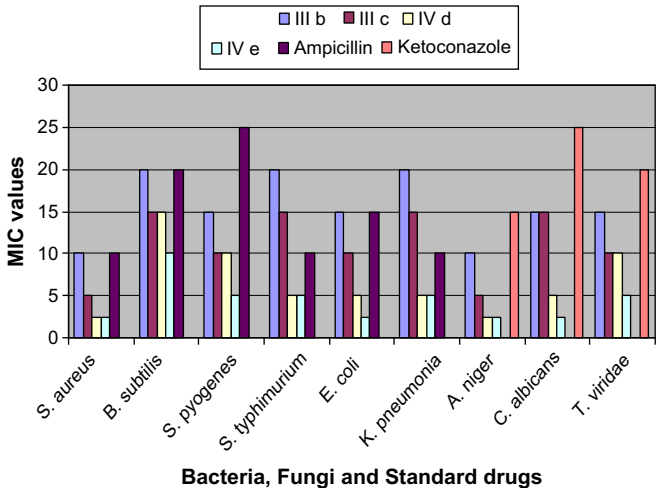
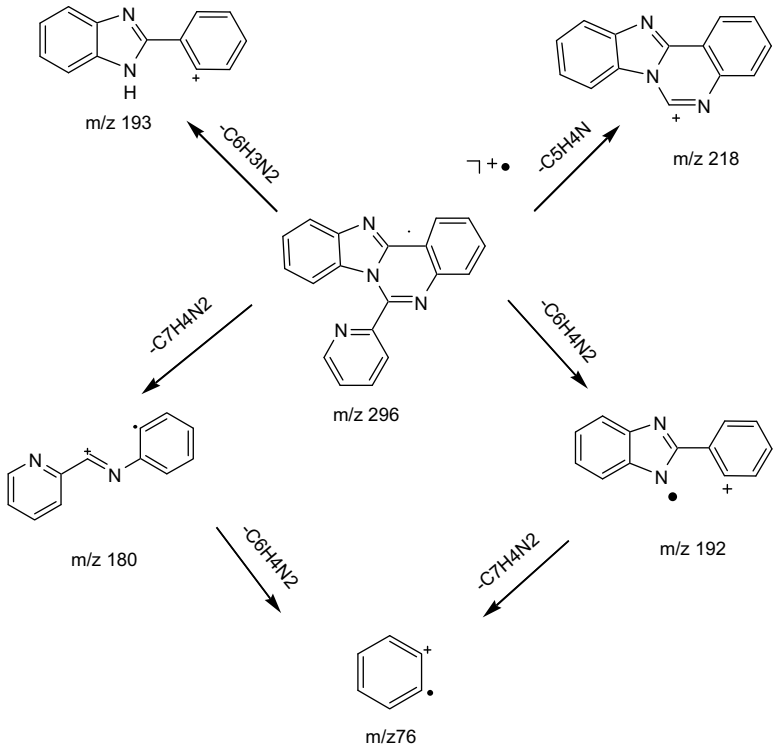


Fig. 5. Comparison of MIC values (in μg/ml) of quinazolines and standard drugs against different bacteria and fungi.

5. Conclusion

Benzimidazo[1,2-*c*]quinazolines have been used widely in the pharmaceutical industry and medicine because of their antimicrobial, antipyretic, anti-inflammatory, and anticancer properties. We have described the synthesis, characterization and antimicrobial studies of several mono, bis-benzimidazo[1,2-*c*]quinazolines. All are newly synthesized compounds, except one (**IIIa**) [23]. Furthermore, we have chosen six bacterial and three fungal strains for microbial studies of these entire quinazoline compounds. From this study it is evident that mono-benzimidazo[1,2-*c*]quinazoline compounds are showing better activity when compared to Ampicillin and Ketoconazole towards inhibiting all tested bacterial strains (except *S. typhimurium*, *K. pneumonia*) and all fungal strains respectively. Furthermore, the most potent activity was observed in bis-benzimidazo[1,2-*c*]quinazolines (**IVd** and **IVe**) against all bacterial and fungal strains when compared to respective standard drugs Ampicillin and Ketoconazole. From the results it could be deduced that among the compounds those containing pyridyl, thienyl at the C_6 position of the benzimidazo[1,2-*c*]quinazoline moiety showed better activities against the test bacteria and fungi than those containing other aromatic substituents. Especially, the compounds with two quinazoline moieties (**IVd**, **IVe**) were more active than all mono-benzimidazo[1,2-*c*]quinazolines. A possible explanation for this result is that the antibacterial and antifungal activity of compounds may be depending on the basic skeleton of molecule as well as on the nature of substituents. Hence we



Scheme 2. FAB mass fragmentation pattern of 6-(2-pyridyl)benzo[4,5]imidazo[1,2-*c*]quinazoline (**IIIb**).

conclude that although all quinazolines itself were observed active the activity was further enhanced by the presence of basic bis-benzimidazo[1,2-*c*]quinazoline structure and as well as pyridyl, thienyl groups.

6. Experimental protocols

Analar grade reagents and freshly distilled solvents were used throughout the investigations. Purity of the compounds was checked by TLC using Merck 60F254 silica gel plates. Micro analytical (C, N, H) data was obtained by using a Perkin–Elmer 2400 CHN elemental analyzer. The IR spectra were recorded in KBr pellets on Perkin–Elmer-283 spectrophotometer. Brucker 400 MHz and Brucker 67.93 MHz spectrometers were used for ^{1}H NMR and ^{13}C NMR measurements. ESI and FAB MS were used to obtain mass spectra. Hot air oven (Instrument and equipment Pvt. Ltd., Mumbai), incubator (Instrument and equipment Pvt. Ltd., Mumbai), laminar airflow unit (Clas laminar technologies Pvt. Ltd. Secunderabad), autoclave (Medica instrument Mfg. Co., Mumbai) were used in the present investigations. Organisms like *S. aureus*, *B. subtilis*, *S. pyogenes* (Gram positive), *S. typhimurium*, *E. coli*, *K. pneumonia* (Gram negative) bacteria and *A. niger*, *C. albicans*, *T. viridae* fungi were used in the present investigations.

*6.1. Procedure for preparation of mono-2-*o*-(arylideneamino) phenyl benzimidazoles (**Ia**–**e**)*

2-(*o*-Aminophenyl) benzimidazole (A, 0.418 g, 2 mmol) was dissolved in 20 ml of ethanol. To this solution, 20 ml of Ar–CHO solution (2 mmol, viz., 0.212 g of benzaldehyde, 0.214 g of 2-formyl pyridine, 0.224 g of 2-formyl thiophene, 0.192 g of 2-formyl furan or 0.190 g of 2-formyl pyrrole in ethanol) and acetic acid (2–3 drops) were added. The reaction mixture was refluxed under heat for 2 h after which the compound was obtained. The product was filtered and recrystallized from a dichloromethane–methanol mixture (2:1, v/v).

*6.2. Procedure for preparation of bis-2-*o*-(arylideneamino) phenyl benzimidazoles (**IIa**–**e**)*

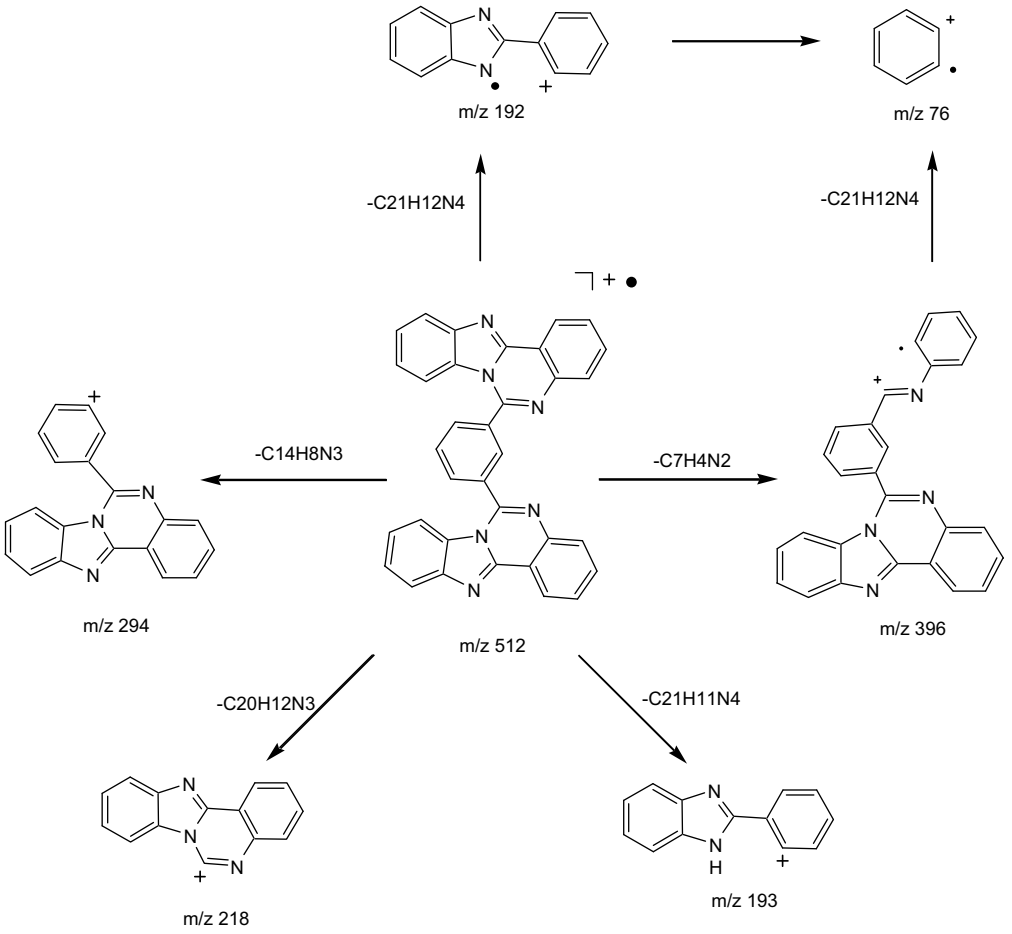
2-(*o*-Aminophenyl) benzimidazole (A, 0.837 g, 4 mmol) was dissolved in 20 ml of ethanol. To this solution, 20 ml of CHO–Ar–CHO solution (2 mmol, viz., 0.268 g of *o*-pthalaldehyde, 0.268 g of *m*-pthalaldehyde, 0.268 g of *p*-pthalaldehyde, 0.270 g of 2,6-diformyl pyridine or 0.280 g of 2,5-diformyl thiophene in ethanol) and acetic acid (2–3 drops) were added. The reaction mixture was refluxed under heat for 2 h after which the compound was obtained. The product was filtered and recrystallized from a dichloromethane–methanol mixture (2:1, v/v).

*6.3. Procedure for preparation of mono, bis-6-substituted benzimidazo[1,2-*c*]quinazolines[BIQ–Ar and BIQ–Ar–BIQ] (**IIIa**–**e** and **IVa**–**e**)*

All the benzimidazo[1,2-*c*]quinazolines (**IIIa**–**e** and **IVa**–**e**) were prepared from azomethines **Ia**–**e** and **IIa**–**e** through oxidative cyclisation method reported by Davis and Mann [23]. Appropriate amount of powdered potassium permanganate was added to a solution of the azomethyne compound (**Ia**–**e**, **IIa**–**e**) in 50–75 ml of acetone. The mixture was boiled under reflux for 30 min. it was then filtered hot, and an equal volume of hot water added to the filtrate. The quinazolines **(IIIa**–**e** and **IVa**–**e**) rapidly separated, and was collected by filtration and recrystalized from dimethylformamide.

6.3.1. N-*[2-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl]*-N-*[(*E*)-1-phenylmethylidene]amine (**Ia**)*

Yield 69%; mp 221–223 °C (lit. mp 222 °C); IR 3300, 1655, 1590, 1575, 1130 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 2H, Ar), 7.12–7.18 (m, 2H, Ar), 7.22–7.25 (m, 3H, Ar); 7.30–7.32 (m, 3H, Ar), 7.63 (d, 1H, Ar, $J = 7.3$ Hz), 7.95 (d, 1H, Ar, $J = 7.3$ Hz), 8.22 (s, 1H, CH═N), 8.89 (s, 1H,



Scheme 3. FAB mass fragmentation pattern of 6-(3-benzo[4,5]imidazo[1,2-*c*]quinazoline-6-ylphenyl)benzo[4,5]imidazo[1,2-*c*]quinazoline (**IVb**).

imidazolyl –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.1, 118.2, 123.1, 123.5, 126.2, 130.5, 130.8, 131.2, 132.2, 136.5, 141.2, 142,6, 143.8, 146.3, 165.7 (20C, Ar–C); Anal. Found: C, 81.08; H, 5.07; N, 14.15%. Calcd for $C_{20}H_{15}N_3$: C, 80.78; H, 5.08; N, 14.13%. MS: $[M]^+$ at *m*/*z* 297.

6.3.2. N-*[2-(1*H-*Benzo[*d*]imidazol-2-yl)phenyl]*-N-*[(*Z*)-1-(2-pyridyl)methylidene]amine (**Ib**)*

Yield 79%; mp 228–230 °C; IR 3300, 1630, 1610, 1580, 1100 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (t, 1H, Ar, J = 7.4 Hz), 7.06–7.10 (m, 2H, Ar), 7.12–7.18 (m, 2H, Ar), 7.29–7.30 (m, 2H, Ar); 7.62 (d, 1H, Ar, J = 7.3 Hz), 7.75–7.79 (m, 1H, Ar), 7.82 (t, 1H, Ar, J = 8.0 Hz), 7.94 (d, 1H, Ar, J = 7.3 Hz), 8.24 (s, 1H, CH═N), 8.72 (d, 1H, J = 5.25 Hz), 8.89 (s, 1H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.1, 118.2, 123.1, 123.5, 126.2, 130.5, 130.8, 131.2, 132.2, 136.5, 141.2, 142,8, 143.5, 146.3, 149.40, 154.48, 166.1 (19C, Ar–C), Anal. Found: C, 76.02; H, 5.07; N, 18.80%. Calcd for $C_{19}H_{14}N_4$: C, 76.49; H, 4.73; N, 18.78%. MS: $[M]^+$ at *m*/*z* 298.

6.3.3. N-*[2-(1*H-*Benzo[*d*]imidazol-2-yl)phenyl]*-N-*[(*E*)-1-(2-thienyl)methylidene]amine (**Ic**)*

Yield 80%; mp 220–222 °C; IR 3300, 1635, 1608, 1590, 1130, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 6.82 (t, 1H, Ar, J = 7.4 Hz), 7.27 (d, 2H, Ar, J = 3.8 Hz), 7.36–7.43 (m, 4H, Ar), 7.45–7.48 (m, 2H, Ar), 7.63 (d, 1H, Ar, J = 7.3 Hz), 7.94 (d, 1H, Ar, J = 7.3 Hz), 8.02 (s, 1H, CH═N), 8.89 (s, 1H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.2, 118.2, 121.1, 123.3, 126.2, 130.5, 131.2, 132.2, 132.4, 133.5, 141.1, 143.5, 146.5, 146.8, 147.40, 148.5 (18C, Ar–C); Anal. Found: C, 71.29; H, 4.59; N, 13.80%. Calcd for $C_{18}H_{13}N_3S$: C, 71.26; H, 4.32; N, 13.85%. MS: $[M]^+$ at *m*/*z* 303.

6.3.4. N-*[2-(1*H-*Benzo[*d*]imidazol-2-yl)phenyl]*-N-*[(*E*)-1-(2-furyl)methylidene]amine (**Id**)*

Yield 78%; mp 214–216 °C; IR 3300, 1638, 1605, 1585, 1128 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 6.67 (dd, 1H, Ar, J = 3.62, 1.81 Hz furanyl C_4H), 6.82 (t, 1H, Ar, J = 7.4 Hz), 6.95 (d, 1H, J = 3.62 Hz, furanyl C_3H), 7.36–7.42 (m, 3H, Ar), 7.44–7.48 (m, 3H, Ar), 7.63 (d, 1H, Ar, J = 7.3 Hz), 7.94 (d, 1H, Ar, J = 7.3 Hz), 8.02 (s, 1H, CH═N), 8.85 (s, 1H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 111.6, 114.0, 114.3, 121.6, 123.4, 123.5, 126.2, 130.5, 130.8, 131.1, 132.0, 141.1, 142,3, 143.5, 146.1, 154.2 (18C, Ar–C), Anal. Found: C, 76.02; H, 5.01; N, 14.80%. Calcd for $C_{18}H_{13}N_3O$: C, 75.25; H, 4.56; N, 14.62%. MS: $[M]^+$ at *m*/*z* 287.

6.3.5. N-*[2-(1*H-*Benzo[*d*]imidazol-2-yl)phenyl]*-N-*[(*E*)-1-(1*H-*2-pyrrolyl)methylidene] amine (**Ie**)*

Yield 75%; mp 210–212 °C; IR 3300, 3215, 1640, 1605, 1580, 1130 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 5.93 (dd, 1H, J = 3.94, 2.88 Hz, pyrrolyl C_4–H), 6.63 (d, 1H, J = 2.88 Hz, pyrrolyl C_5–H), 6.82 (t, 1H, Ar, J = 7.4 Hz), 6.95 (d, 1H, J = 3.94 Hz, pyrrolyl C_3–H), 7.36–7.42 (m, 3H, Ar), 7.43–7.49 (m, 2H, Ar), 7.63 (d, 1H, Ar, J = 7.3 Hz), 7.94 (d, 1H, Ar, J = 7.3 Hz), 7.98 (s, 1H, CH═N), 8.85 (s, 1H, –NH), 9.77 (s, 1H, pyrrolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 113.6, 114.4, 118.2, 121.7, 123.5, 126.2, 130.5, 130.8, 131.2, 132.5, 136.5, 141.1, 141.2, 144,8, 146.3, 149.40 (18C, Ar–C); Anal. Found: C, 75.49; H, 5.01; N, 19.55%. Calcd for $C_{18}H_{14}N_4$: C, 75.51; H, 4.93; N, 19.57%. MS: $[M]^+$ at *m*/*z* 286.

6.3.6. N*1-(*Z*)-1-[2-([2-(1*H*-Benzo[*d*]imidazol-2-l)phenyl]iminomethyl)phenyl]methylidene-2-(1*H*-benzo[*d*]imidazol-2-yl)aniline (**IIa**)*

Yield 75%; mp 224–225 °C; IR 3300, 1640, 1605, 1585, 1120 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ in ppm 6.81 (d, 2H, Ar, $J = 8.4$ Hz), 6.83 (t, 2H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 4H, Ar), 7.12–7.18 (m, 4H, Ar), 7.32 (t, 2H, Ar, $J = 7.6$ Hz), 7.35–7.44 (m, 4H, Ar), 7.95 (d, 2H, Ar, $J = 7.3$ Hz), 8.31 (s, 2H, CH═N), 8.89 (s, 2H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.1,118.2, 123.1, 123.5, 125.2, 127.3, 128.3, 130.5, 130.8, 131.2, 132.2, 133.5, 138.3, 141.1, 142,6, 144.2, 146.6, 158.2 (34C, Ar–C); Anal. Found: C, 80.08; H, 5.02; N, 16.02%. Calcd for $C_{34}H_{24}N_6$: C, 79.05; H, 4.68; N, 16.27%. MS: $[M]^+$ at *m*/*z* 516.

6.3.7. N*1-(*E*)-1-[3-([2-(1*H*-Benzo[*d*]imidazol-2-yl) phenyl]iminomethyl)phenyl]methylidene-2-(1*H*-benzo[*d*]imidazol-2-yl)aniline (**IIb**)*

Yield 68%; mp 216–218 °C; IR 3300, 1625, 1605, 1575, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ in ppm 6.81 (d, 2H, Ar, $J = 8.4$ Hz), 6.83 (t, 2H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 4H, Ar), 7.12–7.18 (m, 4H, Ar), 7.35–7.42 (m, 2H, Ar), 7.62 (t, 2H, Ar, $J = 6.4$ Hz), 7.93 (t, 2H, Ar, $J = 8.8$ Hz), 7.95 (d, 2H, Ar, $J = 7.3$ Hz), 8.07 (s, 2H, CH═N), 8.89 (s, 2H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.3, 114.4, 123.1, 123.4, 124.2, 126.4, 127.3, 128.3, 130.7, 130.9, 132.2, 134.3, 133.5, 135.3, 141.1, 142,6, 144.5, 146.6, 146.8, 168.2 (34C, Ar–C); Anal. Found: C, 80.08; H, 4.69; N, 16.15%. Calcd for $C_{34}H_{24}N_6$: C, 79.05; H, 4.68; N, 16.27%. MS: $[M]^+$ at *m*/*z* 516.

6.3.8. N*1-(*E*)-1-[4-([2-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl]iminomethyl)phenyl]methylidene-2-(1*H*-benzo[*d*]imidazol-2-yl)aniline (**IIc**)*

Yield 71%; mp 220–222 °C; IR 3300, 1621, 1600, 1580, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ in ppm 6.80 (d, 2H, Ar, $J = 8.4$ Hz), 6.82 (t, 2H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 4H, Ar), 7.13–7.18 (m, 4H, Ar), 7.39–7.40 (m, 2H, Ar), 7.42 (t, 4H, Ar, $J = 7.8$ Hz), 7.95 (d, 2H, Ar, $J = 7.3$ Hz), 8.21 (s, 2H, CH═N), 8.87 (s, 2H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.1, 114.2, 123.1, 123.5, 125.2, 127.3, 128.3,130.5, 130.8, 131.2, 132.2, 137.3, 141.1, 142,6, 144.2, 146.6, 168.3 (34C, Ar–C); Anal. Found: C, 80.02; H, 5.07; N, 16.24%. Calcd for $C_{34}H_{24}N_6$: C, 79.05; H, 4.68; N, 16.27%. MS: $[M]^+$ at *m*/*z* 516.

6.3.9. N*1-(*E*)-1-[6-([2-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl]iminomethyl)-2-pyridyl]methylidene-2-(1*H*-benzo[*d*]imidazol-2-yl)aniline (**IId**)*

Yield 81%; mp 255–257 °C; IR 3300, 1640, 1605, 1580, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ in ppm 6.80 (d, 2H, Ar, $J = 8.4$ Hz), 6.82 (t, 2H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 4H, Ar), 7.13–7.18 (m, 4H, Ar), 7.20 (d, 2H, Ar, $J = 7.8$ Hz), 7.39–7.42 (m, 2H, Ar), 7.89 (t, 1H, Ar, $J = 7.8$ Hz), 7.95 (d, 2H, Ar, $J = 7.3$ Hz), 8.12 (s, 2H, CH═N), 8.87 (s, 2H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.2, 114.4, 121.6, 123.5, 124.6, 127.3, 128.3,130.5, 131.8, 132.6, 141.8, 142,6, 144.2, 146.6, 146.8, 154.2, 168.4 (33C, Ar–C); Anal. Found: C, 76.59; H, 4.52; N, 18.75%. Calcd for $C_{33}H_{23}N_7$: C, 76.58; H, 4.48; N, 18.94%. MS: $[M]^+$ at *m*/*z* 517.

6.3.10. N*1-(*Z*)-1-[5-([2-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl]iminomethyl)-2-thienyl]methylidene-2-(1*H*-benzo[*d*]imidazol-2-yl)aniline (**IIe**)*

Yield 79%; mp 226–228 °C; IR 3300, 1620, 1605, 1585, 1130, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ in ppm 6.80 (d, 2H, Ar, $J = 8.4$ Hz), 6.82 (t, 2H, Ar, $J = 7.4$ Hz), 7.05–7.09 (m, 4H, Ar), 7.13–7.18 (m, 4H, Ar), 7.20 (s, 2H, thienyl $-C_3-H$, C_4-H), 7.39–7.42 (m, 2H, Ar), 7.90 (s, 2H, CH═N), 7.95 (d, 2H, $J = 7.3$ Hz), 8.87 (s, 2H, –NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 113.4, 114.1, 121.6, 123.4, 125.4, 126.3, 128.3, 130.3, 131.1, 132.3, 141.8, 142,6, 144.2, 145.6, 146.5, 146.8, 151.2, (32C, Ar–C) Anal. Found: C, 73.59; H, 4.57; N, 16.29%. Calcd for $C_{32}H_{22}N_6S$: C, 73.54; H, 4.24; N, 16.08%. MS: $[M]^+$ at *m*/*z* 522.

*6.3.11. 6-Phenylbenzo[4,5]imidazo[1,2-c]quinazoline (**IIIa**)*

Yield 81%; mp 241–242 °C (lit. mp 242 °C); IR 1621, 1585, 1529, 1457, 1380, 773, 741, 732 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ in ppm 6.65 (d, 1H, Ar, $J = 8.2$ Hz), 6.81 (d, 1H, Ar, $J = 8.4$ Hz), 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.17–8.03 (m, 9H, Ar), 8.75 (dd, 1H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 112.4, 120.1, 121.5, 122.4, 125.8, 126.4, 129.3, 129.5, 130.7, 131.5, 132.6, 133.2, 145.6, 146.5, 146.8, 151.5, (20C, Ar–C) Anal. Found: C, 81.35; H, 4.58; N, 14.25%. Calcd for $C_{20}H_{13}N_3$: C, 81.34; H, 4.44; N, 14.23%. MS: $[M]^+$ at *m*/*z* 295.

*6.3.12. 6-(2-Pyridyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IIIb**)*

Yield 67%; mp 228–230 °C; IR 1621, 1610, 1524, 1459, 1378, 775, 744, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ in ppm 6.65 (d, 1H, Ar, $J = 8.2$ Hz), 6.81 (d, 1H, Ar, $J = 8.4$ Hz), 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.03 (t, 1H, Ar, $J = 7.4$ Hz), 7.20–8.03 (m, 6H, Ar), 8.75 (dd, 1H, $J = 6.9$, 2.5 Hz) 8.80 (dd, 1H, $J = 4.93$, 1.7 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 112.4, 120.3, 121.6, 122.8, 125.8, 126.4, 129.8, 130.7, 131.5, 133.3, 138.4, 143.1, 145.6, 146.5, 146.8, 150.2, 151.5, 152.4 (19C, Ar–C); Anal. Found: C, 77.08; H, 5.02; N, 18.90%. Calcd for $C_{19}H_{12}N_4$: C, 77.01; H, 4.08; N, 18.91%. MS: $[M]^+$ at *m*/*z* 296.

*6.3.13. 6-(2-Thienyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IIIc**)*

Yield 74%; mp 232–234 °C; IR 1640, 1605, 1580, 1459, 1385, 775, 745, 749, 690 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ in ppm 6.62 (d, 1H, $J = 3.63$ Hz), 6.66 (d, 1H, Ar, $J = 8.2$ Hz), 6.80 (d, 1H, Ar, $J = 8.4$ Hz), 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.08 (d, 1H, $J = 5.25$ Hz), 7.20–7.50 (m, 4H, Ar), 7.52 (d, 1H, $J = 5.25$ Hz), 8.74 (dd, 1H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 111.4, 119.3, 121.6, 121.7, 123.0, 124.8, 125.4, 126.8, 130.7, 131.5, 132.3, 138.4, 143.6, 145.4, 146.1, 147.8, 149.2, (18C, Ar–C) Anal. Found: C, 71.76; H, 4.02; N, 13.95%. Calcd for $C_{18}H_{11}N_3$: C, 71.74; H, 3.68 N, 13.94%. MS: $[M]^+$ at *m*/*z* 301.

*6.3.14. 6-(2-Furyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IIId**)*

Yield 81%; mp 225–227 °C; IR 1638, 1615, 1575, 1465, 1380, 770, 742, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ in ppm 6.46 (d, 1H, furyl C_3–H, $J = 3.53$ Hz), 6.55 (d, 1H, furyl C_4–H, $J = 3.5$ Hz), 6.65 (d, 1H, Ar, $J = 8.2$ Hz), 6.80 (d, 1H, Ar, $J = 8.4$ Hz), 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.20–7.51 (m, 4H, Ar), 7.59 (s, 1H, furyl C_5–H), 8.75 (dd, 1H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 111.5, 112.3, 120.6, 121.7, 122.8, 124.8, 126.4, 127.8, 130.7, 131.2, 132.6, 137.2, 143.6, 145.6, 146.1, 147.8, 148.2, (18C, Ar–C) Anal. Found: C, 76.80; H, 3.92; N, 14.90%. Calcd for $C_{18}H_{11}N_3O$: C, 75.78; H, 3.89; N, 14.73%. MS: $[M]^+$ at *m*/*z* 285.

*6.3.15. 6-(1H-2-Pyrrolyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IIIe**)*

Yield 72%; mp 217–219 °C; IR 3215, 1640, 1620, 1580, 1475, 1382, 770, 745, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ in ppm 5.73 (d, 1H, $J = 3.8$ Hz), 6.38 (d, 1H, $J = 3.8$ Hz), 6.65 (d, 1H, Ar, $J = 8.2$ Hz), 6.80 (d, 1H, Ar, $J = 8.4$ Hz), 6.83 (t, 1H, Ar, $J = 7.4$ Hz), 7.14 (s, 1H), 7.21–7.54 (m, 4H, Ar), 8.74 (dd, 1H, $J = 6.9$, 2.5 Hz) 10.6 (s, 1H, pyrrolyl NH); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 111.6, 114.6, 119.6, 121.9, 122.8, 124.6, 125.4, 126.3, 129.7, 131.8, 132.5, 142.2, 143.6, 145.6, 146.1, 147.8, 149.2, (18C, Ar–C) Anal. Found: C, 76.08; H, 5.01; N, 19.80%. Calcd for $C_{18}H_{12}N_4$: C, 76.04; H, 4.25; N, 19.71%. MS: $[M]^+$ at *m*/*z* 284.

*6.3.16. 6-(2-Benzo[4,5]imidazo[1,2-c]quinazolin-6-ylphenyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IVa**)*

Yield 78%; mp 235–237 °C; IR 1635, 1605, 1460, 1380, 1130, 775, 740, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 6.64 (d, 2H, Ar,

$J = 8.2$ Hz), 6.81 (d, 2H, Ar, $J = 8.4$ Hz), 6.82 (t, 2H, Ar, $J = 7.4$ Hz), 7.20–7.85 (m, 10H, Ar), 8.70–8.78 (m, 4H, Ar); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 113.0, 114.7, 120.5, 121.4, 122.7, 125.4, 126.3, 128.7, 131.8, 133.5, 135.5, 142.2, 143.5, 144.2, 145.1, 152.5, 154.2, (34C, Ar–C) Anal. Found: C, 80.08; H, 4.07; N, 16.15%. Calcd for $C_{34}H_{20}N_6$: C, 79.67; H, 3.93; N, 16.40%. MS: $[M]^+$ at m/z 512.

*6.3.17. 6-(3-Benzo[4,5]imidazo[1,2-c]quinazolin-6-ylphenyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IVb**)*

Yield 71%; mp 242–244 °C; IR 1620, 1610, 1459, 1385, 1128, 779, 758, 740 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63 (d, 2H, Ar, $J = 8.2$ Hz), 6.81 (d, 2H, Ar, $J = 8.4$ Hz), 6.83 (t, 2H, Ar, $J = 7.4$ Hz), 7.19–7.28 (m, 4H, Ar), 7.53 (d, 2H, Ar, $J = 7.6$ Hz), 7.81 (t, 2H, $J = 7.6$ Hz), 8.10–8.25 (m, 4H, Ar), 8.76 (dd, 2H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 113.0, 114.5, 120.4, 121.9, 122.4, 124.6, 126.5, 128.7, 131.7, 133.5, 142.2, 143.5, 144.2, 145.1, 152.2, 156.2, (34C, Ar–C); Anal. Found: C, 81.08; H, 4.07; N, 16.21%. Calcd for $C_{34}H_{20}N_6$: C, 79.67; H, 3.93; N, 16.40%. MS: $[M]^+$ at m/z 512.

*6.3.18. 6-(4-Benzo[4,5]imidazo[1,2-c]quinazolin-6-ylphenyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IVc**)*

Yield 75%; mp 280–282 °C; IR 1625, 1601, 1457, 1388, 1125, 779, 773, 732 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.65 (d, 2H, Ar, $J = 8.2$ Hz), 6.80 (d, 2H, Ar, $J = 8.4$ Hz), 6.83 (t, 2H, Ar, $J = 7.4$ Hz), 7.20–8.02 (m, 8H, Ar), 8.38 (s, 4H, Ar), 8.74 (dd, 2H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 112.3, 120.3, 121.7, 122.8, 124.3, 126.9, 129.7, 131.3, 133.4, 135.2, 138.4, 141.2, 143.8, 145.2, 149.1, 152.2, 156.2, (34C, Ar–C); Anal. Found: C, 79.81; H, 4.02; N, 16.45%. Calcd for $C_{34}H_{20}N_6$: C, 79.67; H, 3.93; N, 16.40%. MS: $[M]^+$ at m/z 512.

*6.3.19. 6-(6-Benzo[4,5]imidazo[1,2-c]quinazolin-6-yl-2-pyridyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IVd**)*

Yield 72%; mp 354–356 °C; IR 1625, 1605, 1590, 1380, 1130, 747, 738, 732 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.64 (d, 2H, Ar, $J = 8.2$ Hz), 6.81 (d, 2H, Ar, $J = 8.4$ Hz), 6.82 (t, 2H, Ar, $J = 7.4$ Hz), 7.12–7.82 (m, 10H, Ar), 7.90 (t, 1H, Ar, $J = 7.8$ Hz) 8.74 (dd, 2H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 111.2, 112.2, 120.3, 121.5, 122.8, 123.3, 124.3, 126.6, 129.7, 132.3, 133.3, 135.2, 138.4, 141.2, 143.8, 145.2, 147.7, 151.1, 153.2, 156.2, (33C, Ar–C); Anal. Found: C, 77.17; H, 3.91; N, 18.75%. Calcd for $C_{33}H_{19}N_7$: C, 77.18; H, 3.73; N, 19.09%. MS: $[M]^+$ at m/z 513.

*6.3.20. 6-(5-Benzo[4,5]imidazo[1,2-c]quinazolin-6-yl-2-thienyl)benzo[4,5]imidazo[1,2-c]quinazoline (**IVe**)*

Yield 73%; mp 314–316 °C; IR 1635, 1608, 1457, 1388, 1129, 775, 742, 731, 693 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.65 (d, 2H, Ar, $J = 8.2$ Hz), 6.80 (d, 2H, Ar, $J = 8.4$ Hz), 6.83 (t, 2H, Ar, $J = 7.4$ Hz), 6.95 (s, 2H, Ar) 7.17–8.01 (m, 8H, Ar), 8.74 (dd, 2H, $J = 6.9$, 2.5 Hz); ^{13}C NMR (67.93 MHz, DMSO-d_6) δ 110.3, 111.8, 112.5, 119.6, 120.7, 121.5, 123.3, 124.7, 126.2, 129.7, 130.3, 132.8, 133.5, 138.2, 141.2, 143.3, 145.2, 147.7, 150.1, 152.3 (32C, Ar–C); Anal. Found: C, 75.08; H, 4.02; N, 16.15%. Calcd for $C_{32}H_{18}N_6S$: C, 74.11; H, 3.50; N, 16.21%. MS: $[M]^+$ at m/z 518.

6.4. Antimicrobial testing by agar diffusion method

Antimicrobial testing was performed by cup plate method [29,30]. All cultures were routinely maintained on NA (nutrient agar) and incubated at 37 °C. The inoculums of bacteria were performed by growing the culture in NA broth at 37 °C for overnight. 27 ml of molten agar was added to sterile Petri dishes and allowed to solidify for 1 h. Then 50 ml of the bacterial culture suspension was spread with the help of sterile cotton swab on NA plates uniformly. Six millimetre wide bores were made on the agar using a borer. The solutions of test compounds (1000 μg/ml) were added into each of the bores using a sterile tip with micropipette. A similar plate was prepared by replacing quinazoline by Ampicillin. This was taken as a standard against bacteria. The plates were then incubated at 37 °C for 24 h. The fungal strains were grown and maintained on Sabouraud glucose agar plates. A similar test compounds and standard Ketoconazole plates were prepared for antifungal screening. The plates were incubated at 26 °C for 72 h. The zone of the clearance around each bore after the incubation period, confirms the antimicrobial activity of the respective quinazoline. Each experiment was carried out in three replicates with each replication consisting of three test tubes for quantitative analysis. The clear zones formed around each bore were measured and average diameter of the inhibition zone was calculated and expressed in millimeter. It was used to determine the antimicrobial activity of quinazolines. The results were analyzed using ANOVA one way at $p \leq 0.05$.

The minimum inhibitory concentration required was also found when a series of dilutions were tested. The minimum inhibitory concentration at which no growth was observed was taken as the MIC values.

6.5. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration [31,32] of **IIIb**, **IIIc** and **IVd**, **IVe** against *S. aureus*, *B. subtilis*, *S. pyogenes*, *S. typhimurium*, *E. coli*, *K. pneumonia* (bacterial strains), *A. niger*, *C. albicans*, *T. viridae* (fungal strains) was determined by liquid dilution method. Stock solutions of tested compounds with 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μg/ml concentrations were prepared with appropriate solvent. The solutions of standard drugs, Ampicillin and Ketoconazole were prepared in the same concentrations. Inoculums of the bacterial and fungal culture were also prepared. To a series of tubes containing 1 ml each of quinazoline compound solution with different concentrations and 0.2 ml of the inoculum was added. Further 3.8 ml of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h and observed for the presence of turbidity. This method was repeated by changing quinazoline compounds with standard drug Ampicillin (in the case of bacteria) and with Ketoconazole (in the case of fungi) for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC values.

References

[1] Y. Zhang, Z. Chen, Y. Lou, Y. Yu, Eur. J. Med. Chem. 44 (2009) 448–452.
[2] A. Kumar, C.S. Rajput, Eur. J. Med. Chem. 44 (2009) 83–90.
[3] R. Arienzo, S. Cramp, H.J. Dyke, P.M. Lockey, D. Norman, A.G. Roach, P. Smith, M. Wong, S.P. Wren, Bioorg. Med. Chem. Lett. 17 (2007) 1403–1407.
[4] P. Kung, M.D. Casper, K.L. Cook, L.W. Lingardo, L.M. Risen, T.A. Vickers, R. Ranken, L.B. Blyn, J.R. Wyatt, P. Dan Cook, D.J. Ecker, J. Med. Chem. 42 (1999) 4705–4713.
[5] A.R. Genady, Eur. J. Med. Chem. 44 (2009) 409–416.
[6] V. Alagarsamy, M. Rupeshkumar, K. Kavitha, S. Meena, D. Shankar, A.A. Siddiqui, R. Rajesh, Eur. J. Med. Chem. 43 (2008) 2331–2337.
[7] P.M. Chandrika, T. Yakaiah, A.R. Rao, B. Narsaiah, N.C. Reddy, V. Sridhar, J.V. Rao, Eur. J. Med. Chem. 43 (2008) 846–852.
[8] S. Grasso, N. Micale, A.M. Monforte, P. Monforte, S. Polimeni, M. Zappal, Eur. J. Med. Chem. 35 (2000) 1115–1119.
[9] T. Herget, M. Freitag, M. Morbitzer, R. Kupfer, T. Stamminger, M. Marschall, Antimicrob. Agents Chemother. 48 (2004) 4154–4162.
[10] V.G. Chapoulaud, N. Ple, A. Turck, G. Queguiner, Tetrahedron 56 (2000) 5499–5507.
[11] C.J. Daly, C.M. Milligan, G. Milligan, J.F. Mackenzie, J.C. Mcgrath, J. Pharmacol. Exp. Ther. 286 (1998) 984–990.
[12] K.P. Chan, H. Yang, A.S. Hay, J. Polym. Sci. 34 (1996) 1923–1931.
[13] G.D. Galarcei, R.E. Foncea, A.M. Edwards, H. Pessoamahana, C.D.P. Mahana, R.A. Ebenspergeri, Biol. Res. 41 (2008) 43–50.
[14] L.D. Via, O. Gia, S.M. Mango, A.D. Settimo, A.M. Marini, G. Primofiore, F.D.A. Settimo, S. Salerno, Farmaco 56 (2001) 159–167.

[15] C. Lamazzi, S. Leonce, B. Pfeiffer, P. Renard, G. Guillaumet, C.W. Rees, T. Besson, Bioorg. Med. Chem. 10 (2000) 2183–2185.
[16] W.H.W. Lunn, R.W. Harper, R.L. Stone, J. Med. Chem. 14 (1971) 1069–1071.
[17] M.F. Brana, J.M. Castellano, G. Keilhauer, A. Machuca, Y. Martin, C. Redondo, E. Schlick, N. Walker, Anticancer. Drug Des. 9 (1994) 527–538.
[18] A.A. Spasov, I.N. Yozhitsa, L.I. Bugaeva, V.A. Anisimova, Pharma. Chem. J. 33 (1999) 232–243.
[19] B. Fernández, J. Castellano, M. Redondo, Eur. Pat. Appl. 331 (1989) 093.
[20] B.A. Insuasty, H. Torres, J. Quiroga, R. Abonia, R. Rodriguez, M. Nogueras, A. Sanchez, C. Saitz, S.L. Alvarez, S.A. Zacchino, J. Chil. Chem. Soc. 51 (2006) 927–932.
[21] L.N. Vostrova, T.A. Voronina, T.L. Karaseva, S.A. Gernega, É.I. Ivanov, A.M. Kirichenko, M. Yu, Totrova, Pharma. Chem. J. 20 (1986) 404–406.
[22] D.W. Hein, R.J. Alheim, J.J. Leavitt, J. Am. Chem. Soc. 79 (1957) 427–429.
[23] M. Davis, F.G. Mann, J. Chem. Soc. (1962) 945–954.
[24] S. Pareek, S. Vyas, G. Seth, P.C. Vyas, Heteroat. Chem. 19 (2008) 154–157.
[25] A.A. Fadda, H.M. Refat, M.E.A. Zaki, E. Monir, Synth. Commun. 31 (2001) 3537–3545.
[26] P. Amudha, P. Akilan, M. Kandaswamy, Polyhedron 18 (1999) 1355–1362.
[27] V.V. Korshak, A.L. Rusanov, T.G. Iremashvili, L.K. Plieva, T.V. Lekae, Die Makromolekulare Chemie 176 (1973) 1271.
[28] H.J. Jung, N. Singh, D.O. Jang, Tetrahedron Lett. 49 (2008) 2960–2964.
[29] P.M. Reddy, Y.P. Ho, K. Shanker, R. Rohini, V. Ravinder, Eur. J. Med. Chem., in press.
[30] S. Alam, J. Chem. Sci. 166 (2004) 325–332.
[31] U. Omrum, S. Arikan, S. Kocago, B. Semeak, S. Unala, Diagn. Microbiol. Infect. Dis. 38 (2000) 101–107.
[32] M. Malue, J.M. Bastide, A. Biancard, Int. J. Antimicrob. Agents 25 (2005) 321–328.
[33] R. Cruickshank, J.P. Duguid, B.P. Marmion, R.H.A. Swain, Medical Microbiology, 12th ed., vol. II, (1975).