European Journal of Medicinal Chemistry 54 (2012) 900–906

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Synthesis, characterization and antimicrobial studies of some new quinoline incorporated benzimidazole derivatives

B. Garudachari [a], M.N. Satyanarayana [b], B. Thippeswamy [c], C.K. Shivakumar [c], K.N. Shivananda [d], Gurumurthy Hegde [e], Arun M. Isloor [a,*]

[a] Medicinal Chemistry Laboratory, Department of Chemistry, National Institute of Technology Karnataka, Surathkal, Mangalore 575 025, India
[b] Department of Physics, National Institute of Technology Karnataka, Surathkal, Mangalore 575 025, India
[c] Department of P.G. Studies and Research in Microbiology, Jnanasahyadri, Kuvempu University, Shankaraghatta 577 451, India
[d] Schulich Faculty of Chemistry, Technion Israel Institute of Technology, Haifa, Israel
[e] Faculty of Industrial Science and Technology, Universiti Malaysia Pahang, LebuhrayaTunRazak, 26300, Gambang, Kuantan, Paghang Darul Makmur, Malaysia

ARTICLE INFO

Article history:
Received 20 October 2011
Received in revised form
17 May 2012
Accepted 22 May 2012
Available online 1 June 2012

Keywords:
Benzimidoquinolines
Antibacterial studies
Antifungal studies

ABSTRACT

Two new series of quinoline incorporated benzimidazole derivatives (**4a**–**i** and **8a**–**f**) were synthesized from substituted aniline and isatin through multi-step reaction. 6-substituted-4-carboxyquinolines (**3a**,**b** and **7**) were synthesized by multi component one pot reactions (via Doebner reaction and Pfitzinger reaction respectively) and the targeted benzimidazole derivatives were obtained by the reaction of 6-substituted-4-carboxyquinolines (**3a**,**b** and **7**) with substituted aromatic diamines in acidic media. All the newly synthesized compounds were characterized by IR, NMR mass spectral study and also by C, H, N analyses. The final compounds were screened for their *in-vitro* antibacterial and antifungal activity by well plate method (zone of inhibition). The results revealed that, compounds **4c**, **4d**, **8c** and **8d** showed significant antibacterial activity. The compound **8b** was found to be potent antifungal agent. **4a**, **8a** and **8f** showed moderate to good antimicrobial activity as compared to the standard drugs against all tested microbial strains.

1. Introduction

In recent years, the mounting threat of bacterial resistance has heightened the urgency to discover and develop anti-ineffective agents with novel mechanism of action and enhanced activity profile. Clinical use of potent drugs is limited in many cases due to their systemic side effects. To overcome the clinical limitation, considerable research efforts have been directed to the discovery of high potency local antimicrobial agents with reduced or without systemic adverse effects.

The development of potent and effective antimicrobial agent is most important to overcome the emerging multi-drug resistance strains of bacteria and fungi such as Methicillin resistant *Staphylococcus aureus* (MRSA) [1,2]. The heterocyclic compounds play an important role in developing new antimicrobial, anticancer, antimalarial, anticonvulsant agents. Recent observations suggested that, quinolines and substituted benzimidazoles have wide range of applications in the field of pharmaceuticals as antimalarial [3,4], anti-tuberculosis [5], antitumour [6,7], anticancer [8], analgesic, anti-inflammatory [9,10], antimicrobial and antiviral [11–13] agents. The fused heterocyclic rings which include Saquinavir (antiviral), Aripiprazole (antipsychotic), are some of drugs which contain quinoline as a core moiety (Figs. 1 and 2).

Azole class of drugs particularly fused imidazoles occupy prominent place in medicinal chemistry because of their broad spectrum of pharmacological activities such as anti-inflammatory, analgesic, anticancer, antiulcer, antimicrobial, antiviral, pesticidal, cytotoxicity and anti-arrhythmic [14–17] activities. Omeprazole, Mebendazole, Pimobendan and Albendazole are well-known drugs in the market which contain fused imidazoles as active core moiety.

Literature review revealed that, insertion of pharmacophore at position 4 of quinoline with substituted amines enhances its anti-tuberculosis, antimicrobial activity [18]. Substituted quinolines at 2 and 3 positions with various benzimidazole derivatives showed excellent pharmacological properties like antimicrobial, antifungal and antitumour activities [19,20]. Prompted by these observations and in continuation of our research on biologically active heterocycles [21–24], we hereby report the synthesis of some new benzimidazole derivatives containing substituted quinoline nucleus. These compounds were evaluated for their antimicrobial properties.

* Corresponding author. Tel.: +91 824 2474000; fax: +91 824 2474033.
E-mail address: isloor@yahoo.com (A.M. Isloor).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2012.05.027

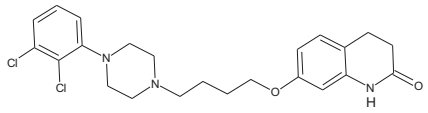


Fig. 1. Structure of Aripiprazole (antipsychotic).

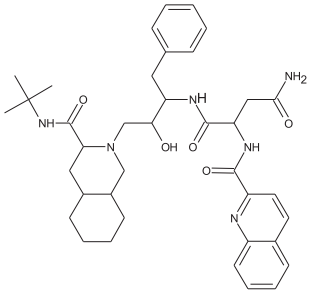


Fig. 2. Structure of Saquinavir (antiviral).

2. Results and discussion

2.1. Chemistry

6-Substituted-4-carboxyquinolines (**3a**, **b**) were synthesized by Doebner reaction using substituted aniline (**1**), 4-fluorobenzaldehyde (**2**), pyruvic acid and catalytic amount of trifluoroacetic acid in ethanol media. The versatile Pfitzinger reaction was utilized to synthesize 4-carboxyquinoline (**7**) in satisfactory yields by reacting isatin (**5**) with α-methylketone (**6**) in aqueous ethanol [25–27]. The targeted quinoline incorporated benzimidazole derivatives (**4a**–**i** and **8a**–**f**) were synthesized by reacting 6-substituted-4-carboxyquinolines (**3a**, **b** and **7**) with various aromatic-1,2-diamines in polyphosphoric acid media. The crude products were purified by column chromatography. The reaction pathway has been summarized in Schemes 1 and 2. Newly synthesized compounds were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

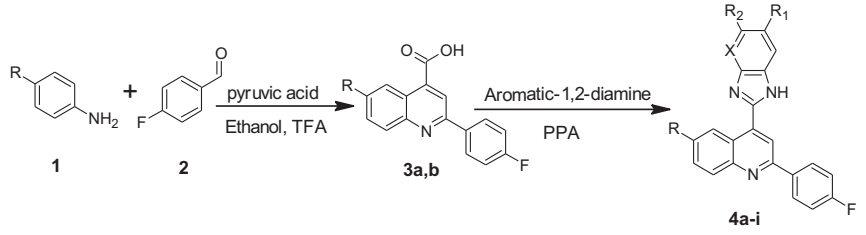
Formation of 6-substituted-4-carboxyquinolines (**3a**, **b** and **7**) was confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The formation of quinoline acid (**3a**) was confirmed by the peak at 3330 cm^{-1} in IR spectrum which is due to the −OH stretching of carboxylic acid. Band at 1718 cm^{-1} is due to C═O stretch of acid group. The ^{1}H NMR spectrum of **3a** showed a singlet at δ 13.77 corresponds to acid −OH proton of quinoline. A singlet at δ 8.49 is due to quinoline-3H proton, 4-fluorophenyl aromatic protons appeared as multiplet at δ 7.02–8.25 further confirmed the structure of the compound. The mass spectrum of **3a** showed molecular ion peak at m/z = 302 (M + 1), which is in agreement with the molecular formula $C_{16}H_9ClFNO_2$.

Formation of 4-(1*H*-benzimidazol-2-yl)-6-chloro-2-(4-fluorophenyl)quinoline (**4a**) was confirmed by the presence of absorption peak at 3356 cm^{-1} in IR spectrum which is due to −NH stretching of benzimidazole. Band at 1621 and 1597 is due to C═N and C═C of benzimidazole respectively. The ^{1}H NMR spectrum of compound **4a** showed broad singlet at δ 13.35 (D_2O exchangeable) which is due to benzimidazole −NH. Singlet at δ 8.67 is due to quinoline-3H, multiplet at δ 7.66–7.77 is due to aromatic protons of benzimidazole ring and multiplet at δ 7.28–7.47 is due to protons of 4-fluorophenyl moiety conformed the structure. The mass spectrum of **4a** showed molecular ion peak at m/z = 374 (M + 1), which is in agreement with the molecular formula $C_{22}H_{13}ClN_3$. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in Table 1.

2.2. Antimicrobial studies

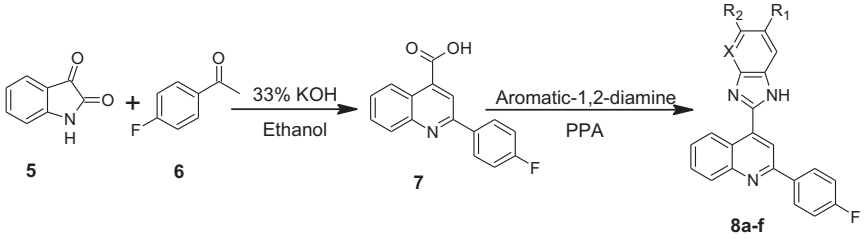
2.2.1. Antibacterial studies

The newly synthesized compounds (**4a**–**i** and **8a**–**f**) were screened for their *in-vitro* antibacterial activity against *S. aureus*,



Where R= H, Cl, F; R_1= Cl, F; R_2= H, Cl; X= CH, N

Scheme 1. Synthetic route for the compounds **4a**–**i**.



Where R_1= H, Cl, F; R_2=,H Cl; X= CH, N

Scheme 2. Synthetic route for the compounds **8a**–**f**.

Table 1
Characterization data of the compounds **4a**–**i** and **8a**–**f**.

Comp. No	R	R_1	R_2	X	Mol. Wt	Mol. Formula	M.p. (°C)	Yield (%)
4a	Cl	H	H	H	373.82	$C_{22}H_{13}ClN_3$	241–241	60
4b	Cl	Cl	H	H	408.26	$C_{22}H_{12}Cl_2FN_3$	197–198	50
4c	Cl	H	H	N	374.80	$C_{21}H_{12}ClFN_4$	266–267	58
4d	Cl	Cl	Cl	H	442.70	$C_{22}H_{11}Cl_3FN_3$	165–166	40
4e	F	H	H	H	373.81	$C_{22}H_{13}F_2N_3$	218–219	54
4f	F	Cl	H	H	391.80	$C_{22}H_{12}ClF_3N_3$	139–139	52
4g	F	H	H	N	358.34	$C_{21}H_{12}F_2N_4$	132–133	51
4h	F	Cl	Cl	H	426.25	$C_{22}H_{11}Cl_2F_2N_3$	108–109	42
4i	F	F	H	H	375.35	$C_{22}H_{12}F_3N_3$	97–98	40
8a	H	H	H	H	339.38	$C_{22}H_{14}FN_2$	164–165	78
8b	H	Cl	H	H	408.26	$C_{22}H_{13}ClFN_3$	234–235	65
8c	H	H	H	N	340.35	$C_{21}H_{13}FN_4$	128–129	68
8d	H	Cl	Cl	H	408.26	$C_{22}H_{12}Cl_2FN_3$	205–206	57
8e	H	F	H	H	357.36	$C_{22}H_{13}ClF_2N_3$	77–78	55
8f	H	OMe	H	H	369.39	$C_{23}H_{16}FN_3O$	74–75	25

Escherichia coli, *Xanthomonas* sp. and *Salmonella* sp. (recultured) using Ciprofloxacin as standard by well plate method (zone of inhibition) [28,29]. The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 6.25 and 12.5 μg/mL.

The antibacterial screening revealed that, few of the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Among the synthesized compounds, **4c** and **8d** showed significant antibacterial activity against *E. coli* and *S. aureus*. Compound **8c** is found to be more potent against *S. aureus*. Compounds **4d** and **8d** exhibited excellent antibacterial activity against *Salmonella* and *Xanthomonas* sp. respectively. Remaining compounds showed moderately good antibacterial activity against all the tested bacterial strains than compared to standard drug Ciprofloxacin. Compounds **4c** and **8c** contain fused pyridine ring in benzimidazole moiety and a 4-fluorophenyl group on second position of quinoline ring which has accounted for the enhanced activity. Compounds **4d** and **8d** contain two chlorine atoms on benzimidazole ring and 4-fluorophenyl group on second position of quinoline ring which are responsible for the moderately good inhibition. Results of antibacterial studies have been presented in Table 2.

2.2.2. Antifungal studies

The *in-vitro* antifungal activities of newly synthesized compounds (**4a**–**i** and **8a**–**f**) were determined by well plate method [30,31]. In this work *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Penicillium* sp. (recultured) were used to investigate the activity. The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 6.25 and 12.5 μg/mL.

The result indicated that, among the tested compounds, **8b** showed significant antifungal activity against *A. niger* and *A. flavus* compared to standard drug Flucanazole. Compounds 4**a**, **8a** and **8f** were active against *A. terreus* and *Penicillium* sp. fungal strains respectively. The enhanced activity of the compound **8b** may be due to the presence of a chlorine atom on benzimidazole ring and 4-fluorophenyl group on second position of quinoline ring. All other compounds showed less inhibition against all the tested micro organisms as compared to the standard drug. Table 3 depicts the antifungal screening results of the final compounds.

3. Conclusion

Two new series of quinoline incorporated benzimidazole derivatives (**4a**–**i** and **8a**–**f**) were synthesized, characterized by IR, NMR, mass spectra and C, H, N analyses. Final compounds were investigated for their *in-vitro* antimicrobial and antifungal activities by well plate method. Among the screened samples, **4c**, **4d**, **8c** and **8d** showed excellent inhibition of bacterial growth. Compound **8b** showed significant inhibition of fungal strain as compared with standard drug Fluconazole.

Table 2
Antibacterial activity of the compounds **4a**–**i** and **8a**–**f**.

Zone of inhibition in mm (mean ± S.D.) $n = 3$								
Compound No.	*Staphylococcus aureus*		*Escherichia coli*		*Xanthomonas* sp		*Salmonella* sp	
Concn. (μg/mL)	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5
Standard Ciprofloxacin	18 ± 0.87	20 ± 0.50	19 ± 1.00	21 ± 0.50	18 ± 0.50	22 ± 0.50	19 ± 0.50	31 ± 0.50
Control	00	00	00	00	00	00	00	00
4a	12 ± 0.50	13 ± 1.00	01 ± 0.50	13 ± 0.50	01 ± 0.50	10 ± 0.50	01 ± 0.50	9 ± 0.50
4b	16 ± 1.00	16 ± 0.50	12 ± 0.50	12 ± 0.50	01 ± 0.50	10 ± 0.50	10 ± 0.50	13 ± 0.50
4c	14 ± 0.50	18 ± 1.00	16 ± 0.50	16 ± 0.50	15 ± 1.00	16 ± 0.50	9 ± 0.50	20 ± 0.50
4d	10 ± 0.50	12 ± 0.50	12 ± 0.50	17 ± 0.50	14 ± 0.50	16 ± 0.50	20 ± 0.50	28 ± 0.50
4e	14 ± 1.00	15 ± 0.50	10 ± 0.50	15 ± 0.50	14 ± 0.50	14 ± 0.50	15 ± 0.50	15 ± 0.50
4f	12 ± 0.50	12 ± 0.50	10 ± 0.50	11 ± 0.50	11 ± 0.50	12 ± 0.50	01 ± 0.50	11 ± 0.50
4g	12 ± 0.50	12 ± 1.00	01 ± 0.50	13 ± 0.50	15 ± 0.50	16 ± 0.50	13 ± 0.50	13 ± 0.50
4h	14 ± 0.50	15 ± 0.50	01 ± 0.50	12 ± 0.50	12 ± 0.50	16 ± 0.50	9 ± 0.50	12 ± 0.50
4i	12 ± 0.50	12 ± 0.50	16 ± 0.50	16 ± 0.50	01 ± 0.50	14 ± 0.50	9 ± 0.50	13 ± 0.50
8a	12 ± 1.00	13 ± 1.00	11 ± 1.00	16 ± 0.50	11 ± 0.50	12 ± 0.50	9 ± 0.50	15 ± 0.50
8b	12 ± 1.00	13 ± 0.50	01 ± 0.50	16 ± 0.87	15 ± 0.50	15 ± 0.50	9 ± 0.50	17 ± 0.50
8c	16 ± 1.00	16 ± 1.00	14 ± 0.50	14 ± 0.50	10 ± 0.50	14 ± 0.50	9 ± 0.50	12 ± 0.50
8d	12 ± 0.50	16 ± 0.50	12 ± 0.50	16 ± 0.50	20 ± 1.00	22 ± 0.50	11 ± 0.50	12 ± 0.50
8e	10 ± 1.00	16 ± 0.50	10 ± 0.50	11 ± 0.50	01 ± 0.50	13 ± 0.50	10 ± 0.50	11 ± 0.50
8f	12 ± 0.50	12 ± 0.50	12 ± 0.50	14 ± 0.87	12 ± 0.50	14 ± 0.50	10 ± 1.00	11 ± 0.50

Table 3
Antifungal activity of the compounds **4a**–**i** and **8a**–**f**.

Zone of inhibition in mm (mean ± S.D.) $n = 3$								
Compound No.	*Aspergillus niger*		*Aspergillus flavus*		*Penicillium* sp.		*Aspergillus terreus*	
Concn. (μg/mL)	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5
Standard Flucanazole	14 ± 0.50	18 ± 1.00	15 ± 0.50	20 ± 0.50	19 ± 0.50	21 ± 0.50	16 ± 0.50	18 ± 0.50
Control	00	00	00	00	00	00	00	00
4a	10 ± 1.00	12 ± 0.50	01 ± 0.50	01 ± 0.50	16 ± 0.50	18 ± 0.50	13 ± 0.50	14 ± 0.50
4b	01 ± 1.00	10 ± 0.50	01 ± 0.50	17 ± 1.00	12 ± 1.00	17 ± 0.50	9 ± 0.50	9 ± 1.00
4c	12 ± 0.50	14 ± 0.50	01 ± 0.50	10 ± 0.50	01 ± 0.50	13 ± 1.00	9 ± 0.50	15 ± 0.50
4d	12 ± 1.00	12 ± 1.00	13 ± 0.50	13 ± 0.50	12 ± 0.50	19 ± 0.50	9 ± 0.50	10 ± 0.50
4e	10 ± 0.50	12 ± 1.00	01 ± 0.50	10 ± 0.50	12 ± 0.50	12 ± 1.00	9 ± 0.50	9 ± 1.00
4f	01 ± 0.50	10 ± 0.50	01 ± 0.50	10 ± 0.50	15 ± 1.00	17 ± 0.50	10 ± 0.87	14 ± 0.50
4g	11 ± 1.00	13 ± 0.50	11 ± 0.50	14 ± 0.50	14 ± 0.87	15 ± 0.50	10 ± 0.50	15 ± 0.50
4h	10 ± 1.00	13 ± 0.50	12 ± 1.00	15 ± 0.50	01 ± 1.00	12 ± 0.50	01 ± 0.50	09 ± 0.50
4i	10 ± 0.50	17 ± 0.50	01 ± 0.50	01 ± 0.50	13 ± 0.50	13 ± 1.00	10 ± 0.50	9 ± 0.50
8a	11 ± 1.00	12 ± 1.00	11 ± 1.00	12 ± 0.50	15 ± 0.87	17 ± 0.50	12 ± 0.50	14 ± 0.50
8b	15 ± 1.00	18 ± 1.00	11 ± 0.50	16 ± 0.50	12 ± 0.50	12 ± 0.50	10 ± 0.87	16 ± 1.00
8c	14 ± 1.00	16 ± 1.00	01 ± 0.50	11 ± 0.50	16 ± 0.50	17 ± 0.50	9 ± 0.50	10 ± 0.50
8d	10 ± 0.50	12 ± 0.50	10 ± 1.00	10 ± 0.50	12 ± 0.50	12 ± 1.00	10 ± 0.50	11 ± 0.50
8e	01 ± 0.50	14 ± 1.00	01 ± 0.50	14 ± 0.50	12 ± 0.50	15 ± 0.50	12 ± 0.50	12 ± 0.50
8f	10 ± 1.00	12 ± 0.50	01 ± 0.50	10 ± 1.00	16 ± 0.50	18 ± 1.00	12 ± 0.50	13 ± 0.50

The enhanced antibacterial activity of compounds **4c**, **8c** is due to the presence of fused pyridine ring in benzimidazole moiety as well as 4-fluorophenyl group on second position of quinoline ring. The presence of two chlorine atoms on benzimidazole ring along with 4-fluorophenyl group on second position of quinoline ring is the reason for enhanced activity of **4d** and **8d**. The compound **8b** contains a chlorine atom on benzimidazole ring and 4-fluorophenyl group on second position of quinoline which is responsible for the enhanced antifungal activity.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antimicrobial properties, it showed varied pharmacological activity. Probably in this case the nature of the heterocyclic ring is not so important for antimicrobial activity. Moreover, the presence of different substituents causes a certain change of activity. It can be concluded that the combination of two different heterocyclic moieties namely quinoline and benzimidazole has enhanced the biological activity and hence they are ideally suited for father notifications to obtain more efficient antimicrobial compounds.

4. Experimental

4.1. Chemistry

All the chemicals were purchased from Sigma Aldrich, Merck and S. D. Fine chemicals-India. Commercial grade solvents were used and were distilled before use. Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer and Bruker (400 MHz) spectrometer was used to record ^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6) using TMS as internal standard. Chemical shift values were given in δ (ppm) scales. The mass spectra were recorded on LC–MS-Agilent 1100 series and elemental analysis was performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reactions was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254).

4.2. General procedure for the synthesis of 6-substituted-4-carboxyquinoline (***3a***–***b***)

An equimolar mixture of 4-substituted aniline (**1**) (10.0 g, 0.078 mol), 4-fluorobenzaldehyde (**2**) (9.72 g, 0.078 mol) in ethanol (100 mL) were refluxed for 1 h, pyruvic acid (10.30 g, 0.117 mol) and trifluoroacetic acid (1 mL) were then added to the reaction mass and further refluxed for 12 h. After completion of the reaction, the reaction mixture was poured into ice-cold water. The solid product obtained was filtered, washed with water and recrystallized using ethanol.

4.2.1. 6-Chloro-2-(4-fluorophenyl)quinoline-4-carboxylic acid (***3a***)

Yield: 15.20 g, 64.4%; M.p: 210–211 °C; IR (KBr ν_{max} cm^{-1}): 3384 (O–H-str), 3062, 2983 (C–H-str), 1718 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.98–7.25 (m, 4H, 4-fluorophenyl), 8.49 (d, J = 8.2 Hz, 2H, quinoline), 8.53 (s, 1H, quinoline), 8.74 (m, 1H, quinoline), 14.77 (s, 1H, COOH); MS: m/z = 302 (M + 1); Anal. calcd. For $C_{16}H_9ClFNO_2$: C, 67.70; H, 3.01; N, 4.64. Found: C, 67.68; H, 3.00; N, 4.67%.

4.2.2. 6-Fluoro-2-(4-fluorophenyl)quinoline-4-carboxylic acid (***3b***)

Yield: 9.5 g, 36.8%; M.p: 187–188 °C; IR (KBr ν_{max} cm^{-1}): 3400 (O–H-str), 3068, 2981 (C–H-str), 1681 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ6.90–8.18 (m, 4H, 4-fluorophenyl), 8.49–8.53 (m, 2H, quinoline), 8.54 (s, 1H, quinoline), 8.54 (m, 1H, quinoline), 14.10 (s, 1H, COOH); MS: m/z = 385; Anal. calcd. For $C_{16}H_9F_2NO_2$: C, 67.37; H, 3.18; N, 4.91. Found: C, 67.70; H, 3.16; N, 4.68%.

4.3. Preparation of 2-(4-fluorophenyl)quinoline-4-carboxylic acid (***7***)

A mixture of isatin (**5**) (10.0 g, 0.067 mol), 100 mL 33% alcoholic potassium hydroxide solution and 4-fluoroacetophenone (**6**) (10.32 g, 0.074 mol) were refluxed for 48 h. After completion of reaction, the reaction mass was concentrated by distillation and the residue obtained was acidified with 15% acetic acid. The solid precipitated was filtered, washed with 5% acetic acid, ethanol, hexane and crystallized in methanol.

Yield: 11.30 g, 62.2%; M.p: 155–156 °C; IR (KBr ν_{max} cm^{-1}): 3400 (O–H-str), 3051, 2989 (C–H-str), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.09–7.64 (m, 2H, 4-fluorophenyl), 7.68–7.88 (m, 2H, quinoline), 8.76 (d, J = 8.3 Hz, 2H, 4-fluorophenyl), 8.34 (m, 1H, quinoline), 8.45 (s, 1H, quinoline), 8.59–8.61 (m, 1H, quinoline), 14.11 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.4, 167.2, 162.2, 160.8, 147.9, 134.0, 131.3, 131.0, 130.5, 129.8, 129.6, 129.5, 129.3, 125.6, 123.1, 114.2; MS: m/z = 268 (M + 1); Anal. calcd. For $C_{16}H_9ClFNO_2$: C, 71.91; H, 3.77; N, 5.24. Found: C, 72.10; H, 2.98; N, 5.23%.

4.4. General method for the preparation of 4-substituted benzimidoquinoline (**4a–i** and **8a–f**)

A mixture of 6-substituted-4-carboxyquinoline (**3a**,**b** and **7**) (0.01 mol), aromatic-1,2-diamine (0.01 mol) and fresh polyphosphoric acid (PPA) (2 mL) were heated to 250 °C for 2 h. The reaction mixture was then poured into 20 mL of 30% sodium carbonate solution in water. The solid product obtained was filtered and dried. The crude products were purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent.

4.4.1. 4-(1H-Benzimidazol-2-yl)-6-chloro-2-(4-fluorophenyl) quinoline (**4a**)

IR (KBr ν_{max} cm^{-1}): 3356 (−NH stretching for benzimidazole ring), 3087, 2927 (C−H-str), 1621 (C═N), 1597 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28−7.46 (m, 2H, 4-fluorophenyl), 7.67−7.73 (m, 2H, benzimidazole), 7.75−7.88 (m, 2H, 4-fluorophenyl), 8.18 (d, J = 8.2 Hz, 2H, quinoline), 8.44−8.48 (m, 2H, benzimidazole), 8.63 (s, 1H, quinoline), 9.46 (s, 1H, quinoline), 13.45 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 374 (M + 1). Anal. calcd. For $C_{22}H_{13}FN_3$; Calc: C, 70.69; H, 3.51; N, 11.24; found: C, 70.70; H, 3.50; N, 11.26%.

4.4.2. 6-Chloro-4-(5-chloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (4**b**)

IR (KBr ν_{max} cm^{-1}): 3342 (−NH stretching for benzimidazole ring), 3082, 2935 (C−H-str), 1597 (C═N), 1556 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45−7.87 (m, 4H, 4-fluorophenyl), 7.90 (d, J = 8.2 Hz, 2H, benzimidazole), 8.45 (s, 1H, quinoline), 8.72 (s, 1H, benzimidazole), 8.72 (s, 1H, quinoline), 8.69 (s, d = 8.2 Hz, 2H, quinoline), 13.44 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 409 (M + 1). Anal. calcd. For $C_{22}H_{12}Cl_2FN_3$; Calc: C, 64.72; H, 2.96; N, 10.29; found: C, 64.75; H, 2.99; N, 10.20%.

4.4.3. 6-Chloro-2-(4-fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl)quinoline (**4c**)

IR (KBr ν_{max} cm^{-1}): 3400 (−NH stretching for benzimidazole ring), 3057, 2929 (C−H-str), 1592 (C═N), 1538 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45−7.90 (m, 4H, 4-fluorophenyl), 8.48−8.55 (m, 2H, benzimidazole), 8.73 (s, 1H, quinoline), 8.82 (s, 1H, quinoline), 9.62−9.66 (m, 1H, benzimidazole), 9.84 (d, J = 8.2 Hz, 2H, quinoline), 13.59 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 375 (M + 1). Anal. calcd. For $C_{21}H_{12}ClFN_4$; Calc: C, 67.30; H, 3.23; N, 14.95; found: C, 67.34; H, 3.26; N, 14.93%.

4.4.4. 6-Chloro-4-(5,6-dichloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (4**d**)

IR (KBr ν_{max} cm^{-1}): 3362 (−NH stretching for benzimidazole ring), 3054, 2911 (C−H-str), 1627 (C═N), 1550 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47−7.90 (m, 4H, 4-fluorophenyl), 8.22 (s, 1H, quinoline), 8.45 (s, 1H, quinoline), 8.48 (d, J = 8.3 Hz, 2H, quinoline), 9.58 (s, 1H, benzimidazole), 9.61 (s, 1H, benzimidazole), 13.46 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 444 (M + 1). Anal. calcd. For $C_{22}H_{11}Cl_3FN_3$; Calc: C, 59.69; H, 2.50; N, 9.49; found: C, 59.71; H, 2.51; N, 9.55%.

4.4.5. 4-(1H-Benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl) quinoline (**4e**)

IR (KBr ν_{max} cm^{-1}): 3409, (−NH stretching for benzimidazole ring), 3066, 2925 (C−H-str), 1605 (C═N), 1552 (C═C); ^{1}H NMR (DMSO-d_6) δ: 7.28−7.47 (m, 4H, 4-fluorophenyl), 7.66−7.75−7.81 (m, 2H, benzimidazole), 7.82−7.86 (m, 2H, benzimidazole), 8.45−8.47 (m, 2H, quinoline), 8.76 (s, 1H, quinoline), 9.33−9.40 (m, 1H, quinoline), 13.53 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 358 (M + 1). Anal. calcd. For $C_{22}H_{13}F_2N_3$; Calc: C, 73.94; H, 3.67; N, 11.76; found: C, 73.97; H, 3.68; N, 11.77%.

4.4.6. 4-(5-Chloro-1H-benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl)quinoline (**4f**)

IR (KBr ν_{max} cm^{-1}): 3354 (−NH stretching for benzimidazole ring), 3020, 2979 (C−H-str), 1603 (C═N), 1507 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34−7.70 (m, 4H, 4-fluorophenyl), 8.26−8.28 (m, 1H, quinoline), 8.47 (s, 1H, quinoline), 8.49 (d, J = 8.5 Hz, 2H, benzimidazole), 8.76 (s, 1H, benzimidazole), 9.42−9.45 (m, 2H, quinoline), 13.45 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 392 (M + 1). Anal. calcd. For $C_{22}H_{12}ClF_2N_3$; Calc: C, 67.44; H, 3.09; N, 10.72; found: C, 67.42; H, 3.08; N, 10.75%.

4.4.7. 6-Fluoro-2-(4-fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl)quinoline (**4g**)

IR (KBr ν_{max} cm^{-1}): 3403 (−NH stretching for benzimidazole ring), 3028, 2921 (C−H-str), 1623 (C═N), 1540 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.39−7.48 (m, 4H, 4-fluorophenyl), 7.50−7.53 (m, 2H, quinoline), 7.83−7.85 (m, 1H, quinoline), 8.28−8.48 (m, 2H, benzimidazole), 8.51 (s, 1H, quinoline), 9.38−9.43 (m, 1H, benzimidazole), 13.73 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 359 (M + 1). Anal. calcd. For $C_{21}H_{12}F_2N_4$; Calc: C, 70.39; H, 3.38; N, 15.65; found: C, 70.41; H, 3.35; N, 15.66%.

4.4.8. 4-(5,6-Dichloro-1H-benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl)quinoline (**4h**)

IR (KBr ν_{max} cm^{-1}): 3355 (−NH stretching for benzimidazole ring), 3057, 2932 (C−H-str), 1660 (C═N), 1539 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.38−7.83 (m, 4H, 4-fluorophenyl), 8.26 (s, 1H, quinoline), 8.46−8.48 (m, 2H, quinoline), 8.75−8.76 (m, 1H, quinoline), 9.42 (s, 1H, benzimidazole), 9.46 (s, 1H, benzimidazole), 13.42 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 427 (M + 1). Anal. calcd. For $C_{22}H_{11}Cl_2F_2N_3$; Calc: C, 61.99; H, 2.60; N, 9.86; found: C, 61.98; H, 2.55; N, 9.89%.

4.4.9. 6-Fluoro-4-(5-fluoro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (**4i**)

IR (KBr ν_{max} cm^{-1}): 3400 (−NH stretching for benzimidazole ring), 3057, 2928 (C−H-str), 1584 (C═N), 1540 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.17−7.73 (m, 4H, 4-fluorophenyl), 7.98−8.28 (m, 2H quinoline), 8.45−8.48 (m, 2H, benzimidazole), 8.74 (s, 1H, quinoline), 9.33−9.40 (m, 2H, benzimidazole), 13.53 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 375 (M + 1). Anal. calcd. For $C_{22}H_{12}F_3N_3$; Calc: C, 70.40; H, 3.22; N, 11.20; found: C, 70.45; H, 3.20; N, 11.23%.

4.4.10. 4-(1H-Benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (**8a**)

IR (KBr ν_{max} cm^{-1}): 3396 (−NH stretching for benzimidazole ring), 3055, 2986 (C−H-str), 1619 (C═N), 1594 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30−7.36 (m, 2H, 4-fluorophenyl), 7.38−7.47 (m, 2H, benzimidazole), 7.70−7.89 (m, 2H, quinoline), 7.90−8.19 (m, 2H, benzimidazole), 8.44−8.48 (m, 2H, quinoline), 8.72 (s, 1H, quinoline), 13.43 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 340 (M+1). Anal. calcd. For $C_{22}H_{14}FN_3$; Calc: C, 77.86; H, 4.16; N, 12.38; found: C, 77.89; H, 4.14; N, 12.40%.

4.4.11. 4-(5-Chloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl) quinoline (**8b**)

IR (KBr ν_{max} cm^{-1}): 3325 (−NH stretching for benzimidazole ring), 3066, 2928 (C−H-str), 1601 (C═N), 1508 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30−7.73 (m, 4H, 4-fluorophenyl), 7.76−8.44 (m, 4H, quinoline), 8.45−8.48 (m, 2H, benzimidazole), 8.63 (s, 1H, quinoline), 9.46 (s, 1H, benzimidazole), 13.35 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 374 (M + 1). Anal. calcd. For $C_{22}H_{13}ClFN_3$; Calc: C, 70.69; H, 3.51; N, 11.24; found: C, 70.67; H, 3.53; N, 11.25%.

4.4.12. 2-(4-Fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl) quinoline (**8c**)

IR (KBr ν_{max} cm^{-1}): 3400 (−NH stretching for benzimidazole ring), 3021, 2925 (C−H-str), 1660 (C═N), 1539 (C═C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.39–7.77 (m, 4H, 4-fluorophenyl), 7.91–8.31 (m, 2H, benzimidazole), 8.49–8.52 (m, 4H, quinoline), 8.74 (s, 1H, quinoline), 9.48 (m, 1H, benzimidazole), 13.63 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 341 (M + 1). Anal. calcd. For $C_{21}H_{13}FN_4$; Calc: C, 74.11; H, 3.85; N, 16.46; found: C, 74.15; H, 3.82; N, 16.47%.

4.4.13. 4-(5,6-Dichloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl) quinoline (**8d**)

IR (KBr ν_{max} cm^{-1}): 3453 (−NH stretching for benzimidazole ring), 3102, 3015 (C−H-str), 1622 (C═N), 1539 (C═C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.45–7.76 (m, 4H, 4-fluorophenyl), 7.88–8.47 (m, 4H, quinoline), 8.65 (s, 1H, quinoline), 9.36 (s, 1H, benzimidazole), 9.38 (s, 1H, benzimidazole), 13.66 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 409 (M + 1). Anal. calcd. For $C_{22}H_{12}Cl_2FN_3$; Calc: C, 64.72; H, 2.96; N, 10.29; found: C, 64.70; H, 2.92; N, 10 35%.

4.4.14. 4-(5-Fluoro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl) quinoline (**8e**)

IR (KBr ν_{max} cm^{-1}): 3444 (−NH stretching for benzimidazole ring), 3057, 2919 (C−H-str), 1568 (C═N), 1539 (C═C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.46–7.77 (m, 4H, 4-fluorophenyl), 7.87–8.20 (m, 4H, quinoline), 8.46–8.49 (m, 1H, benzimidazole), 8.64 (s, 1H, quinoline), 9.39–9.44 (m, 2H, benzimidazole), 13.53 (s, 1H, −NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.53, 162.67, 155.20, 149.05, 130.74, 130.15, 130.08, 130.00, 127.91, 127.19, 126.68, 124.16, 119.84, 118.74, 116.45, 116.23. MS: m/z = 358 (M + 1). Anal. calcd. For $C_{22}H_{13}F_2N_3$; Calc: C, 73.94; H, 3.67; N, 11.76; found: C, 73.97; H, 3.65; N, 11.77%.

4.4.15. 2-(4-Fluorophenyl)-4-(6-methoxy-1H-benzimidazol-2-yl) quinoline (**8f**)

IR (KBr ν_{max} cm^{-1}): 3359 (−NH stretching for benzimidazole ring), 3015, 2924 (C−H-str), 1660 (C═N), 1540 (C═C); ^1H NMR (400 MHz, DMSO-d_6): δ 4.01 (s, 3H, $-OCH_3$), 7.41–7.77 (m, 4H, 4-fluorophenyl), 7.89–8.18 (m, 2H, benzimidazole), 8.20–8.41 (m, 3H, quinoline), 8.51 (s, 1H, benzimidazole), 9.06 (s, 1H, quinoline), 9.46–9.49 (m, 1H, quinoline), 13.36 (s, 1H, −NH, D_2O-exchangeble); MS: m/z = 370 (M + 1). Anal. calcd. For $C_{23}H_{16}FN_3O$; Calc: C, 74.78; H, 4.37; N, 11.38; found: C, 74.75; H4.39; N, 11.39%.

4.5. Antibacterial studies

The *in-vitro* antibacterial activity of newly synthesized compounds **4a**–**i** and **8a**–**f** were evaluated for their antibacterial against *S. aureus*, *E. coli*, *Xanthomonas* sp. and *Salmonella* sp. bacterial strains by well plate method. A number of antimicrobial discs were placed on the agar for the purpose of producing zones of inhibition in the bacterial lawn. 15–20 mL of agar media were poured into each petri dish. Agar containing plates were dried by placing in a laminar air flow at 37 ± 2 °C for an hour. Using an agar punch, wells were made on the seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labelled wells. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 ± 2 °C for 24–48 h. Activities were determined by measuring the diameter of inhibition zone (mm). Ciprofloxacin was used as standard. Experiments were triplicates and standard deviation was calculated.

4.6. Antifungal studies

Antifungal studies of newly synthesized compounds (**4a**–**i** and **8a**–**f**) were carried out against *A. niger*, *A. flavus*, *A. terreus* (NCIM 1325) and *Penicillium* sp. (recultured). Sabourands agar media was prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h. Using a puncher, wells were made on the seeded agar plates. The different concentrations of the test compounds in DMSO were added into each labelled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicates and maintained at 25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with flucanazole as standard. Zones of inhibition (mm) were determined for compounds (**4a**–**i** and **8a**–**f**).

Acknowledgements

AMI thanks Department of Atomic Energy, Board for Research in Nuclear Sciences, Government of India for the 'Young Scientist' award. AMI and SMN thanks Department of Information & technology, Government of India, New Delhi for the financial support.

References

[1] T. Chua, C.L. Moore, M.B. Perri, S.M. Donabedian, W. Masch, D. Vager, S.L. Davis, K. Lulek, B. Zimnicki, M.J. Zervos, J. Clin. Microbiol. 46 (2008) 2345–2352.
[2] D.T.W. Chu, J.J. Plattner, L. Katz, J. Med. Chem. 39 (1996) 3853–3874.
[3] K. Kaur, M. Jain, R.P. Reddy, R. Jain, Eur. J. Med. Chem. 45 (2010) 3245–3264.
[4] P. Nasveld, S. Kitchener, Trans. R. Soc. Trop. Med. Hyg. 99 (2005) 2–5.
[5] S. Eswaran, A.V. Adhikari, I.H. Chowdhury, N.K. Pal, K.D. Thomas, Eur. J. Med. Chem. 45 (2010) 3374–3383.
[6] L.C. Chou, M.T. Tsai, M.H. Hsu, S.H. Wang, T.D. Way, C.H. Huang, H.Y. Lin, K. Qian, Y. Dong, K.H. Lee, L.J. Huang, S.C. Kuo, J. Med. Chem. 53 (2010) 8047–8058.
[7] M. Yamato, Y. Takeuchi, K. Hashigaki, Y. Ikeda, M.R. Chang, K. Takeuchi, M. Matsushima, T. Tsuruo, T. Tashiro, J. Med. Chem. 32 (1989) 1295–1300.
[8] Y.L. Chen, H.M. Hung, C.M. Lu, K.C. Li, C.C. Tzeng, Bioorg. Med. Chem. 12 (2004) 6539–6546.
[9] M. Gaba, D. Singh, S. . Si ngh, V. Sharma, P. Gaba, Eur. J. Med. Chem. 45 (2010) 2245–2249.
[10] K.C.S. Achar, K.M. Hosamani, H.R. Seetharamareddy, Eur. J. Med. Chem. 45 (2010) 2048–2054.
[11] R.V. Shingalapur, K.M. Hosamani, R.S. Keri, Eur. J. Med. Chem. 44 (2009) 4244–4248.
[12] P.R. Vachharajani, M.J. Solanki, G.G. Dubal, V.H. Shah, Der Pharma Chemica 3 (2011) 280–285.
[13] S. Eswaran, A.V. Adhikari, N.S. Shetty, Eur. J. Med. Chem. 44 (2009) 4637–4647.
[14] J. Mann, A. Baron, Y.O. Boahen, E. Johansson, G. Parkinson, L.R. Kelland, S. Neidle, J. Med. Chem. 44 (2001) 138–144.
[15] D.A. Horton, G.T. Bourne, M.L. Smythe, Chem. Rev. 103 (2003) 893–930.
[16] S.M. Sondhi, S. Rajvanshi, M. Johar, N. Bharti, A. Azam, A.K. Singh, Eur. J. Med. Chem. 37 (2002) 835–843.
[17] A. Gellis, H. Kovacic, N. Boufatah, P. Vanelle, Eur. J. Med. Chem. 43 (2008) 1858–1864.
[18] S. Eswaran, A.V. Adhikari, R.A. Kumar, J. Med. Chem. 45 (2010) 957–966.
[19] D. Mungra, M. Patel, R. Patel, Med. Chem. Res. 20 (2011) 782–789.
[20] N. Perin, L. Uzelac, I. Piantanida, G.K. Zamola, M. Kralj, M. Hranjec, Bioorg. Med. Chem. 19 (2011) 6329–6339.
[21] A.M. Isloor, B. Kalluraya, M. Rao, J. Saudi Chem. Soc. 4 (3) (2000) 265–270.
[22] A.M. Isloor, B. Kalluraya, P. Shetty, Eur. J. Med. Chem. 44 (2009) 3784–3787.
[23] A.M. Vijesh, A.M. Isloor, V. Prabhu, S. Ahmad, S. Malladi, Eur. J. Med. Chem. 45 (2010) 5460–5464.
[24] B. Chandrakantha, P. Shetty, V. Nambiyar, N. Isloor, A.M. Isloor, Eur. J. Med. Chem. 45 (2010) 1206–1210.

[25] A.V. Ivachtchenko, A.V. Khvat, V.V. Kobak, V.M. Kysil, C.T. Williams, Tetrahedron Lett. 45 (2004) 5473–5476.
[26] A. Zarghi, R. Ghodsi, E. Azizi, B. Daraie, M. Hedayati, O.G. Dadrass, Bioorg. Med. Chem. 17 (2009) 5312–5317.
[27] K.A. Metwally, L.M.A. Aziz, E.S.M. Lashine, M.I. Husseinyb, R.H. Badawya, Bioorg. Med. Chem. 14 (2006) 8675–8682.
[28] B.A. Arthington-Skaggs, M. Motley, D.W. Warnock, C.J. Morrison, J. Clin. Microbiol. 38 (2000) 2254–2260.
[29] L. Rocha, A. Marston, O. Potterat, M.A.C. Kaplan, H. Stoeckli-Evans, K. Hostettmann, Phytochemistry 40 (5) (1995) 1447–1452.
[30] D.J.Mac. Lowry, M.J. Jaqua, S.T. Selepak, Appl. Microbiol. 20 (1970) 46–53.
[31] A. Portillo, R. Vila, B. Freixa, T. Adzet, S. Can-igueral, J. Ethnopharmacol. 76 (1) (2001) 93–98.