Design and Synthesis of New Benzimidazole Derivatives as Potential Antimicrobial Agents

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Abstract: A new series of 3,6-dialkyl-6,9-dihydro-2-methyl-9-oxo-3*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids, 3-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-1*H*-1,2,4-triazol-5(4*H*)-ones, 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-3-substituted-1,3,4-oxadiazole-2(3*H*)-thiones and 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-2-(alkylthio)-1,3,4-oxadiazoles has been synthesized and tested for antibacterial and antifungal activities. Among the tested compounds,**7e** and **7g** showed the highest antibacterial activity against *Escherichia coli*, while compound **7i** showed antimicrobial activity against *Bacillus subtilis*. In addition, a significant antimicrobial activity against *Staphylococcus aureus* was exhibited by compound **13a**, while both compounds **13b** and **13c** showed good antifungal activity. Detailed syntheses, spectroscopic and biological data are reported.

[El-Sherbeny MA, Maarouf AR, Hassan AHE, Abdel-Aziz NI. **Design and Synthesis of New Benzimidazole Derivatives as Potential Antimicrobial Agents.** *J Am Sci* 2012;8(12):785-798]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 110

Keywords: Benzimidazoles; imidazoquinolines; antimicrobial agents

1. Introduction

Numerous quinolones that contain the common 1-substituted-4-oxo-1,4-dihydroquinoline-3-carboxylic acid skeleton and condensed with different five membered heterocyclic rings were found to exhibit potent antimicrobial activity than the parent quinolones (Kaminsky and Meltzer 1968, Kaminsky 1970, Albrecht 1972, Kaodya et al., 1976, Kamentani et al., 1977, Serman et al., 1997). On this basis, it was found interesting to synthesize a new series of 3,6-dialkyl-6,9-dihydro-2-methyl-9-oxo-3*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids to be evaluated for antimicrobial activity and in order to study the effect of different substituents on both antibacterial and antifungal activities.

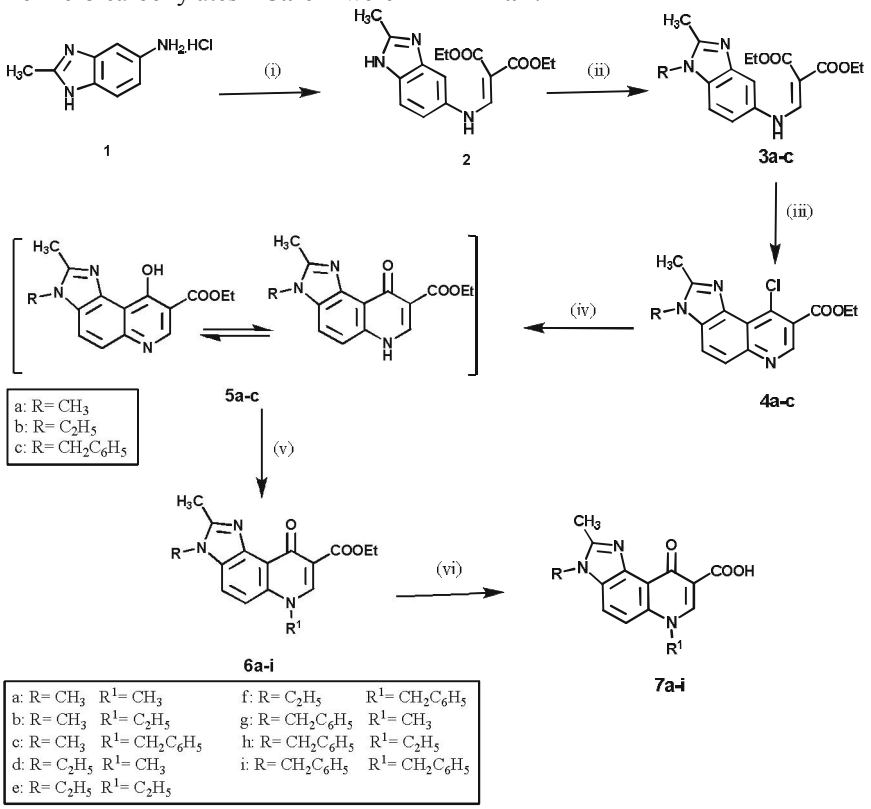
In addition, benzimidazole nucleus has received considerable attention of medicinal chemists where many of its substituted derivatives have pronounced antibacterial (Habib et al., 1989, Habib et al., 1997, El-Masry et al., 2000, He et al., 2004, Pawar et al., 2004, Göker et al., 2005, Datong et al., 2009, Prabal et al., 2011), antifungal (Göker et al., 2002, Ryu et al., 2003, Yildiz-Oren et al., 2004), antiviral (Cheng et al., 2005, Michele et al., 2010), antiprotozoal (Navarrete-Vâzquez et al., 2001, Andrzejewska et al., 2002, Navarrete-Vázquez et al., 2006), anthelmintic (Pilyugin et al., 2003, Mavrova et al., 2006) and anticancer activities (Boufatah et al., 2004, Nawrocka et al., 2005, Nawrocka et al., 2006, Hanan et al., 2010). Regarding the antimicrobial activity, the literature survey has pointed to the positive role of incorporating an aryl substituents such as thiazole (Pawar et al., 2004), substituted phenyl (Göker et al., 2002) and pyridyl moieties at position 2 of benzimidazole nucleus (Chenget al., 2005) to obtain effective antimicrobial agents. In addition, the antimicrobial activity of some moieties such as thiosemicarbazide (Dogan et al., 1998, Pandeya et al., 1999), triazole (Katica et al., 2001, Ulusoy et al., 2001, Hacer et al., 2010) and oxadiazole was also reported (Diana 2003, Cacic et al., 2006, Mallikarjuna et al., 2009). Moreover, the hybrid molecules that contain benzimidazole nucleus condensed in the same frame with thiosemicarbazide, triazole or oxadiazole afforded compounds that were reported to exhibit good antimicrobial activity (Habib et al., 1989, Habib et al., 1997, El-Masry et al., 2000, Bahaa et al., 2006). Furthermore, structure-activity relationship studies of substituted benzimidazoles demonstrated that the presence of electron withdrawing groups at position 5 and/or 6 generated compounds with potent antimicrobial activity (Yildiz-Oren et al., 2004). The above mentioned remarks have prompted us to synthesize a new series of benzimidazole derivatives. In these compounds, some functional groups which are believed to contribute to the antimicrobial activity were incorporated at position 1, 2 and 5 with the objective of determining the influence of these substituents upon the antimicrobial activity.

2. Results and discussion

Chemistry

The desired target compounds are depicted in schemes 1 and 2. The reported enamine, diethyl 2-((2-methyl-1*H*-benzimidazole-5-ylamino)methylene) malonate **2** (Renault et al., 1982), was prepared by reacting equimolar quantities of 5(6)-amino-2-methyl-1*H*-benzimidazole hydrochloride **1** (Bapat

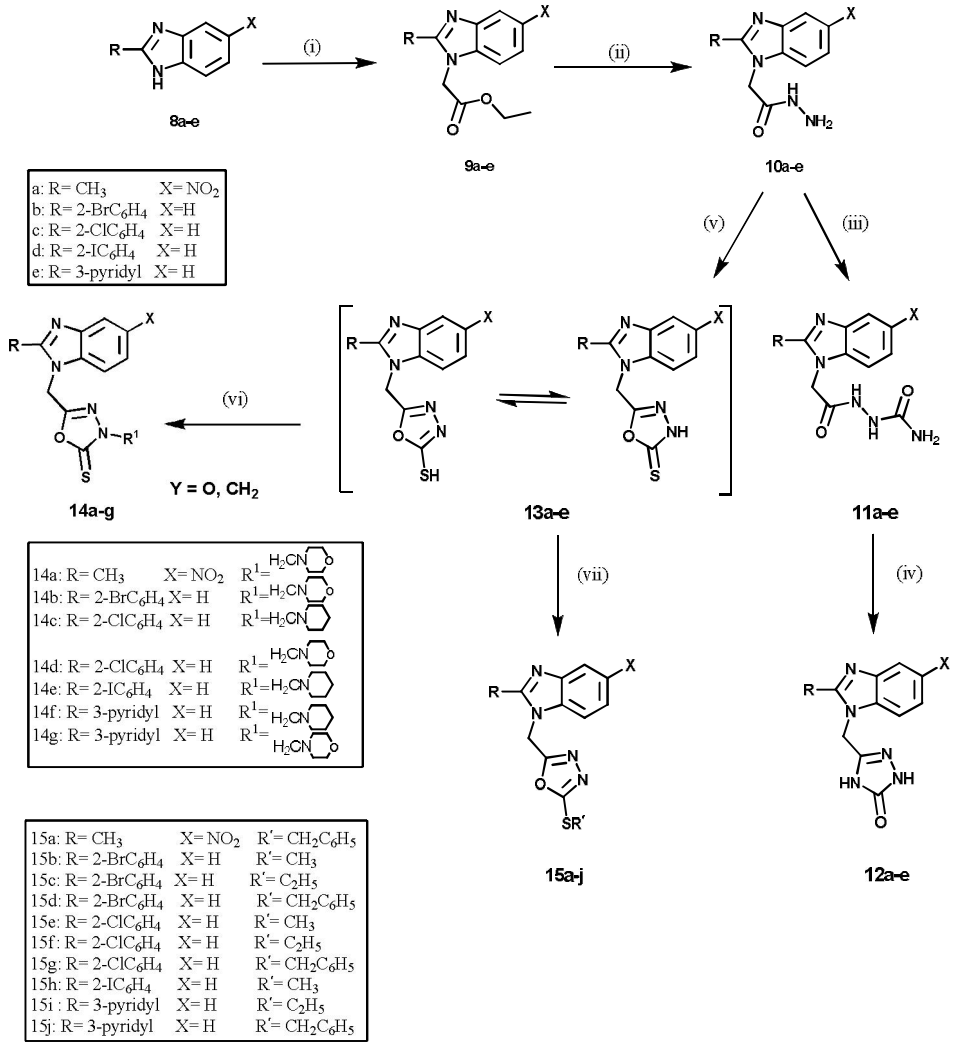
and Shirsat 1965) and diethyl ethoxymethylenemalonate in ethanol in the presence of sodium methoxide. Alkylation of compound **2** with the appropriate alkyl halides in DMF and in the presence of sodium methoxide afforded the corresponding diethyl 2-((1-alkyl-2-methyl-1*H*-benzimidazol-5-ylamino)methylene)malonates **3a-c**. Imidazo[4,5-*f*]quinoline derivatives **4a-c** were successfully obtained *via* cyclization of the intermediate enamines **3a-c** with phosphorus oxychloride. Ethyl 3-alkyl-9-hydroxy-2-methyl-3H-imidazo[4,5-*f*]quinoline-8-carboxylates **5a-c** were synthesized in analogy to the method of May and Baker (May and Baker Ltd., 1968) using a mixture of acetic acid and anhydrous sodium acetate as a catalyst. *N*-Alkylation of compounds **5a-c** was achieved by using the appropriate alkyl halide in DMF and in the presence of anhydrous potassium carbonate to yield the corresponding alkylated derivatives **6a-i**. Base-catalyzed hydrolysis of the later compounds using aqueous sodium hydroxide was achieved, followed by acidification with hydrochloric acid to afford the target carboxylic acids **7a-i**.



Scheme 1 Reaction protocol for the synthesis of **3,4,5 a-c** and **6,7 a-i** : (i) diethyl ethoxymethylenemalonate $NaOCH_3$, C_2H_5OH (ii) alkyl halide, $NaOCH_3$, DMF (iii) $POCl_3$ (iv) CH_3COONa, CH_3COOH (v) alkyl halide, K_2CO_3, DMF (vi) NaOH, HCl

Moreover, ethyl 2-(2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)acetates **9a-e** were prepared either by heating under reflux 1*H*-benzimidazole derivatives **8a-e** with ethyl chloroacetate in dry acetone in the presence of dry potassium carbonate, or by stirring with ethyl chloroacetate in DMF in the presence of sodium methoxide at room temperature. Carboxylic acid hydrazides **10a-e** were prepared by reacting the ethyl esters **9a-e** with hydrazine hydrate in absolute ethanol. Interaction of carboxylic acid hydrazides **10a-e** with urea in aqueous medium afforded 1-(2-(2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)acetyl)semicarbazides **11a-e**. The appropriate semicarbazides were cyclized using 10% sodium hydroxide to give 3-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-1*H*-1,2,4-triazol-5(4*H*)-ones **12a-e.** However 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl) methyl)-1,3,4-oxadiazole-2-thiols **13a-e** were prepared via treatment of carboxylic acid hydrazides **10a-e** with carbon disulfide in ethanolic potassium hydroxide followed by acidification with hydrochloric acid. Treatment of the compounds **13a-e** with one equivalent of paraformaldehyde and one equivalent of piperidine or morpholine in ethanol at room temperature afforded the corresponding Mannich bases **14a-g**. Thioetherification of compounds **13a-e**

with three equivalents of the appropriate alkyl halide in aqueous ethanolic sodium hydroxide at room temperature gave 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl) methyl)-2-(alkylthio)-1,3,4-oxadiazoles **15a-j**. The purity of the compounds was monitored by TLC and the structures of all the derivatives were supported by spectral data. The IR, ^{1}H NMR and mass spectra are in agreement with the proposed structures.



Scheme 2. Reaction protocol for the synthesis of **9,10,11,12,13 a-e, 14a-g** and **15a-j** : (i) $ClCH_2COOC_2H_5$, CH_3COCH_3, K_2CO_3 or $ClCH_2COOC_2H_5$, $NaOCH_3$, DMF (ii) $NH_2NH_2.H_2O$, C_2H_5OH (iii) NH_2CONH_2 (iv) 10% NaOH (v) CS_2, KOH, HCl (vi) CH_2O, morpholine or piperidine, C_2H_5OH (vii) alkyl halide, NaOH, C_2H_5OH

Microbiology

Twenty one of the newly synthesized compounds of the general structures **A**, **B**, **C, D, E** and **F** (Figure 1) were tested for their antimicrobial activity using disc diffusion method (Cavalieri et al., 2005) against Gram-negative bacteria; *Escherichia coli* (*E. coli,* ATCC 8739), Gram-positive bacteria; *Bacillus subtilis* (*B. subtilis* ATCC 31784) and *Staphylococcus aureus* (*S. aureus* ATCC 6538), andpathogenic fungi; *Candida albicans* (ATCC 10231).

Levofloxacin was used as a reference standard for antibacterial activity, while the antifungal activity was compared with tioconazole. The results of the antimicrobial activity screening of the tested compounds are summarized in Table 1. The most active tested compounds in the disc diffusion assay were subjected to a quantitative assay in order to determine their MICs using the two fold serial broth dilution technique (Cavalieri et al., 2005). The results of MICs of the tested compounds are summarized in Table 2.

The data obtained from the biological screening of the tested compounds with general structure **A** indicated that compounds **7e** and **7g** exhibited the highest activity against *E. coli* among the tested compounds. Moreover, good activity was noticed for compound **7i** against *B. subtilis*, MIC value 1.15. None of the tested compounds with general structure **B**, showed activity against *E. coli*. Compounds **11e** and **11b** showed good activity against *B. subtilis*, MIC values 2.33, 2.53 µg/ml, respectively. In case of compounds with the general structure **C**, none of the tested compounds showed activity against *E. coli* nor *Candida albicans*. However, compound **12b** showed antimicrobial activity against *S. aureus*, MIC value 2.44. For structure **D**, there was no observed activity against *E. coli*. Meanwhile, compound **13c** exhibited good activity against *B. subtilis* with MIC value 2.43. µg/ml. In addition, the activity against *S. aureus* was observed by compound **13a,** MIC value 1.65. µg/ml.. Moreover, all the tested compounds belonging to structure **D** showed good antifungal activity, especially compounds **13b** and **13c**, MIC values 2.15, 2.23 µg/ml, respectively. For structures **E** and **F**, no observed activity was noticed for all the tested compounds belonging to these structures against all the tested micro-organisms.

Table 1 Antimicrobial screening results of the tested compounds

Compounds	General Structure	Inhibition zone in mm			
		E. coli	*B. subtilis*	*S. aureus*	*Candida albicans*
7b	A	19	-	12	14
7e	A	20	16	14	16
7g	A	20	-	12	-
7h	A	18	20	12	14
7i	A	17	25	18	19
11a	B	-	-	-	-
11b	B	-	16	-	14
11e	B	-	21	12	13
12b	C	-	17	19	-
12e	C	-	19	14	-
13a	D	-	17	19.5	19
13b	D	-	19	-	20
13c	D	-	21	18.5	19
13d	D	-	19.5	-	19
13e	D	-	-	-	17
14b	E	-	-	-	-
14c	E	-	-	-	-
14d	E	-	-	-	-
15e	F	-	-	-	-
15f	F	-	-	-	-
15g	F	-	-	-	-
Levofloxacin		29	28	32	-
Tioconazole		-	-	-	23

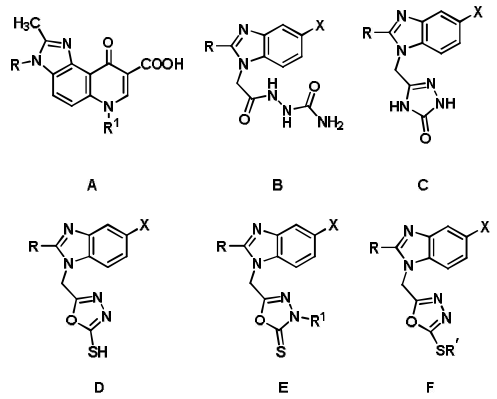


Figure 1. General formulae of the tested compounds

Table 2. MICs (µg/ml) of the tested compounds

Compounds	MIC (µg/ml)			
	E. coli	*B. subtilis*	*S. aureus*	*Candida albicans*
7b	2.73	-	-	-
7e	2.40	4.55	3.77	3.34
7g	2.54	-	-	-
7h	2.95	2.82	-	-
7i	3.23	1.15	3.54	2.54
11b	-	2.53	-	6.67
11e	-	2.33	-	-
12b	-	-	2.44	-
12e	-	-	4.43	-
13a	-	-	1.65	2.45
13b	-	3.55	-	2.15
13c	-	2.43	3.23	2.23
13d	-	3.11	-	2.61
Levofloxacin	0.425	0.165	0.225	-
Tioconazole	-	-	-	0.39

3- Conclusion

Among the tested compounds, only imidazoquinolines **7** exhibited antibacterial activity against E.coli. Compounds **7i** and **13a** showed significant activity against *B. subtilis* and *S. aureus*, respectively. In addition, 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiols **13a-e** showed good antifungal activity. However formation of Mannich bases **14a-g** or thioethers **15a-j** abolish this activity and these results might indicate the importance of the thiol group for the antifungal activity of these compounds.

4- Experimental

Elemental analyses (C, H, N) were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT. USA) at the microanalytical unit of Cairo University. Melting points were determined in open capillaries on an electrothermal L A 9000 series
(Electrothermal Engineering Ltd., Essex, UK) and are uncorrected. TLC chromatography was performed on precoated silica gel ^{60}F254 plates (Merk Co., Sofia, Bulgaria). Infrared spectra were recorded on Pye Unicam sp 1000 IR spectrophotometer (Thermoelectron, Egelsbach, Germany). ^{1}H-NMR spectra were recorded on Varian Gemini EM-300 MH_Z NMR spectrophotometer (Varian, Fort Collins, Co, USA). TMS was used as internal standard and chemical shifts were measured in δ ppm. Mass spectra were recorded on Varian MAT 311-A 70 eV (Varian). 5(6)-Amino-2-methyl-1*H*-benzimidazole hydrochloride **1**(Bapat and Shirsat 1965), diethyl 2-((2-methyl-1*H*-benzimidazol-5(6)-ylamino)methylene)malonate **2** (Renault et al., 1982), ethyl 9-hydroxy-2,3-dimethyl-3*H*-imidazo[4,5-*f*] quinoline-8-carboxylate **5a** (Forbes et al., 1990), 2-methyl-5(6)-nitro-1*H*-benzimidazole **8a** (Efros 1952) and 2-substituted-1*H*-benzimidazoles **8b-e** (Phillips 1928, Rope et al., 1952, Hein et al., 1957) were prepared following the procedures reported in the literature.

Synthesis

*Diethyl 2-((1-alkyl-2-methyl-1H-benzimidazol-5-ylamino)methylene)malonates **(3a-c)***

To a stirred suspension of diethyl 2-((2-methyl-1*H*-benzimidazol-5(6)-ylamino)methylene)malonate (**2**) (6.34 g, 0.02 mol) in DMF (25 mL), sodium methoxide (1.08 g, 0.02 mol) was added. Stirring was continued for 1 hour and then the appropriate alkyl halide (0.02 mol) was added. The reaction mixture was stirred at room temperature for 5 hours, poured into water and extracted with dichloromethane (3×100 mL). The organic extract was dried over anhydrous sodium sulphate and chromatographed over silica gel using ethyl acetate/petroleum ether grade 60-80 (1:1) as an eluting system to afford compounds **3a-c** as pure products.

*Diethyl 2-((1,2-dimethyl-1H-benzimidazol-5-ylamino)methylene)malonate **(3a)***

Yield, 85%; mp 192-193ºC. ^{1}H-NMR ($CDCl_3$); δ 1.29-1.41 (t, *J*=8.03 Hz, 6H, $OCH_2C\underline{H}_3$), 2.59 (s, 3H, $C\underline{H}_3$), 3.63 (s, 3H, $NC\underline{H}_3$), 4.25-4.35 (q, *J*=8.01 Hz, 4H, $OC\underline{H}_2CH_3$), 6.91-7.00 (d, *J*=7.50 Hz , 1H, C6-H), 7.29 (s, 1H, C4-H), 7.62-7.65 (d, *J*=7.51 Hz, 1H, C7-H), 8.49-8.53 (d, 1H, NHC<u>H</u>=), 11.03-11.08 (d, 1H, N<u>H</u>). Anal. Calcd for $C_{17}H_{21}N_3O_4$ (%): C,61.62; H,6.39; N,12.68. Found: C,62.01; H,5.99; N,12.21.

*Diethyl 2-((1-ethyl-2-methyl-1H-benzimidazol-5-ylamino)methylene)malonate **(3b)***

Yield, 82%; mp 205-207ºC. ^{1}H-NMR ($CDCl_3$); δ 1.12-1.21 (t, *J*=8.03 Hz, 3H, $NCH_2C\underline{H}_3$), 1.28-1.31 (t, *J*=8.03 Hz, 6H, $OCH_2C\underline{H}_3$), 2.55 (s, 3H, $C\underline{H}_3$), 4.00-4.15 (q, *J*=8.01 Hz, 2H, $NC\underline{H}_2CH_3$), 4.22-4.32 (q, *J*=8.01 Hz, 4H, $OC\underline{H}_2CH_3$), 6.90-7.10 (d, *J*=7.50 Hz , 1H, C6-H), 7.29 (s, 1H, C4-H), 7.63-7.66 (d, *J*=7.51 Hz, 1H, C7-H), 8.48-8.52 (d, 1H, NHC<u>H</u>=), 11.13-11.18 (d, 1H, N<u>H</u>). Anal. Calcd for $C_{18}H_{23}N_3O_4$ (%): C, 62.59; H, 6.71; N, 12.17. Found: C, 62.21; H, 6.91; N, 12.30.

*Diethyl 2-((1-benzyl-2-methyl-1H-benzimidazol-5-ylamino)methylene)malonate **(3c)***

Yield, 90%; mp 136-138ºC. ^{1}H-NMR ($CDCl_3$); δ 1.23-1.36 (t, *J*=8.03 Hz, 6H, $OCH_2C\underline{H}_3$), 2.52 (s, 3H, $C\underline{H}_3$), 4.16-4.31 (q, *J*=8.01 Hz, 4H, $OC\underline{H}_2CH_3$), 5.02 (s, 2H, $C\underline{H}_2$), 6.96-7.02 (d, *J*=7.50 Hz , 1H, C6-H), 7.05-7.23 (m, 5H, benzyl-H), 7.37 (s, 1H, C4-H), 7.82-7.85 (d, *J*=7.51 Hz, 1H, C7-H), 8.49-8.53 (d, 1H, NHC<u>H</u>=), 11.03-11.08 (d, 1H, N<u>H</u>). Anal. Calcd for $C_{23}H_{25}N_3O_4$ (%): C, 67.80; H, 6.18; N, 10.31. Found: C, 68.00; H, 6.40; N, 10.51.

*Ethyl 3-alkyl-9-chloro-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylates **(4a-c)***

A mixture of diethyl 2-((1-alkyl-2-methyl-1*H*-benzimidazol-5-ylamino)methylene)malonates **3a-c** (0.003 mol) and phosphorus oxychloride (10 mL) was heated under reflux for 12 hours. The reaction mixture was poured into crushed ice (50 g), neutralized with potassium carbonate and extracted with dichloromethane (3×50 mL). The organic extract was dried over anhydrous sodium sulphate, evaporated under reduced pressure and the obtained residues were crystallized from methanol/water.

*Ethyl 9-chloro-2,3-dimethyl-3H-imidazo[4,5-f]quinoline-8-carboxylate **(4a)***

Yield, 78%; mp 171-173ºC. ^{1}H-NMR (DMSO-d_6); δ 1.44-1.50 (t, *J*=8.05 Hz, 3H, $OCH_2C\underline{H}_3$), 2.51 (s, 3H, $C\underline{H}_3$), 3.55 (s, 3H, $NC\underline{H}_3$), 4.34-4.46 (q, *J*=8.02 Hz, 2H, $OC\underline{H}_2CH_3$), 7.88-7.93 (d, *J*=7.51 Hz, 1H, C4-H), 8.21-8.26 (d, *J*=7.51 Hz, 1H, C5-H), 9.46 (s, 1H, C7-H). Anal. Calcd for $C_{15}H_{14}ClN_3O_2$ (%): C, 59.31; H, 4.65; N, 13.83. Found: C, 59.60; H, 4.90; N, 14.03.

Ethyl 9-chloro-3-ethyl-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(4b)***

Yield, 68%; mp 210-211ºC.[1]H-NMR (DMSO-d_6); δ 1.31-1.39 (t, *J*=8.03 Hz, 3H, $NCH_2C\underline{H}_3$), 1.47-1.55 (t, *J*=8.05 Hz, 3H, $OCH_2C\underline{H}_3$), 2.55 (s, 3H, $C\underline{H}_3$), 3.71-3.86 (q, *J*=8.02 Hz, 2H, $NC\underline{H}_2CH_3$), 4.34-4.47 (q, *J*=8.02 Hz, 2H, $OC\underline{H}_2CH_3$), 7.89-7.93 (d, *J*=7.51 Hz, 1H, C4-H), 8.22-8.27 (d, *J*=7.51 Hz, 1H, C5-H), 9.48 (s, 1H, C7-H). Anal. Calcd for $C_{16}H_{16}ClN_3O_2$ (%): C,60.47; H,5.08; N,13.22. Found: C,60.01; H,5.50; N,12.89.

Ethyl 3-benzyl-9-chloro-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(4c)***

Yield, 89%; mp 185-186ºC.[1]H-NMR (DMSO-d_6); δ 1.29-1.37 (t, *J*=8.03 Hz, 3H, $OCH_2C\underline{H}_3$), 2.59 (s, 3H, $C\underline{H}_3$), 4.32-4.44 (q, *J*=8.02 Hz, 2H, $OC\underline{H}_2CH_3$), 4.99 (s, 2H, $C\underline{H}_2$), 6.94-7.23 (m, 5H, benzyl-H), 7.85-7.89 (d, *J*=7.51 Hz, 1H, C4-H), 8.19-8.23 (d, *J*=7.51 Hz, 1H, C5-H), 9.43 (s, 1H, C7-H). Anal. Calcd for $C_{21}H_{18}ClN_3O_2$(%): C, 66.40; H, 4.78; N, 11.06. Found: C, 66.70; H, 4.40; N, 11.33.

Ethyl 3-alkyl-9-hydroxy-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylates ***(5b-c)***

A mixture of ethyl 3-alkyl-9-chloro-2-methyl-3*H*-imidazo[4,5-*f*]quinoline-8-carboxylates **4b-c** (0.006 mol) and anhydrous sodium acetate (3.3 g 0.04 mol) in glacial acetic acid (33 mL) was heated under reflux for 7 hours. The reaction mixture was concentrated under reduced pressure and the obtained residues were crystallized from the appropriate solvent.

Ethyl 3-ethyl-9-hydroxy-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(5b)***

Crystallization solvent, DMF; yield, 38%; mp 283-284ºC. [1]H-NMR (DMSO-d_6); δ 1.29-1.37 (t, *J*=8.03 Hz, 3H, $-OCH_2C\underline{H}_3$), 1.47-1.55 (t, *J*=8.05 Hz, 3H, $NCH_2C\underline{H}_3$), 2.59 (s, 3H, $C\underline{H}_3$), 3.71-3.86 (q, *J*=8.02 Hz, 2H, $NC\underline{H}_2CH_3$), 4.38-4.51 (q, *J*=8.02 Hz, 2H, $OC\underline{H}_2CH_3$), 5.39 (s, 1H, O<u>H</u>), 7.82-7.86 (d, *J*=7.51 Hz, 1H, C4-H), 8.19-8.23 (d, *J*=7.51 Hz, 1H, C5-H), 9.36 (s, 1H, C7-H). Anal. Calcd for $C_{16}H_{17}N_3O_3$ (%): C,64.20; H,5.72; N,14.04. Found: C,64.50; H,5.20; N,14.24.

Ethyl 3-benzyl-9-hydroxy-2-methyl-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(5c)***

Crystallization solvent, n-butanol; yield, 82%; mp 224-225ºC. [1]H-NMR (DMSO-d_6); δ 1.24-1.29 (t, *J*=8.03 Hz, 3H, $-OCH_2C\underline{H}_3$), 2.50 (s, 3H, $C\underline{H}_3$), 4.32-4.44 (q, *J*=8.02 Hz, 2H, $OC\underline{H}_2CH_3$), 4.99 (s, 2H, $C\underline{H}_2$ benzyl), 5.35 (s, 1H, O<u>H</u>), 6.95-7.23 (m, 5H, benzyl-H), 7.77-7.81 (d, *J*=7.51 Hz, 1H, C4-H), 8.13-8.17 (d, *J*=7.51 Hz, 1H, C5-H), 9.32 (s, 1H, C7-H). MS (m/z, %); 362 [M^++1] (10.3), 361 [M^+] (31.4), 315 (35.5), 300 (33.4), 238 (43.7), 91 (100). Anal. Calcd for $C_{21}H_{19}N_3O_3$ (%): C, 69.79; H, 5.30; N, 11.63. Found: C, 69.83; H, 5.40; N, 11.50.

Ethyl 3,6-dialkyl-6,9-dihydro-2-methyl 9-oxo-3H-imidazo[4,5-f] quinoline-8-carboxylates ***(6a-i)***

The appropriate alkyl halide (methyl iodide, ethyl iodide or benzyl chloride, 0.003 mol) was added dropwise while stirring over a period of 15 minutes to a suspension of ethyl 3-alkyl-9-hydroxy-2-methyl-3*H*-imidazo[4,5-*f*]quinoline-8-carboxylates **5a-c** (0.001 mol) and anhydrous potassium carbonate (0.15 g, 0.001 mol) in dry DMF (2.5 mL). The reaction mixture was heated under reflux for 1 hour, cooled, poured into ice (50 g) and extracted with chloroform (3×100 mL). The organic extract was dried over anhydrous sodium sulphate, evaporated under reduced pressure and the obtained residue was crystallized from the appropriate solvent.

Ethyl 6,9-dihydro-2,3,6-trimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6a)***

Crystallization solvent, ethanol; yield, 85%; mp 229-230ºC. [1]H-NMR (DMSO-d_6); δ 1.32-1.40 (t, *J*=8.03 Hz, 3H, $OCH_2C\underline{H}_3$), 2.59 (s, 3H, C2-$C\underline{H}_3$), 3.30 (s, 3H, N3-$C\underline{H}_3$), 3.63 (s, 3H, N6-$C\underline{H}_3$), 4.23-4.35 (q, 2H, *J*=8.01 Hz, $OC\underline{H}_2CH_3$), 6.60-6.64 (d, *J*=7.49 Hz, 1H, C5-H), 7.74- 7.78 (d, *J*=7.49 Hz, 1H, C4-H), 7.99 (s, 1H, C7-H). Anal. Calcd for $C_{16}H_{17}N_3O_3$ (%): C,64.20; H,5.72; N,14.04. Found: C,64.21; H,5.43; N,13.84.

Ethyl 6-ethyl-6,9-dihydro-2,3-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6b)***

Crystallization solvent, ethanol; yield, 73%; mp 202-203ºC. [1]H-NMR (DMSO-d_6); δ 1.19-1.25 (t, *J*=8.06 Hz, 3H, $NCH_2C\underline{H}_3$), 1.38-1.47 (t, *J*=8.03 Hz, 3H, $OCH_2C\underline{H}_3$), 2.54 (s, 3H, $C\underline{H}_3$), 3.04-3.16 (q, *J*=8.04 Hz, 2H, $NC\underline{H}_2CH_3$), 3.47 (s, 3H, $C\underline{H}_3$), 4.18-4.30 (q, *J*=8.01 Hz, 2H, $OC\underline{H}_2CH_3$), 6.66-6.70 (d, *J*=7.49 Hz, 1H, C5-H), 7.80-7.84 (d, *J*=7.49 Hz, 1H, C4-H), 8.07 (s, 1H, C7-H). Anal. Calcd for $C_{17}H_{19}N_3O_3$ (%): C,65.16; H,6.11; N,13.41. Found: C,65.45; H,6.15; N,13.80.

Ethyl 6-benzyl-6,9-dihydro-2,3-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6c)***

Crystallization solvent, ethanol; yield, 54%; mp 264-265ºC. [1]H-NMR (DMSO-d_6); δ 1.30-1.42 (t, *J*=8.03 Hz, 3H, $OCH_2C\underline{H}_3$), 2.49 (s, 3H, C2-$C\underline{H}_3$), 3.90 (s, 3H, N3-$C\underline{H}_3$), 4.27-4.40 (q, *J*=8.01 Hz, 2H, $OC\underline{H}_2CH_3$), 4.98 (s, 2H, $C\underline{H}_2$ benzyl), 6.66-6.71 (d, *J*=7.49 Hz, 1H, C5-H), 6.95-7.23 (m, 5H, benzyl-H), 7.76- 7.81 (d, *J*=7.49 Hz, 1H, C4-H), 7.96 (s, 1H, C7-H). Anal. Calcd for $C_{22}H_{21}N_3O_3$ (%): C, 70.38; H, 5.64; N, 11.19. Found: C, 69.99; H, 5.75; N, 11.50.

Ethyl 3-ethyl-6,9-dihydro-2,6-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6d)***

Crystallization solvent, ethanol; yield, 75%; mp 226-227ºC. [1]H-NMR (DMSO-d_6); δ 1.18-1.24 (t, *J*=8.06 Hz, 3H, $NCH_2C\underline{H}_3$), 1.37-1.46 (t, *J*=8.03 Hz, 3H, $OCH_2C\underline{H}_3$), 2.55 (s, 3H, $C\underline{H}_3$), 3.14-3.17 (q, *J*=8.04 Hz, 2H, $NC\underline{H}_2CH_3$), 3.45 (s, 3H, $C\underline{H}_3$), 4.19-4.31 (q, *J*=8.01 Hz, 2H, $OC\underline{H}_2CH_3$), 6.67-6.70 (d, *J*=7.49 Hz, 1H, C5-H), 7.81-

7.84 (d, *J*=7.49 Hz, 1H, C4-H), 8.17 (s, 1H, C7-H). Anal. Calcd for $C_{17}H_{19}N_3O_3$ (%): C, 65.16; H, 6.11; N, 13.41. Found: C, 64.80; H, 5.71; N, 13.38.

Ethyl 3,6-diethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6e)***

Crystallization solvent, ethanol/acetone; yield, 73%; mp 214-215ºC. ^{1}H-NMR (DMSO-d_6); δ 1.10-1.18 (t, *J*=8.06 Hz, 3H, CH_2CH_3), 1.33-1.45 (t, *J*=8.05 Hz, 3H, OCH_2CH_3), 1.47-1.55 (t, *J*=8.05 Hz, 3H, CH_2CH_3), 2.52 (s, 3H, CH_3), 3.04-3.16 (q, *J*=8.04 Hz, 2H, CH_2CH_3), 3.71-3.83 (q, *J*=8.02 Hz, 2H, CH_2CH_3), 4.24-4.37 (q, 2H, *J*=8.01 Hz, OCH_2CH_3), 6.63-6.68 (d, *J*=7.49 Hz, 1H, C5-H), 7.72- 7.75 (d, *J*=7.49 Hz, 1H, C4-H), 8.01 (s, 1H, C7-H). Anal. Calcd for $C_{18}H_{21}N_3O_3$ (%): C, 66.40; H, 6.47; N, 12.84. Found: C, 66.00; H, 6.07; N, 13.30.

Ethyl 6-benzyl-3-ethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6f)***

Crystallization solvent, methanol; yield, 55%; mp 207-208ºC. ^{1}H-NMR (DMSO-d_6); δ 1.18-1.24 (t, *J*=8.06 Hz, 3H, NCH_2CH_3), 1.37-1.46 (t, *J*=8.03 Hz, 3H, OCH_2CH_3), 2.55 (s, 3H, CH_3), 3.14-3.17 (q, *J*=8.04 Hz, 2H, NCH_2CH_3), 4.19-4.31 (q, *J*=8.01 Hz, 2H, OCH_2CH_3), 4.98 (s, 2H, CH_2 benzyl), 6.66-6.71 (d, *J*=7.49 Hz, 1H, C5-H), 6.95-7.23 (m, 5H, benzyl-H), 7.76- 7.81 (d, *J*=7.49 Hz, 1H, C4-H), 7.96 (s, 1H, C7-H). Anal. Calcd for $C_{23}H_{23}N_3O_3$(%): C, 70.93; H, 5.95; N, 10.79. Found: C, 70.63; H, 6.05; N, 11.00.

Ethyl 3-benzyl-6,9-dihydro-2,6-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6g)***

Crystallization solvent, ethanol; yield, 90%; mp 126-127ºC. ^{1}H-NMR (DMSO-d_6); δ 1.31-1.43 (t, *J*=8.03 Hz, 3H, OCH_2CH_3), 2.42 (s, 3H, C2-CH_3), 3.80 (s, 3H, N6-CH_3), 4.26-4.39 (q, *J*=8.01 Hz, 2H, OCH_2CH_3), 4.97 (s, 2H, CH_2 benzyl), 6.65-6.70 (d, *J*=7.49 Hz, 1H, C5-H), 6.96-7.23 (m, 5H, benzyl-H), 7.75- 7.80 (d, *J*=7.49 Hz, 1H, C4-H), 7.94 (s, 1H, C7-H). Anal. Calcd for $C_{22}H_{21}N_3O_3$(%): C, 70.38; H, 5.64; N, 11.19. Found: C, 70.00; H, 5.90; N, 11.40.

Ethyl 3-benzyl-6-ethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6h)***

Crystallization solvent, ethanol; yield, 95%; mp 236-238ºC. ^{1}H-NMR (DMSO-d_6); δ 1.12-1.19 (t, *J*=8.06 Hz, 3H, CH_2CH_3), 1.31-1.43 (t, *J*=8.03 Hz, 3H, OCH_2CH_3), 2.58 (s, 3H, CH_3), 3.11-3.23 (q, *J*=8.04 Hz, 2H, CH_2CH_3), 4.28-4.41 (q, *J*=8.01 Hz, 2H, OCH_2CH_3), 4.99 (s, 2H, CH_2 benzyl), 6.67-6.72 (d, *J*=7.49 Hz, 1H, C5-H), 6.95-7.23 (m, 5H, benzyl-H), 7.76- 7.81 (d, *J*=7.49 Hz, 1H, C4-H), 7.95 (s, 1H, C7-H). Anal. Calcd for $C_{23}H_{23}N_3O_3$ (%): C,70.93; H,5.95; N,10.79. Found: C,70.45; H,5.85; N,11.11.

Ethyl 3,6-dibenzyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylate ***(6i)***

Crystallization solvent, acetic acid/DMF; yield, 83%; mp 297-299ºC. ^{1}H-NMR (DMSO-d_6); δ 1.38-1.46 (t, *J*=8.03 Hz, 3H, OCH_2CH_3), 2.63 (s, 3H, CH_3), 4.22-4.34 (m, 4H, OCH_2CH_3+ CH_2 benzyl), 5.02 (s, 2H, CH_2 benzyl), 6.63-6.67 (d, *J*=7.49 Hz, 1H, C5-H), 6.90-7.29 (m, 10H, benzyl-H), 7.74- 7.78 (d, *J*=7.49 Hz, 1H, C4-H), 7.99 (s, 1H, C7-H). Anal. Calcd for $C_{28}H_{25}N_3O_3$ (%): C,74.48; H,5.58; N,9.31. Found: C,74.10; H,5.90; N,8.98.

3,6-Dialkyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acids ***(7a-i)***

A mixture of compounds **6a-i** (0.001 mol) and NaOH (0.08 g, 0.002 mol) in water (10 ml) was heated under reflux for 1 hour. After cooling, the reaction mixture was filtered and acidified by dropwise addition of 4N HCl. The formed precipitate was collected by filtration, washed with water, dried and crystallized from the appropriate solvent.

6,9-Dihydro-2,3,6-trimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7a)***

Crystallization solvent, DMF; yield, 89%; mp 296-298ºC. ^{1}H-NMR (DMSO-d_6); δ 2.57 (s, 3H, C2-CH_3), 3.29 (s, 3H, N3-CH_3), 3.63 (s, 3H, N6-CH_3), 6.60-6.63 (d, *J*=7.49 Hz, 1H, C5-H), 7.66- 7.78 (d, *J*=7.49 Hz, 1H, C4-H), 7.98 (s, 1H, C7-H), 11.06 (s, 1H, OH). Anal. Calcd for $C_{14}H_{13}N_3O_3$ (%): C, 61.99; H, 4.83; N, 15.49. Found: C, 62.09; H, 5.03; N, 15.59.

6-Ethyl-6,9-dihydro-2,3-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7b)***

Crystallization solvent, DMF; yield, 70%; mp 271-272ºC. ^{1}H-NMR (DMSO-d_6); δ 1.11-1.15 (t, *J*=8.06 Hz, 3H, CH_2CH_3), 2.55 (s, 3H, C2-CH_3), 3.28 (s, 3H, N3-CH_3), 3.14-3.17 (q, *J*=8.04 Hz, 2H, CH_2CH_3), 6.62-6.68 (d, *J*=7.49 Hz, 1H, C5-H), 7.70- 7.75 (d, *J*=7.49 Hz, 1H, C4-H), 7.91 (s, 1H, C7-H), 11.03 (s, 1H, OH). Anal. Calcd for $C_{15}H_{15}N_3O_3$ (%): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.09; H, 5.60; N, 14.60.

6-Benzyl-6,9-dihydro-2,3-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7c)***

Crystallization solvent, methanol /DMF; yield, 81%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 2.59 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 4.32 (s, 2H, CH_2 benzyl), 6.60-6.64 (d, *J*=7.49 Hz, 1H, C5-H), 6.99-7.21 (m, 5H, benzyl-H), 7.74- 7.78 (d, *J*=7.49 Hz, 1H, C4-H), 7.96 (s, 1H, C7-H), 11.04 (s, 1H, OH). Anal. Calcd for $C_{20}H_{17}N_3O_3$ (%): C,69.15; H,4.93; N,12.10. Found: C,69.50; H,5.23; N,12.51.

3-Ethyl-6,9-dihydro-2,6-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7d)***

Crystallization solvent, acetic acid/DMF; yield, 86%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 1.47-1.55 (t, *J*=8.05 Hz, 3H, CH_2CH_3), 2.56 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 3.71-3.83 (q, *J*=8.02 Hz, 2H, CH_2CH_3), 6.62-6.64 (d, *J*=7.49 Hz, 1H, C5-H), 7.76- 7.79 (d, *J*=7.49 Hz, 1H, C4-H), 7.98 (s, 1H, C7-H), 11.06 (s, 1H, OH). Anal. Calcd for $C_{15}H_{15}N_3O_3$ (%):C, 63.15; H, 5.30; N, 14.73.Found: C,63.55; H,5.72; N,14.31.

3,6-Diethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7e)***

Crystallization solvent, acetic acid/DMF; yield, 86%; mp 283-284ºC. ^{1}H-NMR (DMSO-d_6); δ 1.12-1.17 (t, *J*=8.06 Hz, 3H, $CH_2C\underline{H}_3$), 1.45-1.53 (t, *J*=8.05 Hz, 3H, $CH_2C\underline{H}_3$), 2.54 (s, 3H, $C\underline{H}_3$), 3.04-3.16 (q, *J*=8.04 Hz, 2H, $C\underline{H}_2CH_3$), 3.69-3.81 (q, *J*=8.02 Hz, 2H, $C\underline{H}_2CH_3$), 6.63-6.69 (d, *J*=7.49 Hz, 1H, C5-H), 7.71- 7.76 (d, *J*=7.49 Hz, 1H, C4-H), 7.92 (s, 1H, C7-H), 11.03 (s, 1H, OH). Anal. Calcd for $C_{16}H_{17}N_3O_3$ (%): C, 64.20; H, 5.72; N, 14.04. Found: C, 64.60; H, 5.92; N, 14.40.

6-Benzyl-3-ethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7f)***

Crystallization solvent, DMF; yield, 76%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 1.50-1.58 (t, *J*=8.05 Hz, 3H, $CH_2C\underline{H}_3$), 2.63 (s, 3H, $C\underline{H}_3$), 3.79-3.91 (q, *J*=8.02 Hz, 2H, $C\underline{H}_2CH_3$), 4.38 (s, 2H, $C\underline{H}_2$ benzyl), 6.70-6.74 (d, *J*=7.49 Hz, 1H, C5-H), 6.95-7.16 (m, 5H, benzyl-H), 7.75- 7.79 (d, *J*=7.49 Hz, 1H, C4-H), 8.01 (s, 1H, C7-H), 11.18 (s, 1H, OH). Anal. Calcd for $C_{21}H_{19}N_3O_3$ (%): C,69.79; H,5.30; N,11.63. Found: C,70.01; H,5.72; N,11.91.

3-Benzyl-6,9-dihydro-2,6-dimethyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7g)***

Crystallization solvent, DMF; yield, 91%; mp 268-269ºC. ^{1}H-NMR (DMSO-d_6); δ 2.56 (s, 3H, C2-$C\underline{H}_3$), 3.53 (s, 3H, N6-$C\underline{H}_3$), 4.31 (s, 2H, $C\underline{H}_2$ benzyl), 6.61-6.63 (d, *J*=7.49 Hz, 1H, C5-H), 6.98-7.20 (m, 5H, benzyl-H), 7.73- 7.77 (d, *J*=7.49 Hz, 1H, C4-H), 7.96 (s, 1H, C7-H), 11.06 (s, 1H, OH). Anal. Calcd for $C_{20}H_{17}N_3O_3$ (%): C, 69.15; H, 4.93; N, 12.10. Found: C, 69.27; H, 4.44; N, 11.90.

3-Benzyl-6-ethyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f] quinoline-8-carboxylic acid ***(7h)***

Crystallization solvent, DMF; yield, 91%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 1.11-1.19 (t, *J*=8.06 Hz, 3H, $CH_2C\underline{H}_3$), 2.61 (s, 3H, $C\underline{H}_3$), 3.04-3.16 (q, *J*=8.04 Hz, 2H, $C\underline{H}_2CH_3$), 4.99 (s, 2H, $C\underline{H}_2$ benzyl), 6.61-6.65 (d, *J*=7.49 Hz, 1H, C5-H), 7.00-7.21 (m, 5H, benzyl-H), 7.73- 7.86 (d, *J*=7.49 Hz, 1H, C4-H), 7.96 (s, 1H, C7-H), 11.09 (s, 1H, OH). Anal. Calcd for $C_{21}H_{19}N_3O_3$ (%): C,69.79; H,5.30; N,11.63. Found: C,70.20; H,5.71; N,11.43.

3,6-Dibenzyl-6,9-dihydro-2-methyl-9-oxo-3H-imidazo[4,5-f]quinoline-8-carboxylic acid ***(7i)***

Crystallization solvent, DMF; yield, 93%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 2.69 (s, 3H, $C\underline{H}_3$), 4.65 (s, 2H, $C\underline{H}_2$ benzyl), 4.99 (s, 2H, $C\underline{H}_2$ benzyl), 6.81-6.86 (d, *J*=7.49 Hz, 1H, C5-H), 7.09-7.45 (m, 10H, benzyl-H), 7.78-7.82 (d, *J*=7.49 Hz, 1H, C4-H), 8.06 (s, 1H, C7-H), 11.24 (s, 1H, OH). Anal. Calcd for $C_{26}H_{21}N_3O_3$ (%): C,73.74; H,5.00; N,9.92. Found: C,74.00; H,5.20; N,9.52.

Ethyl 2-(2-substituted-5-(un)substituted-1H-benzimidazol-1-yl)acetates ***(9a-e)***

Method A:

To a stirred mixture of the appropriate 2-substituted-1*H*-benzimidazole **8a,b,d** (0.1 mol) and dry potassium carbonate (13.82 g, 0.1 mol) in dry acetone (100 ml), ethyl chloroacetate (12.2 g, 0.1 mol) was added dropwise. The reaction mixture was heated under reflux for 6 hours, filtered and the filtrate was concentrated under reduced pressure. The separated residue was crystallized from ethanol.

Method B:

Sodium methoxide (5.4 g, 0.1 mol) was added to a stirred solution of 2-substituted-1*H*-benzimidazole **8c,e** (0.1 mol) in DMF (100 ml). Ethyl chloroacetate (12.2 g, 0.1 mol) was then added to the reaction mixture and stirring was continued for 6 hours. The solvent was evaporated under reduced pressure and the separated residue was crystallized from ethanol.

Ethyl 2-(2-methyl-5-nitro-1H-benzimidazol-1-yl)acetate ***(9a)***

Method A; yield, 78%; mp 87-88ºC. IR(cm^{-1}): 3100 (aromatic C–H stretch), 2989, 2957, 2849 (aliphatic C–H stretch), 1734 (C=O stretch), 1655 (C=N stretch), 1597 (aromatic C–C stretch). ^{1}H-NMR ($CDCl_3$); δ 1.28-1.38 (t, 3H, $CH_2C\underline{H}_3$), 2.59 (s, 3H, $C\underline{H}_3$), 4.16-4.29 (q, 2H, $OC\underline{H}_2CH_3$), 4.72 (s, 2H, -$C\underline{H}_2CO$), 7.94-7.98 (d, *J*=7.5 Hz, 1H, C7-H), 8.17-8.21 (d, *J*=7.5 Hz, 1H, C6-H), 8.63 (s, 1H, C4-H). Anal. Calcd for $C_{12}H_{13}N_3O_4$ (%): C,54.75; H,4.98; N,15.96. Found: C,55.01; H,4.51; N,16.21.

Ethyl 2-(2-(2-bromophenyl)-1H-benzimidazol-1-yl)acetate ***(9b)***

Method A; yield, 81%; mp 98-100ºC. ^{1}H-NMR ($CDCl_3$); δ 1.25-1.33 (t, 3H, $OCH_2C\underline{H}_3$), 4.15-4.25 (q, 2H, $OC\underline{H}_2CH_3$), 4.70 (s, 2H, -$C\underline{H}_2CO$), 7.70-8.19 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{15}BrN_2O_2$ (%): C,56.84; H,4.21; N,7.80. Found: C,56.44; H,4.27; N,7.48.

Ethyl 2-(2-(2-chlorophenyl)-1H-benzimidazol-1-yl)acetate ***(9c)***

Method B; yield, 83%; mp 72-73ºC. IR(cm^{-1}): 3059 (aromatic C–H stretch), 2989, 2921 (aliphatic C–H stretch), 1750 (C=O stretch), 1655 (C=N stretch), 1596 (aromatic C–C stretch). ^{1}H-NMR ($CDCl_3$); δ 1.26-1.34 (t, 3H, $OCH_2C\underline{H}_3$), 4.13-4.25 (q, 2H, $OC\underline{H}_2CH_3$), 4.69 (s, 2H, -$C\underline{H}_2CO$), 7.70-8.18 (m, 8H, Ar-H). MS (m/z, %); 317 [M^+ + 2] (21.8), 316 [M^+ + 1] (16.5), 314.80 [M^+] (40.1), 241 (58.8), 240 (64.2), 229 (20.4), 228 (36.8), 227 (46.3), 206 (90.1), 205 (100), 204 (30.9), 77 (85), 76 (97.9). Anal. Calcd for $C_{17}H_{15}ClN_2O_2$ (%): C,64.87; H,4.80; N,8.90. Found: C,64.64; H,4.71; N,9.31.

Ethyl 2-(2-(2-iodophenyl)-1H-benzimidazol-1-yl)acetate ***(9d)***

Method A; yield, 74%; mp 76-77ºC. ^{1}H-NMR ($CDCl_3$); δ 1.23-1.31 (t, 3H, CH_2CH_3), 4.09-4.22 (q, 2H, OCH_2CH_3), 4.64 (s, 2H, -CH_2CO), 7.10-7.79 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{15}IN_2O_2$ (%): C,50.26; H,3.72; N,6.90. Found: C,50.56; H,3.91; N,7.35.

Ethyl 2-(2-(3-pyridyl)-1H-benzimidazol-1-yl)acetate ***(9e)***

Method B; yield, 65%; mp 112-113ºC. ^{1}H-NMR ($CDCl_3$); δ 1.25-1.30 (t, 3H, CH_2CH_3), 4.18-4.30 (q, 2H, OCH_2CH_3), 4.74 (s, 2H, -CH_2CO), 7.22-8.81 (m, 8H, Ar-H). Anal. Calcd for $C_{16}H_{15}N_3O_2$ (%): C,68.31; H,5.37; N,14.94. Found: C,68.82; H,5.34; N,15.21.

2-(2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)acetohydrazides ***(10a-e)***

Hydrazine hydrate 98% (5 g, 0.1 mol) was added dropwise to a solution of compounds **9a-e** (0.05 mol) in absolute ethanol (100 ml). The reaction mixture was heated under reflux for 12 hours, cooled to room temperature and poured into water. The separated solid was filtered, dried and crystallized from aqueous ethanol.

2-(2-Methyl-5-nitro-1H-benzimidazol-1-yl)acetohydrazide ***(10a)***

Yield, 95%; mp 157-158ºC. ^{1}H-NMR (DMSO-d_6); δ 2.61 (s, 3H, CH_3), 3.45 (br.s., 2H, -NHNH_2), 4.68 (s, 2H, -CH_2CO), 7.92-8.00 (m, 2H, C7-H + -NHNH_2), 8.18-8.23 (d, *J*=7.5 Hz, 1H, C6-H), 8.65 (s, 1H, C4-H). Anal. Calcd for $C_{10}H_{11}N_5O_3$ (%): C,48.19; H,4.45; N,28.10. Found: C,47.85; H,4.80; N,28.50.

2-(2-(2-Bromophenyl)-1H-benzimidazol-1-yl)acetohydrazide ***(10b)***

Yield, 91%; mp 179-180ºC. ^{1}H-NMR (DMSO-d_6); δ 3.39 (br.s, 2H, -NHNH_2), 4.75 (s, 2H, -CH_2CO), 7.10-7.95 (m, 9H, Ar-H+-NHNH_2). Anal. Calcd for $C_{15}H_{13}BrN_4O$ (%): C,52.19; H,3.80; N,16.23. Found: C,51.07; H,3.67; N,16.26.

2-(2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)acetohydrazide ***(10c)***

Yield, 86%; mp 148-149ºC. ^{1}H-NMR (DMSO-d_6); δ 3.42 (br.s, 2H, -NHNH_2), 4.65 (s, 2H, -CH_2CO), 7.15-7.76 (m, 8H, Ar-H), 8.05 (br.s, 1H, -NHNH$_2$). Anal. Calcd for $C_{15}H_{13}ClN_4O$(%): C,59.91; H,4.36; N,18.63. Found: C,60.09; H,4.06; N,18.29.

2-(2-(2-Iodophenyl)-1H-benzimidazol-1-yl)acetohydrazide ***(10d)***

Yield, 64%; mp 166-167ºC. ^{1}H-NMR (DMSO-d_6); δ 3.52 (br.s, 2H, -NHNH_2), 4.74 (s, 2H, -CH_2CO), 7.18-8.12 (m, 9H, Ar-H+-NHNH_2). Anal. Calcd for $C_{15}H_{13}IN_4O$ (%): C,45.94; H,3.34; N,14.29. Found: C,45.44; H,3.45; N,14.35.

2-(2-(3-Pyridyl)-1H-benzimidazol-1-yl)acetohydrazide ***(10e)***

Yield, 70%; mp 198-199ºC. ^{1}H-NMR (DMSO-d_6); δ 3.49 (br.s, 2H, -NHNH_2), 4.62 (s, 2H, -CH_2CO), 7.26-8.82 (m, 9H, Ar-H+-NHNH_2). Anal. Calcd for $C_{14}H_{13}N_5O$(%): C,62.91; H,4.90; N,26.20. Found: C,63.11; H,5.10; N,26.00.

1-(2-(2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)acetyl)semicarbazides ***(11a-e)***

To a suspension of 2-(2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)acetohydrazides **10a-e** (0.04 mol) in water (16 ml), urea (9.6 g, 0.16 mol) was added and the reaction mixture was heated under reflux for 1 hour. After cooling, the formed precipitate was filtered, dried and crystallized from the appropriate solvent.

1-(2-(2-Methyl-5-nitro-1H-benzimidazol-1-yl)acetyl)semicarbazide ***(11a)***

Crystallization solvent, water; yield, 95%; mp 190-191ºC. IR(cm^{-1}): 3251 (N–H stretch), 3067 (aromatic C–H stretch), 2978 (aliphatic C–H stretch), 1684 (C=O stretch), 1617 (C=N stretch, N–H bend), 1573 (N–H bend).^{1}H-NMR (DMSO-d_6); δ 2.59 (s, 3H, CH_3), 4.62 (s, 2H, CH_2CO), 6.03 (br.s, 3H, NHCONH$_2$, exchangeable with D_2O), 7.94-7.98 (d, *J*=7.50 Hz, 1H, C7-H), 8.01 (br.s, 1H, CH_2CONH, exchangeable with D_2O), 8.17-8.21 (d, *J*=7.50 Hz, 1H, C6-H), 8.63 (s, 1H, C4-H). Anal. Calcd for $C_{11}H_{12}N_6O_4$ (%): C,45.21; H,4.14; N,28.76. Found: C,45.60; H,4.50; N,29.10.

1-(2-(2-(2-Bromophenyl)-1H-benzimidazol-1-yl)acetyl)semicarbazide ***(11b)***

Crystallization solvent, ethanol/water; yield, 95%; mp 229-230ºC. ^{1}H-NMR (DMSO-d_6); δ 4.68 (s, 2H, NCH_2CO), 6.15 (br.s, 3H, NHCONH$_2$, exchangeable with D_2O), 7.10-7.74 (m, 8H, Ar-H), 8.03 (br.s, 1H, CH_2CONH, exchangeable with D_2O). Anal. Calcd for $C_{16}H_{14}BrN_5O_2$ (%): C,49.50; H,3.63; N,18.04. Found: C,49.74; H,4.01; N,18.56.

1-(2-(2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)acetyl)semicarbazide ***(11c)***

Crystallization solvent, ethanol/water; yield, 89%; mp 223-224ºC. IR(cm^{-1}): 3396, 3195 (N–H stretch), 3065 (aromatic C–H stretch), 2931 (aliphatic C–H stretch), 1678 (C=O stretch), 1652 (C=N stretch), 1587 (N–H bend, aromatic C–C stretch). ^{1}H-NMR (DMSO-d_6); δ 4.65 (s, 2H, CH_2CO), 6.11 (br.s, 3H, NHCONH$_2$, exchangeable with D_2O), 7.16-7.74 (m, 8H, Ar-H), 8.05 (br.s, 1H, CH_2CONH, exchangeable with D_2O). Anal. Calcd for $C_{16}H_{14}ClN_5O_2$ (%): C,55.90; H,4.10; N,20.37. Found: C,55.86; H,4.14; N,20.64.

1-(2-(2-(2-Iodophenyl)-1H-benzimidazol-1-yl)acetyl)semicarbazide ***(11d)***

Crystallization solvent, methanol/water; yield, 91%; mp 282-283ºC. ^{1}H-NMR (DMSO-d_6); δ 4.66 (s, 2H, CH_2CO), 6.13 (br.s, 3H, NHCONH$_2$, exchangeable with D_2O), 7.15-7.74 (m, 8H, Ar-H), 8.06 (br.s, 1H, CH_2CONH,

exchangeable with D_2O). Anal. Calcd for $C_{16}H_{14}IN_5O_2$(%): C,44.16; H,3.24; N,16.09. Found: C,44.19; H,3.73; N,16.36.

1-(2-(2-(3-Pyridyl)-1H-benzimidazol-1-yl)acetyl)semicarbazide ***(11e)***

Crystallization solvent, water; yield, 76%; mp 235-236ºC. ^{1}H-NMR (DMSO-d_6); δ 4.67 (s, 2H, C$\underline{H}_2$CO), 6.15 (br.s, 3H, N$\underline{H}$CON$\underline{H}_2$, exchangeable with D_2O), 7.17-7.74 (m, 8H, Ar-$\underline{H}$), 8.09 (br.s, 1H, CH$_2$CON$\underline{H}$, exchangeable with D_2O). Anal. Calcd for $C_{15}H_{14}N_6O_2$(%): C,58.06; H,4.55; N,27.08. Found: C,58.40; H,3.97; N,26.80.

3-((2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-ones ***(12a-e)***

A mixture of 1-(2-(2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)acetyl)semicarbazides (**11a-e**) (0.001 mol) and NaOH (0.4 g, 0.01 mol) in water (4 ml) was heated at 90-95 °C with stirring for 10 hours. The reaction mixture was filtered and the filtrate was neutralized with HCl. The obtained precipitate was filtered, washed with water and crystallized from the appropriate solvent.

3-((2-Methyl-5-nitro-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-one ***(12a)***

Crystallization solvent, ethanol; yield, 82%; mp 293-294ºC. ^{1}H-NMR (DMSO-d_6); δ 2.61 (s, 3H, C$\underline{H}_3$), 3.80 (s, 2H, C$\underline{H}_2$), 6.02 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.11 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.94-7.98 (d, J=7.50 Hz, 1H, C7-$\underline{H}$), 8.17-8.21 (d, J=7.50 Hz, 1H, C6-$\underline{H}$), 8.63 (s, J=1.48 Hz, 1H, C4-$\underline{H}$). Anal. Calcd for $C_{11}H_{10}N_6O_3$(%): C,48.18; H,3.68; N,30.65. Found: C,48.53; H,3.73; N,30.71.

3-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-one **(12b)**

Crystallization solvent, acetone; yield, 85%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 3.89 (s, 2H, C$\underline{H}_2$), 6.11 (s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.18 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.28-7.75 (m, 8H, Ar-$\underline{H}$). Anal. Calcd for $C_{16}H_{12}BrN_5O$ (%): C,51.91; H,3.27; N,18.92. Found: C,51.97; H,3.62; N,19.20.

3-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-one ***(12c)***

Crystallization solvent, acetone; yield, 90%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 3.91 (s, 2H, C$\underline{H}_2$), 6.07 (s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.08 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.18-7.75 (m, 8H, Ar-$\underline{H}$). Anal. Calcd for $C_{16}H_{12}ClN_5O$ (%): C,58.99; H,3.71; N,21.50. Found: C,59.34; H,3.25; N,21.00.

3-((2-(2-Iodophenyl)-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-one ***(12d)***

Crystallization solvent, methanol; yield, 60%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ δ 4.11 (s, 2H, C$\underline{H}_2$), 6.15 (brs, 1H, N$\underline{H}$, exchangeable with D_2O), 7.19 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.12-7.64 (m, 8H, Ar-$\underline{H}$). Anal. Calcd for $C_{16}H_{12}IN_5O$ (%): C,46.06; H,2.90; N,16.79. Found: C,46.42; H,2.54; N,17.10.

3-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-1H-1,2,4-triazol-5(4H)-one ***(12e)***

Crystallization solvent, ethanol; yield, 40%; mp >300ºC. ^{1}H-NMR (DMSO-d_6); δ 3.95 (s, 2H, C$\underline{H}_2$), 6.08 (brs, 1H, N$\underline{H}$, exchangeable with D_2O), 7.06 (br.s, 1H, N$\underline{H}$, exchangeable with D_2O), 7.24-8.85 (m, 8H, Ar-$\underline{H}$). Anal. Calcd for $C_{15}H_{12}N_6O$ (%): C,61.64; H,4.14; N,28.75. Found: C,61.20; H,4.45; N,28.95.

5-((2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiols ***(13a-e)***

To a suspension of 2-(2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)acetohydrazides **10a-e** (0.02 mol) in ethanol (50 mL), potassium hydroxide (1.12 g, 0.02 mol) and carbon disulfide (1.67 g, 0.022 mol) were added while stirring. The reaction mixture was heated under reflux for 8 hours then the solvent was evaporated under reduced pressure. The obtained residue was dissolved in water (200 mL) then treated with conc. hydrochloric acid (2.5 mL) while stirring at 0 °C. The separated solid was filtered, dried and crystallized from the appropriate solvent.

5-((2-Methyl-5-nitro-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol ***(13a)***

Crystallization solvent, acetic acid/water; yield, 90%; mp 209-210ºC. ^{1}H-NMR (DMSO-d_6); δ 2.63 (s, 3H, C$\underline{H}_3$), 3.15 (s, 1H, S$\underline{H}$, exchangeable with D_2O), 5.09 (s, 2H, C$\underline{H}_2$), 7.97-8.01 (d, *J*=7.50 Hz, 1H, C7-$\underline{H}$), 8.17-8.21 (d, *J*=7.50 Hz, 1H, C6-$\underline{H}$), 8.63 (s, 1H, C4-$\underline{H}$). Anal. Calcd for $C_{11}H_9N_5O_3S$ (%): C,45.36; H,3.11; N,24.04. Found: C,45.56; H,3.50; N,24.44.

5-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol ***(13b)***

Crystallization solvent, ethanol; yield, 75%; mp 229-230ºC. ^{1}H-NMR (DMSO-d_6); δ 3.17 (s, 1H, S$\underline{H}$, exchangeable with D_2O), 5.30 (s, 2H, C$\underline{H}_2$), 7.19-7.85 (m, 8H, Ar-$\underline{H}$). Anal. Calcd for $C_{16}H_{11}BrN_4OS$ (%): C,49.62; H,2.86; N,14.47. Found: C,49.84; H,3.20; N,14.93.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol ***(13c)***

Crystallization solvent, ethanol; yield, 83%; mp 250-251ºC. ^{1}H-NMR (DMSO-d_6); δ 3.19 (s, 1H, S$\underline{H}$, exchangeable with D_2O), 5.21 (s, 2H, C$\underline{H}_2$), 7.18-7.82 (m, 8H, Ar-$\underline{H}$). MS (m/z, %); 346 [M^+ + 3] (1.3), 345 [M^+ + 2] (5), 344 [M^+ + 1] (21.9), 342.75 [M^+] (27.3), 77 (100). Anal. Calcd for $C_{16}H_{11}ClN_4OS$ (%): C,56.06; H,3.23; N,16.34; S,9.35. Found: C,55.63; H,3.19; N,16.26; S,9.38.

5-((2-(2-Iodophenyl)-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol ***(13d)***

Crystallization solvent, acetone; yield, 70%; mp 252-253ºC. ^{1}H-NMR (DMSO-d_6); δ 3.20 (s, 1H, SH, exchangeable with D_2O), 5.31 (s, 2H, CH_2), 7.19-7.87 (m, 8H, Ar-H). Anal. Calcd for $C_{16}H_{11}IN_4OS$ (%): C,44.25; H,2.55; N,12.90. Found: C44.31,; H,2.81; N,12.70.

5-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol ***(13e)***

Crystallization solvent, acetic acid/water; yield, 70%; mp 208-209ºC. ^{1}H-NMR (DMSO-d_6); δ 3.12 (s, 1H, SH, exchangeable with D_2O), 4.98 (s, 2H, CH_2), 7.32-8.93 (m, 8H, Ar-H). Anal. Calcd for $C_{15}H_{11}N_5OS$ (%): C,58.24; H,3.58; N,22.64. Found: C,58.60; H,3.80; N,22.60.

5-((2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)methyl)-3-substituted-1,3,4-oxadiazole-2(3H)-thiones ***(14a-g)***

To a suspension of 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiols **13a-e** (0.001 mol) in ethanol (3 mL), paraformaldehyde (0.09 g, 0.001 mol) was added portionwise while stirring for 15 minutes The appropriate secondary amine (0.001 mol) was then added and the reaction mixture was stirred at room temperature for 12 hours. The formed precipitate was filtered, washed with ethanol, dried and crystallized from dichloromethane/n-hexane mixture.

5-((2-Methyl-5-nitro-1H-benzimidazol-1-yl)methyl)-3-morpholinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14a)***

Yield, 60%; mp 184-185ºC. ^{1}H-NMR ($CDCl_3$); δ 2.33-2.40 (t, *J*=7.09 Hz, 4H, morpholine-H), 2.59 (s, 3H, CH_3), 3.63-3.72 (m, 6H, of morpholine-H + CH_2), 3.99 (s, 2H, CH_2), 7.96-8.02 (d, *J*=7.50 Hz, 1H, C7-H), 8.19-8.23 (d, *J*=7.50 Hz, 1H, C6-H), 8.63 (s, 1H, C4-H). Anal. Calcd for $C_{16}H_{18}N_6O_4S$ (%): C,49.22; H,4.65; N,21.53. Found: C,49.24; H,4.35; N,21.16.

5-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-3-morpholinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14b)***

Yield, 58%; mp 180-181ºC. ^{1}H-NMR ($CDCl_3$); δ 2.35-2.43 (t, *J*=7.09 Hz, 4H, morpholine-H), 3.69-3.79 (m, 6H, of morpholine-H + CH_2), 4.05 (s, 2H, CH_2), 7.15-7.78 (m, 8H, Ar-H). Anal. Calcd for $C_{21}H_{20}BrN_5O_2S$ (%): C,51.86; H,4.14; N,14.40. Found: C,51.80; H,4.25; N,14.50.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-3-piperidinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14c)***

Yield, 70%; mp 164-166ºC. ^{1}H-NMR ($CDCl_3$); δ 1.52-1.59 (m, 6H, piperidine-H), 2.19-2.28 (m, 4H, piperidine-H), 3.72 (s, 2H, CH_2), 4.32 (s, 2H, CH_2), 7.16-7.74 (m, 8H, Ar-H). Anal. Calcd for $C_{22}H_{22}ClN_5OS$ (%): C,60.06; H,5.04; N,15.92; S,7.29. Found: C,60.01; H,5.08; N,15.96; S,7.61.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-3-morpholinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14d)***

Yield, 62%; mp 188-190ºC. ^{1}H-NMR ($CDCl_3$); δ 2.34-2.44 (t, *J*=7.09 Hz, 4H, morpholine-H), 3.70-3.78 (m, 6H, of morpholine-H + CH_2), 4.07 (s, 2H, CH_2), 7.16-7.88 (m, 8H, Ar-H). Anal. Calcd for $C_{21}H_{20}ClN_5O_2S$(%): C,57.07; H,4.56; N,15.85. Found: C,57.50; H,4.42; N,15.82.

5-((2-(2-Iodophenyl)-1H-benzimidazol-1-yl)methyl)-3-piperidinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14e)***

Yield, 40%; mp 135-136ºC. ^{1}H-NMR ($CDCl_3$); δ 1.46-1.53 (m, 6H, piperidine-H), 2.25-2.36 (m, 4H, piperidine-H), 3.82 (s, 2H, CH_2), 4.21 (s, 2H, CH_2), 7.12-7.78 (m, 8H, Ar-H). Anal. Calcd for $C_{22}H_{22}IN_5OS$ (%): C,49.72; H,4.17; N,13.18. Found: C,50.00; H,4.27; N,13.08.

5-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-3-piperidinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14f)***

Yield, 63%; mp 139-140ºC. ^{1}H-NMR ($CDCl_3$); δ 1.50-1.54 (m, 6H, piperidine-H), 2.26-2.34 (m, 4H, piperidine-H), 3.72 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 7.15-7.79 (m, 8H, Ar-H). Anal. Calcd for $C_{21}H_{22}N_6OS$ (%): C,62.05; H,5.45; N,20.67. Found: C,61.83; H,5.97; N,20.33.

5-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-3-morpholinomethyl-1,3,4-oxadiazole-2(3H)-thione ***(14g)***

Yield, 50%; mp 155-157ºC. ^{1}H-NMR ($CDCl_3$); δ 2.39-2.47 (t, *J*=7.09 Hz, 4H, morpholine-H), 3.72-3.82 (m, 6H, morpholine-H + CH_2), 4.11 (s, 2H, CH_2), 7.22-8.81 (m, 8H, Ar-H). Anal. Calcd for $C_{20}H_{20}N_6O_2S$ (%): C,58.81; H,4.94; N,20.57. Found: C,58.40; H,4.63; N,20.73.

5-((2-Substituted-5-(un)substituted-1H-benzimidazol-1-yl)methyl)-2-(alkylthio)-1,3,4-oxadiazoles **(15a-j)**

To a suspension of 5-((2-substituted-5-(un)substituted-1*H*-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (**13a-e**) (0.001 mol) in ethanol (3 ml), a solution of NaOH (0.04 g, 0.001 mol) in water (1 ml) was added while stirring. Stirring was continued till clear solution was obtained, then the appropriate alkyl halide (0.003 mol) was added. The reaction mixture was stirred at room temperature for 12 hours, then diluted with water (20 mL). The formed precipitate was filtered, washed with methanol, dried and crystallized from aqueous ethanol.

5-((2-Methyl-5-nitro-1H-benzimidazol-1-yl)methyl)-2-(benzylthio)-1,3,4-oxadiazole ***(15a)***

Yield, 92%; mp 96-98ºC. ^{1}H-NMR ($CDCl_3$); δ 2.54 (s, 3H, CH_3), 4.19 (s, 2H, SCH_2), 4.99 (s, 2H, CH_2), 6.99-7.21 (m, 5H, benzyl-H), 7.92-7.96 (d, *J*=7.50 Hz, 1H, C7-H), 8.15-8.19 (d, *J*=7.50 Hz, 1H, C6-H), 8.62 (s, 1H, C4-H). Anal. Calcd for $C_{18}H_{15}N_5O_3S$ (%): C,56.68; H,3.96; N,18.36. Found: C,55.94; H,3.53; N,18.20.

5-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-2-(methylthio)-1,3,4-oxadiazole ***(15b)***

Yield, 79%; mp 165-167ºC. ^{1}H-NMR ($CDCl_3$); δ 2.47 (s, 3H, SCH_3), 5.03 (s, 2H, CH_2), 7.12-7.83 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{13}BrN_4OS$ (%): C,50.88; H,3.27; N,13.96. Found: C,50.51; H,3.65; N,13.50.

5-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-2-(ethylthio)-1,3,4-oxadiazole ***(15c)***

Yield, 71%; mp 132-134ºC. ^{1}H-NMR ($CDCl_3$); δ 1.19-1.24 (t, *J*=8.07 Hz, 3H, $SCH_2C\underline{H}_3$), 2.81-3.02 (q, *J*=8.00 Hz, 2H, $SC\underline{H}_2CH_3$), 5.00 (s, 2H, $C\underline{H}_2$), 7.13-7.75 (m, 8H, Ar-H). Anal. Calcd for $C_{18}H_{15}BrN_4OS$ (%): C,52.06; H,3.64; N,13.49. Found: C,51.83; H,3.25; N,12.99.

5-((2-(2-Bromophenyl)-1H-benzimidazol-1-yl)methyl)-2-(benzylthio)-1,3,4-oxadiazole ***(15d)***

Yield, 62%; mp 104-106ºC. ^{1}H-NMR ($CDCl_3$); δ 4.29 (s, 2H, $SC\underline{H}_2$), 5.18 (s, 2H, $C\underline{H}_2$), 6.98-8.91 (m, 13H, Ar-H). Anal. Calcd for $C_{23}H_{17}BrN_4OS$ (%): C,57.87; H,3.59; N,11.74. Found: C,58.27; H,3.99; N,11.38.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-2-(methylthio)-1,3,4-oxadiazole ***(15e)***

Yield, 80%; mp 152-154ºC. ^{1}H-NMR ($CDCl_3$); δ 2.52 (s, 3H, $SC\underline{H}_3$), 4.95 (s, 2H, $C\underline{H}_2$), 7.19-7.78 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{13}ClN_4OS$ (%): C,57.22; H,3.67; N,15.70. Found: C,57.33; H,3.57; N,15.20.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-2-(ethylthio)-1,3,4-oxadiazole ***(15f)***

Yield, 78%; mp 185-186ºC. ^{1}H-NMR ($CDCl_3$); δ 1.18-1.26 (t, *J*=8.07 Hz, 3H, $SCH_2C\underline{H}_3$), 2.91-3.03 (q, *J*=8.00 Hz, 2H, $SC\underline{H}_2CH_3$), 4.90 (s, 2H, $C\underline{H}_2$), 7.11-7.73 (m, 8H, Ar-H). Anal. Calcd for $C_{18}H_{15}ClN_4OS$ (%): C,58.30; H,4.08; N,15.11. Found: C,58.21; H,4.21; N,15.33.

5-((2-(2-Chlorophenyl)-1H-benzimidazol-1-yl)methyl)-2-(benzylthio)-1,3,4-oxadiazole ***(15g)***

Yield, 88%; mp 127-128ºC.^{1}H-NMR ($CDCl_3$); δ 4.28 (s, 2H, $SC\underline{H}_2$), 5.06 (s, 2H, $C\underline{H}_2$), 6.98-8.90 (m, 13H, Ar-H). Anal. Calcd for $C_{23}H_{17}ClN_4OS$ (%): C,63.81; H,3.96; N,12.94. Found: C,63.50; H,3.44; N,13.17.

5-((2-(2-Iodophenyl)-1H-benzimidazol-1-yl)methyl)-2-(methylthio)-1,3,4-oxadiazole ***(15h)***

Yield, 55%; mp 156-157ºC. ^{1}H-NMR ($CDCl_3$); δ 2.44 (s, 3H, $SC\underline{H}_3$), 5.12 (s, 2H, $C\underline{H}_2$), 7.10-7.74 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{13}IN_4OS$(%): C,45.55; H,2.92; N,12.50. Found: C,45.72; H,3.11; N,12.90.

5-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-2-(ethylthio)-1,3,4-oxadiazole ***(15i)***

Yield, 60%; mp 143-145ºC. ^{1}H-NMR ($CDCl_3$); δ 1.21-1.28 (t, *J*=8.07 Hz, 3H, $SCH_2C\underline{H}_3$), 2.95-3.06 (q, *J*=8.00 Hz, 2H, $SC\underline{H}_2CH_3$), 4.92 (s, 2H, $C\underline{H}_2$), 7.24-8.86 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{15}N_5OS$ (%): C,60.52; H,4.48; N,20.76. Found: C,60.10; H,4.90; N,20.66.

5-((2-(3-Pyridyl)-1H-benzimidazol-1-yl)methyl)-2-(benzylthio)-1,3,4-oxadiazole ***(15j)***

Yield, 80%; mp 125-127ºC. ^{1}H-NMR ($CDCl_3$); δ 4.27 (s, 2H, $SC\underline{H}_2$), 5.08 (s, 2H, $C\underline{H}_2$), 6.96-8.92 (m, 13H, Ar-H). Anal. Calcd for $C_{22}H_{17}N_5OS$ (%): C,66.15; H,4.29; N,17.53. Found: C,66.25; H,4.33; N,17.40.

Antimicrobial screening

The newly synthesized derivatives were screened for their antimicrobial activities against *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 31784, *Staphylococcus aureus* ATCC 6538 and pathogenic fungi; *Candida albicans* ATCC 10231 by using disc diffusion method. MIC values of tested compounds were determined by two fold serial broth dilution technique.

Disc diffusion assay:

Whatman No. 1 filter paper discs of 6 mm diameter were sterilized by autoclaving for 15 minutes at 121 °C and then they were impregnated with the tested compounds (1000 µg/disc). Three flasks of 75 ml Mueller-Hinton agar, and one of Sabaroud's agar were autoclaved for 15 minutes at 121 °C, cooled to 45-50°C, poured into glass flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. The agar medium was allowed to cool to room temperature. Mueller-Hinton broth cultures of the test bacterial organisms; *Escherichia coli*; *Bacillus subtilis* and *Staphylococcus aureus* and buffered Yeast Nitrogen Base culture of the test fungal organism; *Candida albicans*, were incubated at 35°C until they achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity was then adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standard, this results in a suspension containing approximately $1\text{-}2\times10^8$ colony forming unit/ml. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The surface of Mueller-Hinton agar plates and Sabaroud's agar plates were inoculated with bacteria and fungi respectively, by streaking the swab over the entire sterile agar surface. The impregnated discs were dispensed onto the surface of the inoculated agar plates. Each disc was pressed down to ensure complete contact with the agar surface. Discs were distributed evenly, incubated at 5 ^{o}C for one hour to permit good diffusion and then transferred to an incubator at 37 ^{o}C for 24 hours for bacteria and at 28 ^{o}C for 72 hours for fungi. The inhibition zones caused by various compounds on the tested micro-organisms were examined and measured using a caliper, to the nearest 0.5 mm. levofloxacin (100 µg/disc) was used as a reference standard for bacteria, while tioconazole (100 µg/disc) was used as a reference standard for fungi.

Quantitative assay for determination of the minimum inhibitory concentration (MIC):

Antibacterial and antifungal assays were performed in Mueller-Hinton broth at pH 7.3 and

buffered Yeast Nitrogen Base at pH 7, respectively. Levofloxacin and tioconazole were used as standard drugs for bacteria and fungi respectively. All the tested compounds were dissolved in DMSO. Further dilutions were furnished at the required quantities of the broth used. The concentration range was 128-1 µg/ml for the tested compounds, 32-0.015 µg/ml for levofloxacin and 100-0.05 µg/ml for tioconazole. After inclusion of 100 µl of the broth containing the standard drug or the test compound, 100 µl of bacterial suspensions were inoculated into microplate wells. After incubation for 16-20 hours at 35 ^{o}C, the well containing the lowest concentration of the standard drug or the tested compound that inhibit growth of microorganism as detected by the unaided eye, was recorded to represent the MIC expressed in µg/ml.

Acknowledgement

Authors are thankful to Dr. Khaled Hussein, Associate professor of Microbiology, Faculty of Pharmacy, Mansoura University, for performing the biological screening.

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11/16/2012