ISSN 2321-807X

Synthesis, Characterization and biological evaluation of Novel Carboxamides, Oxadiazoles and Isoindoline-1,3-diones derived from 2-substituted phenylquinoline-4-carbohydrazides.

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ABSTRACT

The new 2-arylquinoline-4-carboxylic acid derivatives **4(a-f)** and **5(a-i)**, were tested by qualitative and quantitative methods on various bacterial and fungal strains and proved to be active at low concentrations against Gram-positive and Gram-negative bacteria as well as fungi. The MIC values were determined for test compounds as well as for reference standards. Compounds **4b** and **5d** showed better antibacterial and antifungal activity than clinically prevalent drugs (Gentamicin, Ampicillin and Fluconazole) against ***Staphylococcus aureus*** and ***Candida albicans***. The structures of newly synthesized compounds have been characterized on the basis of their spectroscopic data. The study revealed the potential of newly synthesized compounds as a novel group of antimicrobials.

Keywords

2-Arylquinoline-4-carbohydrazide, Salicylaldehyde, 2-Hydroxynaphthaldehyde, Phosphorylchloride, Sodium Borohydride and Antimicrobial activity.

Academic Discipline And Sub-Disciplines

Organic and synthetic chemistry.

TYPE (METHOD/APPROACH)

Synthesis of heterocycles and biological activity.

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Chemistry

Vol. 10, No. 8

editorjaconline@gmail.com

www.cirjac.com

ISSN 2321-807X

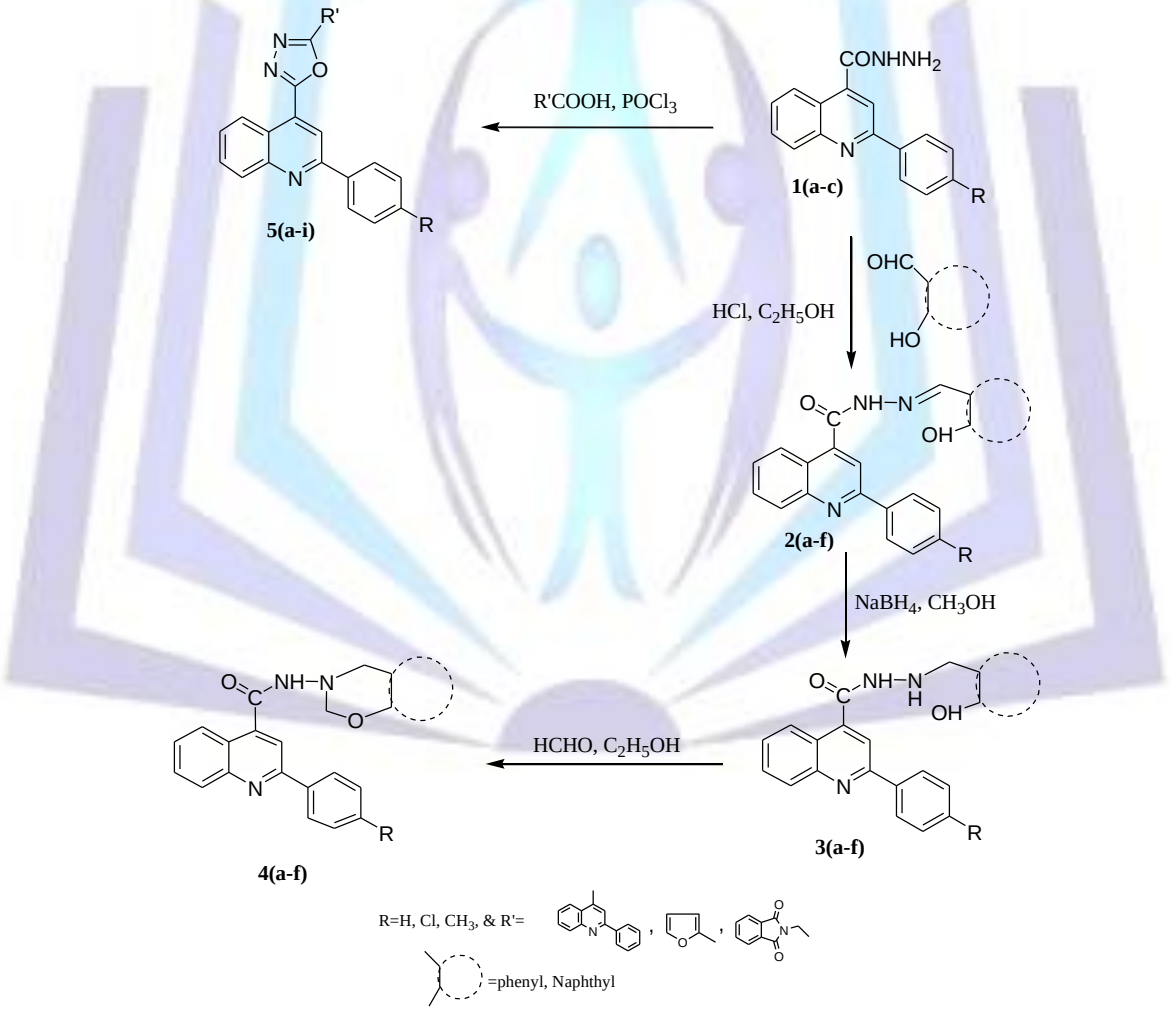
Introduction

The quinoline scaffold is prevalent in a variety of synthetic and natural compounds. Substituted quinolines are one of the oldest known classes of pharmaceutical agents and their relevance in chemotherapy especially against malaria is known [1-6]. Beside antimalarials, a spectrum of other pharmacological activities [7-12] like antimicrobial [13-15], antifungal [16,17] antiamoebic [18] antileishmanial [19,20] antitumor [21-23] hypotensive [24] and antidepressant agents [25,26] has been the major reason for the development of novel and efficient synthesis of quinoline derivatives. Improvement of existing antimicrobial drugs and development of new ones is extremely necessary in today's world, which is witnessing an increasing incidence of bacterial drug resistance. This has also triggered the publications of several simple and elegant derivatives of quinolones [27-32]. Furthermore, several hydrazide-carboxamides [33], oxadiazoles [34] and indolin-2-ones [35,36] have been claimed to exhibit appreciable antimicrobial activity. As a part of our interest in identifying a larger number of bioactive quinolines and in continuation of our previous work [37] we have synthesized some new hydrazide-carboxamides, oxadiazole and isoindoline-1,3-diones of 2-arylquinoline-4-carbohydrazides to evaluate their in vitro antimicrobial activity.

Results And Discussion

Chemistry

The synthetic chemical routes employed in producing **4(a-f)** and **5(a-i)** are portrayed in **Scheme-1**. The starting 2-arylquinoline-4-carboxylic acids were prepared by a literature procedure utilizing well established Pfitzinger reaction from isatin and different α-methyl ketones in satisfactory yields. The acids were subsequently treated with thionyl chloride in refluxing benzene to give corresponding acid chlorides which were used directly to prepare the hydrazides **1(a-c)** through reaction with hydrazine hydrate in refluxing ethanol.



Scheme 1 Synthesis of **4(a-f)** and **5(a-i)** from 2-substituted phenylquinoline-4-carbohydrazides

The hydrazides were characterized by their physical, analytical and spectral data. IR spectra of the hydrazides showed NH and C=O stretching bands at 3264-3356cm^{-1} and 1640-1662cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected region. In the ^{1}HNMR spectra of the hydrazides, the amine(NH_2) proton appeared at 4.7-5.1 ppm as a sharp D_2O exchangeable singlet, whereas a broad more downfield D_2O exchangeable singlet at 9.8-10.1 ppm was characteristic of the NH proton (CONH group), the other protons appeared at the expected chemical shifts and integral values. Reaction of **1(a-c)** with salicylaldehyde or 2-hydroxynapthaldehyde in ethanol in presence of catalytic amount of hydrochloric acid furnished N'-(2-hydroxybenzylidene)-2-(4-substituted phenyl)quinoline-4-carbohydrazides or N'-((3-hydroxynaphthalen-2-yl)methylene)-2-(4-substituted phenyl) quinoline-4-carbohydrazides **2(a-f)**. Then the reduction of **2(a-f)** using sodium borohydride in methanol gave N'-(2-hydroxybenzyl)-2-(4-substitutedphenyl)quinoline-4-carbohydrazides or N'-((3-hydroxy naphthalene-2-yl)methyl-2-(4-substituted phenyl) quinoline-4-carbohydrazides **3(a-f)**. The internal Mannich reaction of **3(a-f)** with formaldehyde in ethanol afforded N-(2H-benzo[e][1,3] oxazin-3(4H)-yl)-2-(4-substitutedphenyl)quinoline-4-carboxamides or N-(2H-naphtho[2,3-e][1,3]oxazin-3(4H)-yl)-2-(4-substitutedphenyl)quinoline-4-carboxamides **4(a-f)**. The structures of all the products were in good agreement with spectral data and elemental analysis. The above series of reactions is suitable for coupling quinazoline moiety with benzoxazine or naphthoxazine moiety through –CONH-bridge.

On the other hand, the condensation of **1(a-c)** with various substituted acids (quinoline-4-carboxylic acids, furoic acid and phthalimidoacetic acid) in the presence of phosphorus oxychloride afforded 2,5-bis(2-substituted phenylquinolin-4-yl)-1,3,4-oxadiazoles or 2-(furan-2-yl)-5-(2-(4-substituted phenyl)quinolin-4-yl)-1,3,4-oxadiazoles or 2-((5-(2-(4-substituted phenyl) quinolin-4-yl)-1,3,4-oxadiazol-2-yl)methyl)isoindoline-1,3-diones **5(a-i)**. The IR spectra of 5 showed disappearance of absorption band at 1640-1662cm^{-1} due to C=O stretching vibration and appearance of a band at 1093-1153cm^{-1} region attributable to C-O-C vibrations providing a strong evidence for the formation of the titled compounds. In the ^{1}HNMR spectrum of **5c**, the signal of CH_2-N of two proton of isoindole appeared as a singlet at 3.89 ppm. The signal of protons of isoindole ring and aromatic protons appeared as a complex multiplet in the region 7.38-8.09 ppm. In the ^{13}CNMR of **5c**, the CH_2-N of isoindole ring carbon appeared at 42.6 ppm, carbonyl carbon peak appeared at 167.2 ppm. The N=C-O of oxadiazole carbon appeared at 162.4 ppm and remaining aromatic carbons peak appeared at region 120-149.8 ppm. The structures of all the products were in good agreement with spectral data and elemental analysis.

Experimental

Melting points were determined with an Electro thermal melting point apparatus and are uncorrected. Reactions were monitored by TLC, performed on silica gel glass plates, visualization on TLC were achieved by iodine indicator. I.R. spectra (potassium bromide) were recorded on Perkin-Elmer FTIR spectrophotometer (v max in cm^{-1}); ^{1}H and ^{13}C-NMR spectra were recorded on Bruker 200/300 MHz instruments using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI MS mass spectra were recorded on a Va 70-70H mass spectrometer (Manchester, UK) at 70 eV, with a trap current of 200 μA and 4 kV of acceleration voltage and ESI mode positive ion trap detector. Elemental Analysis was performed on a Perkin-Elmer 2400 series II elemental CHNS analyzer. All chemicals and reagents were obtained from Aldrich, Lancaster, Merck, Sdfine or Spectrochem Pvt. Ltd and were used without further purification.

2-arylquinoline-4-carboxylic acids [38-40] and 2-arylquinoline-4-carbohydrazides **1(a-c)** [41-45] were prepared according to literature procedures.

General Method for the synthesis of N'-(2-hydroxybenzylidene)-2-(4-substituted phenyl) quinoline-4-carbohydrazide / N'-((3-hydroxynapthalen-2-yl)methylene)-2-(4-substituted phenyl) quinoline-4-carbohydrazide 2(a-f).

2-(4-substituted phenyl) quinoline-4-carbohydrazide **1** (0.01mol) and salicylaldehyde or 2-hydroxynaphthaldehyde (0.01 mol) were refluxed in ethanol (50 mL) containing two drops of concentrated hydrochloric acid for 2 h. Crystalline solids which separated on cooling were collected and recrystallised from ethanol.

N'-(2-hydroxybenzylidene)-2-phenylquinoline-4-carbohydrazide (2a):

Pale yellow solid (Ethanol) (This compound was prepared by the reaction of 2-phenylquinoline-4-carbohydrazide **1a** (0.01mol) and salicylaldehyde (0.01 mol) following the above general procedure. It was obtained as a pale yellow solid.); yield ~87 %; Rf value: 0.46 (9.0:1.0, Benzene: Acetone); mp 212-214^{0}C; IR (KBr) vmax 3402, 3260, 1660, 1620,1588 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 6.88-8.01(m, 14H, ArH), 8.58(s, 1H, CH), 10.01(s, 1H, OH), 11.12(s, 1H, NH); EIMS m/z 367(M+), 290, 232, 204, 163. Anal. Calcd. for $C_{23}H_{17}N_3O_2$: C, 75.20; H, 4.63; N, 11.44. Found: C: 75.00; H, 4.61, N, 11.41.

2-(4-Chlorophenyl)-N'-(2-hydroxybenzylidene)quinoline-4-carbohydrazide (2b):

Pale yellow solid (Ethanol) (This compound was prepared by the reaction of 2-(4-chlorophenyl) quinoline-4-carbohydrazide **1b** (0.01mol) and salicylaldehyde (0.01 mol) following the above general procedure.It was obtained as a pale yellow solid.); yield ~82%; Rf value: 0.43 (9.0: 1.0, Benzene: Acetone); mp 253-254^{0}C; IR (KBr) vmax 3410, 3217, 1662, 1621, 1589, 726 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 7.04-8.23 (m, 13H, ArH), 8.64 (s, 1H, CH), 10.05 (s, 1H, OH), 11.21 (s, 1H, NH); EIMS m/z 401(M+), 403(M+1), 290, 266, 238, 163, 111; Anal. Calcd. for $C_{23}H_{16}ClN_3O_2$: C, 68.82; H, 3.99; N, 10.47. Found: C, 69.00; H, 4.01; N, 10.50.

N'-(2-hydroxybenzylidene)-2-p-tolylquinoline-4-carbohydrazide (2c):

Greenish yellow solid (Ethanol) (This compound was prepared by the reaction of 2-(4-methylphenyl) quinoline-4-carbohydrazide **1c** (0.01mol) and salicylaldehyde (0.01 mol) following the above general procedure. It was obtained as a greenish yellow solid.); yield ~87%; Rf value: 0.42 (8.5: 1.5, Benzene: Acetone); mp 234-236^{0}C; IR (KBr) vmax 3421, 3316, 1664, 1628, 1582, 706 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.28 (s, 3H, CH_3), 6.99-8.17 (m, 13H, ArH), 8.48 (s, 1H, CH), 9.49 (s, 1H, OH), 10.40 (s, 1H, NH). EIMS m/z 381 (M+), 367, 290, 232, 204, 163; Anal. Calcd. for $C_{24}H_{19}N_3O_2$: C, 75.59; H, 4.98; N, 11.02. Found: C, 75.78; H, 5.02; N, 11.05.

N'-((3-hydroxynaphthalen-2-yl)methylene)-2-phenylquinoline-4-carbohydrazide (2d):

Light yellow solid (Ethanol) (This compound was prepared by the reaction of 2-phenylquinoline-4-carbohydrazide **1a** (0.01mol) and 2-hydroxynaphthaldehyde (0.01 mol) following the above general procedure. It was obtained as a light yellow solid.); yield ~89%, Rf value, 0.26 (8.5: 1.5, Benzene: Acetone); mp 226-227^{0}C; IR (KBr) vmax 3400, 3269, 1662, 1625, 1580 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 7.21-8.48 (m, 16H, ArH), 8.61 (s, 1H, CH), 11.18 (s, 1H, OH), 12.28(s, 1H, NH). IR (KBr) vmax: 3400, 3269, 1662, 1625, 1580 cm-1 ; EIMS m/z 417(M+), 340, 232, 213, 204, 185; Anal. Calcd. for $C_{27}H_{19}N_3O_2$: C,77.69; H, 4.55; N, 10.07. Found: C, 77.41; H, 4.51; N, 10.09.

2-(4-chlorophenyl)-N'-((3-hydroxynaphthalen-2-yl)methylene)quinoline-4-carbohydrazide (2e):

Brown yellow crystals (Ethanol) (This compound was prepared by the reaction of 2-(4- chloro phenyl) quinoline-4-carbohydrazide **1b** (0.01mol) and 2-hydroxynaphthaldehyde (0.01 mol) following the above general procedure.It was obtained as a brown yellow crystals.); yield ~78%; Rf value: 0.27 (8.0: 2.0, Benzene: Acetone); mp 290-291^{0}C; IR (KBr) vmax 3406, 3276, 1664, 1625, 1581, 723 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 7.06-8.42 (m, 15H, ArH), 8.78 (s, 1H, CH), 9.98 (s, 1H, OH), 11.2 (s, 1H, NH); EIMS m/z 451(M+), 453(M+1), 266, 238, 213, 185, 111; Anal. Calcd. for $C_{27}H_{18}ClN_3O_2$: C, 71.84; H, 3.99; N, 9.31. Found: C, 72.16; H, 4.08; N, 9.35.

N'-((3-hydroxynaphthalen-2-yl)methylene)-2-p-tolylquinoline-4-carbohydrazide (2f):

Brown yellow crystals (Ethanol) (This compound was prepared by the reaction of 2-(4-methyl phenyl) quinoline-4-carbohydrazide **1c** (0.01mol) and 2-hydroxynaphthaldehyde (0.01 mol) following the above general procedure.It was obtained as a brown yellow crystals.); yield ~76%; Rf value: 0.43 (8.0: 2.0, Benzene: Acetone); mp 244-245^{0}C; IR (KBr) vmax 3413, 3322, 1660, 1620, 1586, 704 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.16 (s, 3H, CH_3), 6.99-8.23 (m, 15H, ArH), 8.70 (s, 1H, CH-N), 9.80 (s, 1H, OH), 10.70 (s, 1H, NH); EIMS m/z 431(M+), 340, 261, 246, 218, 91; Anal. Calcd. for $C_{28}H_{21}N_3O_3$: C, 77.95; H, 4.87; N, 9.74. Found: C, 77.72; H, 4.84; N, 9.92.

General Method for the synthesis of N'-(2-hydroxybenzyl)-2-(4-substituted phenyl)quinoline-4-carbohydrazide/N'-((3-hydroxynapthalen-2-yl)methyl-2-(4-substituted phenyl)quinoline-4-carbohydrazide 3(a-f).

Sodium borohydride (0.01 mol) was added to a solution of N'-(2-hydroxybenzylidene)-2-(4-substituted phenyl)quinoline-4-carbohydrazide or N'-((3-hydroxynaphthalene-2-yl) methylene)-2-(4-substituted phenyl)quinoline-4-carbohydrazide **2** (0.005 mol) in methanol (25 mL) and the reaction mixture was stirred for 4 h. It was then poured in to cold water (50 mL). The product, which separated as a solid, was filtered and washed with water. The crude products were purified by crystallization from ethanol.

N'-(2-hydroxybenzyl)-2-phenylquinoline-4-carbohydrazide (3a):

Brown solid (Ethanol)(To synthesized **3a** sodium borohydride (0.01 mol) was added to a solution of N'-(2-hydroxybenzylidene)-2-phenylquinoline-4-carbohydrazide **2a** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a brown solid.); yield ~79 %; Rf value: 0.34 (9.0: 1.0 Benzene: Acetone); mp 187-189^{0}C; IR (KBr) vmax 3398, 3220, 3135, 1643, 1624, 1540 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 4.01 (s, 2H, CH_2), 5.2 (s, 1H, OH), 6.91-8.11 (m, 14H, ArH), 9.29 (s, 1H, NH-CH_2), 10.08 (s, 1H, NH-CO); Anal. Calcd. for $C_{23}H_{19}N_3O_2$: C, 74.79; H, 5.14; N, 11.38. Found: C, 75.59; H, 5.10; N, 11.35.

2-(4-chlorophenyl)-N'-(2-hydroxybenzyl)quinoline-4-carbohydrazide (3b):

Light brown crystals (Ethanol) (To synthesized **3b** sodium borohydride (0.01 mol) was added to a solution of 2-(4-Chlorophenyl)-N'-(2-hydroxybenzylidene)quinoline-4-carbohydrazide **2b** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a light brown crystals.); yield: 69 %; Rf value: 0.30 (9.0: 1.0 Benzene: Acetone); mp 243-244^{0}C; IR (KBr) vmax 3406, 3227, 3148, 1648, 1598, 1572, 724 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 4.15 (s, 2H, CH_2), 5.94 (s, 1H, OH), 6.81-8.37(m, 13H, ArH), 9.84 (s, 1H, NH-CH_2), 11.01(s, 1H, NH-C-O); Anal. Calcd. for $C_{23}H_{16}ClN_3O_2$: C, 68.48; H, 4.46; N, 10.42. Found: C, 68.65; H, 4.49; N, 10.44.

N'-(2-hydroxybenzyl)-2-p-tolylquinoline-4-carbohydrazide (3c):

Light brown solid (Ethanol) (To synthesized **3c** sodium borohydride (0.01 mol) was added to a solution of N'-(2-hydroxybenzylidene)-2-p-tolylquinoline-4-carbohydrazide **2c** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a light brown solid.); yield ~75 %; Rf value: 0.43 (9.0: 1.0 Benzene: Acetone); mp 232^{0}C; IR (KBr) vmax 3412, 3312, 3167, 1658, 1608, 1578, 702 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.03 (s, 3H, CH_3), 3.98 (s, 2H, CH_2), 6.49 (s, 1H, OH), 6.79-8.27 (m, 13H, ArH), 7.83 (s, 1H, NH-CH_2, D_2O-exchangeable), 9.89 (s, 1H, NH-CO); Anal. Calcd. for $C_{24}H_{12}N_3O_2$: C, 75.19; H, 5.48; N, 10.96. Found: C, 75.00; H, 5.46; N, 10.92.

N'-((3-hydroxynaphthalen-2-yl)methyl)-2-phenylquinoline-4-carbohydrazide (3d):

Light brown solid (Ethanol) (To synthesized **3d** sodium borohydride (0.01 mol) was added to a solution of N'-((3-hydroxynaphthalen-2-yl)methylene)-2-phenylquinoline-4-carbohydrazide **2d** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a light brown solid.); yield ~71 %; Rf value: 0.38 (9.0: 1.0 Benzene: Acetone); mp 196-197^{0}C; IR (KBr) vmax 3396, 3237, 3123, 1668, 1629, 1548 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 4.08 (s, 2H, CH_2), 5.89 (s, 1H, OH), 6.92-8.04 (m, 16H, ArH), 9.69 (s, 1H, NH-CH_2), 10.20 (s, 1H, NH-CO); Anal. Calcd. for $C_{27}H_{21}N_3O_3$: C, 77.32; H, 5.01; N, 10.02. Found: C, 77.51; H, 5.02; N, 10.04.

2-(4-chlorophenyl)-N'-((3-hydroxynaphthalen-2-yl)methyl)quinoline-4-carbohydrazide (3e):

Light brown solid (Ethanol) (To synthesized **3e** sodium borohydride (0.01 mol) was added to a solution of 2-(4-chlorophenyl)-N'-((3-hydroxynaphthalen-2-yl)methylene)quinoline-4-carbohydrazide **2e** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a light brown solid.); yield ~67 %; Rf value: 0.54 (8.0: 2.0 Benzene: Acetone); mp 269-271^{0}C; IR (KBr) vmax 3409, 3225, 3175, 1663, 1612, 1575, 722 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 4.21(s, 2H, CH_2), 6.23 (s, 1H, OH), 6.76-8.19 (m, 15H, ArH), 9.96 (s, 1H, NH-CH_2), 11.12 (s, 1H, NH-CO); Anal. Calcd. for $C_{27}H_{20}ClN_3O_3$: C, 71.53; H, 4.41; N, 9.27. Found: C, 71.36; H, 4.40; N, 9.25.

N'-((3-hydroxynaphthalen-2-yl)methyl)-2-p-tolylquinoline-4-carbohydrazide (3f):

Light brown crystals (Ethanol) (To synthesized **3f** sodium borohydride (0.01 mol) was added to a solution of N'-((3-hydroxynaphthalen-2-yl)methylene)-2-p-tolylquinoline-4-carbohydrazide **2f** (0.005 mol) in methanol (25 mL) and then followed the above general procedure. It was obtained as a light brown crystals.); yield ~79 %; Rf value: 0.49 (9.0: 1.0 Benzene: Acetone); mp 278^{0}C; IR (KBr) vmax 3418, 3316, 3183, 1656, 1612, 1569, 706 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.08 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.63 (s, 1H, OH), 6.84-8.09 (m, 15H, ArH), 7.73 (s, 1H, NH-CH_2, D_2O-exchangeable), 8.87(s, 1H, NH-CO); Anal. Calcd. for $C_{28}H_{23}N_3O_2$: C, 77.59; H, 5.31; N, 9.69. Found: C, 77.41; H, 5.29; N, 9.66.

General Method for the synthesis of N-(2H-benzo[e][1,3]oxazin-3(4H)-yl)-2-(4-substituted phenyl)quinoline-4-carboxamide/N-(2H-naphtho[2,3-e][1,3]oxazin-3(4H)-yl)-2-(4-substituted phenyl)quinoline-4-carboxamide 4(a-f).

Compounds **3** (0.002 mol) and formalin (1ml 37%) were refluxed in ethanol (15ml) for 5 h. The reaction mixture was concentrated under reduced pressure and the resultant solution was poured on to crushed ice. The crude products were recrystallized with appropriate solvents.

N-(2H-benzo[e][1,3]oxazin-3(4H)-yl)-2-phenylquinoline-4-carboxamide (4a):

Brown solid (This compound was prepared by the reaction of N'-(2-hydroxybenzyl)-2-phenylquinoline-4-carbohydrazide (**3a**) (0.002 mol) and formalin (1ml 37%) and then followed the above general procedure. It was obtained as a brown solid.); yield ~78 %; Rf value: 0.44 (9.0: 1.0 Benzene: Acetone); mp 182^{0}C; IR (KBr) vmax 3310, 1667, 1582, 1548, 1115 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 3.93 (s, 2H, N-CH_2-C), 5.09 (s, 2H, N-CH_2-O), 6.87-8.04 (m, 14H, ArH), 8.40 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ = 122.4-148.1 (quinoline and phenyl), 162.8 (C=O), 56.2 (N-CH_2-C), 79.8 (N-CH_2-O); EIMS m/z 381(M+), 247, 232; Anal. Calcd. for $C_{24}H_{19}N_3O_2$: C, 75.59; H, 4.98; N, 11.02. Found: C, 75.98; H, 5.01; N, 11.08.

N-(2H-benzo[e][1,3]oxazin-3(4H)-yl)-2-(4-chlorophenyl)quinoline-4-carboxamide (4b).

Brown crystals (This compound was prepared by the reaction of 2-(4-chlorophenyl)-N'-(2-hydroxybenzyl)quinoline-4-carbohydrazide (**3b**) (0.002 mol) and formalin (1ml 37%) following the above general procedure. It was obtained as a brown crystals.); yield ~74%; Rf value:0.58 (9.0: 1.0 Benzene: Acetone); mp 198^{0}C; IR (KBr) vmax 3182, 1653, 1575, 1545, 1093, 928 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 3.99 (s, 2H, N-CH_2-C), 5.03 (s, 2H, N-CH_2-O), 7.16-8.09 (m, 13H, ArH), 8.96 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ = 123.5-147.9 (quinoline and phenyl), 160.8 (C=O), 54.2 (N-CH_2-C), 83.2 (N-CH_2-O); EIMS m/z 415(M+), 417(M+1), 339, 309, 281, 238; Anal. Calcd. for $C_{24}H_{18}ClN_3O_2$: C, 69.39; H, 4.33; N, 10.02. Found: C, 69.06; H, 4.31; N, 10.07.

N-(2H-benzo[e][1,3]oxazin-3(4H)-yl)-2-p-tolylquinoline-4-carboxamide (4c):

Reddish brown solid (This compound was prepared by the reaction of N'-(2-hydroxybenzyl)-2-p-tolylquinoline-4-carbohydrazide (**3c**) (0.002 mol) and formalin (1ml 37%) following the above general procedure.It was obtained as a reddish brown solid.); yield ~69%; Rf value: 0.52 (9.0: 1.0 Benzene: Acetone); mp 185-186^{0}C; IR (KBr) vmax 3326, 1660, 1595, 1586, 1150, 704 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.50 (s, 2H, CH_3), 3.76 (s, 2H, N-CH_2-C), 5.19 (s, 2H, N-CH_2-O), 8.46 (s, 1H, NH), 6.93-8.01 (m, 13H, ArH); ^{13}CNMR (DMSO-d_6): δ = 20.09 (-CH_3), 52.8 (N-CH_2-N), 87.8 (N-CH_2-O), 121-149.2 (quinoline and phenyl), 159.8 (C=O); EIMS m/z 395(M+), 246, 218, 106; Anal. Calcd. for $C_{25}H_{21}N_3O_2$: C, 75.94; H, 5.31; N, 10.63. Found: C, 75.18; H, 5.26; N, 10.52.

N-(2H-naphtho[2,3-e][1,3]oxazin-3(4H)-yl)-2-phenylquinoline-4-carboxamide (4d):

Reddish brown crystals (This compound was prepared by the reaction of N'-((3-hydroxynaphthalen-2-yl)methyl)-2-phenylquinoline-4-carbohydrazide (**3d**) (0.002 mol) and formalin (1ml 37%) following the above general procedure. It was obtained as a reddish brown crystals.); yield ~66%; Rf value: 0.46 (9.0: 1.0 Benzene: Acetone); mp 224-227^{0}C; IR (KBr) vmax 3276, 1670, 1618, 1542, 1147cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 3.96 (s, 2H, N-CH_2-C), 5.16 (s, 2H, N-CH_2-O), 7.11-8.21 (m, 16H, ArH), 8.92 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ = 49.8 (N-CH_2-C), 91.8 (N-CH_2-O), 109-151.2 (quinoline and phenyl), 162.2 (C=O); EIMS m/z 431(M+), 275, 232, 227, 204; Anal. Calcd. for $C_{28}H_{21}N_3O_2$: C, 77.95; H, 4.87; N, 9.74. Found: C, 78.13; H, 4.88; N, 9.76.

2-(4-chlorophenyl)-N-(2H-naphtho[2,3-e][1,3]oxazin-3(4H)-yl)quinoline-4-carboxamide (4e):

Dark brown crystals (This compound was prepared by the reaction of 2-(4-chlorophenyl)-N'-((3-hydroxynaphthalen-2-yl)methyl)quinoline-4-carbohydrazide (**3e**) (0.002 mol) and formalin (1ml 37%) following the above general procedure. It was obtained as a dark brown crystals.); yield ~74%; Rf value: 0.42 (8.0: 2.0 Benzene: Acetone); mp 218-219^{0}C; IR (KBr) vmax 3306, 1658, 1604, 1548, 1125, 924 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 3.89 (s, 2H, N-CH_2-C), 4.98 (s, 2H, N-CH_2-O), 7.23-8.37 (m, 15H, ArH), 8.98 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ = 51.6 (N-CH_2-C), 88.2 (N-CH_2-O), 104-149.2 (quinoline and phenyl), 158.9 (C=O); EIMS m/z 465(M+), 467(M+1), 354, 295, 266, 238; Anal. Calcd. for $C_{28}H_{20}ClN_3O_2$: C, 72.25; H, 4.30; N, 9.03. Found: C, 72.00; H, 4.33; N, 9.09.

N-(2H-naphtho[2,3-e][1,3] oxazin-3(4H)-yl)-2-p-tolylquinoline-4-carboxamide (4f):

Light brown solid (This compound was prepared by the reaction of N'-((3-hydroxynaphthalen-2-yl)methyl)-2-p-tolylquinoline-4-carbohydrazide (**3f**) (0.002 mol) and formalin (1ml 37%) following the above general procedure. It was obtained as a light brown solid.); yield ~73 %; Rf value: 0.54 (9.0: 1.0 Benzene: Acetone); mp 233-236^{0}C; IR (KBr) vmax 3312, 1667, 1614, 1549, 1123, 701 cm^{-1}; ^{1}HNMR (DMSO-d_6): δ = 2.44 (s, 3H, CH_3), 3.98(s, 2H, N-CH_2-C), 5.10 (s, 2H, N-CH_2-O), 7.33-8.18 (m, 15H, ArH), 8.86 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ = 21.01 (-CH_3), 51.6 (N-CH_2-C), 88.7 (N-CH_2-O), 118-147.9 (quinoline and phenyl), 163.5 (C=O); EIMS m/z 445(M+), 303, 261, 246, 218, 199. Anal. Calcd. for $C_{29}H_{23}N_3O_2$: C, 78.20; H, 5.16; N, 9.43. Found: C, 77.85; H, 5.15; N, 9.39.

General Method for the preparation of 5(a-i).

A mixture of 2-(4-substituted phenyl)-quinoline-4-carbohydrazide **1** (0.01 mol), quinoline-carboxylic acid, furoic acid or phthalimido acetic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h. The reaction mixture was cooled and allowed to stand at room temperature for 2 h. It was then poured on to crushed ice. The solid thus obtained were collected and treated with sodium bicarbonate solution (5%), then with water, filtered and recrystalised from mixture of ethanol and dimethyl formamide (2:1) to get compounds **5(a-i).**

2,5-bis(2-phenylquinolin-4-yl)-1,3,4-oxadiazole (5a):

Light brown crystals [Ethanol and Dimethyl Formamide (2:1)] To synthesized **5a** mixture of 2 -phenyl-quinoline-4-carbohydrazide **1a** (0.01 mol), quinoline-carboxylic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a light brown crystals [Ethanol and Dimethyl Formamide (2:1)]; yield ~78 %; Rf value: 0.34 (8.0: 2.0 Benzene: Acetone); mp 208-209^{0}C; IR (KBr) vmax 3021, 1595, 1529, 1153 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 6.8-8.2 (m, 20H, ArH); ^{13}CNMR ($CDCl_3$): δ = 124-152.4 (quinoline and phenyl), 162.5 (O-C=N of oxadiazloe); EIMS m/z 476(M+), 437, 322, 246, 230, 204; Anal. Calcd. for $C_{32}H_{20}N_4O$: C, 80.67; H, 4.20; N, 11.26. Found: C, 81.01; H, 4.31; N, 11.81.

2-(furan-2-yl)-5-(2-phenylquinolin-4-yl)-1,3,4-oxadiazole (5b):

Dark brown crystals [Ethanol and Dimethyl Formamide (2:1)] (To synthesized **5b** mixture of 2- phenyl-quinoline-4-carbohydrazide **1a** (0.01 mol), furoic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a dark brown crystals.; yield ~73 %; Rf value: 0.26 (9.0: 1.0

Benzene: Acetone); mp 165-167^{0}C; IR (KBr) cm^{-1} vmax 3099, 2920, 1632, 1608, 1108 cm^{-1} ; ^{1}HNMR ($CDCl_3$): δ = 7.17-8.67 (m, 13H, ArH); ^{13}CNMR ($CDCl_3$): δ = 104-154.8 (furan, quinoline and phenyl), 158.5-164.2 (O-C=N of oxadiazole); EIMS m/z 339(M+), 272, 262, 204, 135; Anal. Calcd. for $C_{21}H_{13}N_3O_2$: C, 74.333; H, 3.83; N, 12.38. Found: C, 73.90; H, 3.80; N, 12.31.

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2-((5-(2-phenylquinolin-4-yl)-1,3,4-oxadiazol-2-yl)methyl)isoindoline-1,3-dione (5c):

Dark brown solid [Ethanol and Dimethyl Formamide (2:1)](To synthesized **5c** mixture of 2-phenyl-quinoline-4-carbohydrazide **1a** (0.01 mol), phthalimido acetic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a dark brown solid.; yield ~67 %; Rf value: 0.40 (8.0: 2.0 Benzene: Acetone); mp 224^{0}C; IR (KBr) cm^{-1} 3015, 2993, 1680, 1633, 1582, 1098 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 3.89 (s, 2H, CH_2-N of isoindole), 7.08-8.19 (m, 14H, ArH); ^{13}CNMR ($CDCl_3$): δ = 42.6 (CH_2-N), 120-149.8 (quinolone, isoindoline and phenyl), 162.4 (N=C-O), 167.2 (C=O); EIMS m/z 432(M+), 328, 244, 228, 204, 188; Anal. Calcd. for $C_{24}H_{16}N_4O_3$: C, 72.22; H, 3.70; N, 12.96. Found: C, 72.55; H, 3.72; N, 13.02.

2-(2-(4-chlorophenyl)quinolin-4-yl)-5-(2-phenylquinolin-4-yl)-1,3,4-oxadiazole (5d):

Reddish brown crystals [Ethanol and Dimethyl Formamide (2:1)](To synthesized **5d** mixture of 2-(4-chloro phenyl)-quinoline-4-carbohydrazide **1b** (0.01 mol), quinoline-carboxylic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a reddish brown crystals.); yield ~69 %; Rf value: 0.36 (9.0: 1.0 Benzene: Acetone); mp 212-213^{0}C; IR (KBr) vmax 3094, 1627, 1593, 1142, 740 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 7.08-8.6 (m, 19H, Ar-H); ^{13}CNMR ($CDCl_3$): δ = 120.01-151.8 (quinoline and phenyl), 165.2 (N=C-O of oxadiazole); EIMS m/z 510(M+), 512(M+1), 433, 399, 246, 238, 111; Anal. Calcd. for $C_{32}H_{19}ClN_4O$: C, 75.29; H, 3.72; N, 10.18. Found: C, 75.59; H, 3.74; N, 11.02.

2-(2-(4-chlorophenyl)quinolin-4-yl)-5-(furan-2-yl)-1,3,4-oxadiazole (5e):

Brown crystals [Ethanol and Dimethyl Formamide (2:1)] (To synthesized **5e** mixture of 2-(4-chlorophenyl)-quinoline-4-carbohydrazide **1b** (0.01 mol), furoic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a brown crystals.); yield ~77 %; Rf value: 0.34 (9.0: 1.0 Benzene: Acetone); mp 178^{0}C; IR (KBr) vmax 3082, 1626, 1598, 1148, 732 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 6.98-8.59 (m, 12H, Ar-H); ^{13}CNMR ($CDCl_3$): δ = 110-149.2 (furan, quinoline and phenyl), 153-161.5 (N=C-O of oxadiazole); EIMS m/z 373(M+), 375(M+1), 345, 294, 280, 266, 238; Anal. Calcd. for $C_{21}H_{12}ClN_3O_2$: C, 67.55; H, 3.21; N, 11.26. Found: C, 67.92; H, 3.23; N, 11.32.

2-((5-(2-(4-chlorophenyl)quinolin-4-yl)-1,3,4-oxadiazol-2-yl)methyl) isoindoline-1,3-dione (5f):

Light brown crystals [Ethanol and Dimethyl Formamide (2:1)](To synthesized **5f** mixture of 2-(4- chlorophenyl)-quinoline-4-carbohydrazide **1b** (0.01 mol), phthalimido acetic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a light brown crystals.); yield ~74 %; Rf value: 0.28 (8.5: 1.5 Benzene: Acetone); mp 239^{0}C; IR (KBr) vmax 3078, 2962, 1676, 1629, 1586, 1103, 729 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 3.7 (s, 2H, CH_2-N), 6.13-8.77 (m, 13H, ArH);^{13}CNMR ($CDCl_3$): δ = 40.6 (CH_2-N), 120-150.9 (quinolone, isoindoline and phenyl), 163.1 (N=C-O), 169.2 (C=O); EIMS m/z 466(M+), 468(M+1), 438, 362, 355, 294, 280, 266, 238; Anal. Calcd. for $C_{26}H_{15}ClN_4O_3$: C, 66.95; H, 3.21; N, 11.42. Found: C, 67.38; H, 3.25; N, 12.01.

2-(2-phenylquinolin-4-yl)-5-(2-p-tolylquinoline-4-yl)-1,3,4-oxadiazole (5g):

Reddish brown solid [Ethanol and Dimethyl Formamide (2:1)] (To synthesized **5g** mixture of 2-(4- methyl phenyl)-quinoline-4-carbohydrazide **1c** (0.01 mol), quinoline-carboxylic acid, (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a reddish brown solid.); yield ~81 %; Rf value: 0.26 (9.0: 1.0 Benzene: Acetone); mp 202-203^{0}C; IR (KBr) vmax 3060, 1618, 1588, 1093, 709 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 2.66 (s, 3H, CH_3), 7.06-8.30 (m, 19H, ArH); ^{13}CNMR ($CDCl_3$): δ = 21.09 (CH_3), 121-153 (quinoline and phenyl), 162.6 (N=C-O of oxadiazole); EIMS m/z 490(M+), 462, 399, 387, 286, 260; Anal. Calcd. for $C_{33}H_{22}N_4O$: C, 80.81; H, 4.48; N, 11.42. Found: C, 81.41; H, 4.59; N, 11.49.

2-(furan-2-yl)-5-(2-p-tolylquinolin-4-yl)-1,3,4-oxadiazole (5h):

Light brown crystals [Ethanol and Dimethyl Formamide (2:1)] (To synthesized 5h mixture of 2-(4- methyl phenyl)-quinoline-4-carbohydrazide **1c** (0.01 mol), furoic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a light brown crystals.); yield ~73 %, Rf value, 0.38 (9.0: 1.0 Benzene: Acetone), mp 175-177^{0}C; IR (KBr) vmax 3021, 1637, 1521, 1095, 710 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 2.34 (s, 3H, CH_3), 6.87-6-8.20 (m, 12H, ArH); ^{13}CNMR ($CDCl_3$): δ = 22.09 (CH_3), 123.3-156.1(quinoline and phenyl), 164.5 (N=C-O of

oxadiazole); EIMS m/z 353(M+), 325, 262, 260, 244, 218, 169; Anal. Calcd. for $C_{22}H_{15}N_3O_3$: C, 74.78; H, 4.24; N, 11.89. Found: C, 75.01; H, 4.27; N, 11.96.

2-((5-(2-p-tolylquinolin-4-yl)-1,3,4-oxadiazol-2-yl)methyl)isoindoline1,3-dione(5i):

Light brown crystals [Ethanol and Dimethyl Formamide (2:1)] (To synthesized **5i** mixture of 2-(4- methyl phenyl)-quinoline-4-carbohydrazide **1c** (0.01 mol), phthalimido acetic acid (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed ~ 12h and then followed the above general procedure. It was obtained as a light brown crystals.); yield ~79 %; Rf value: 0.29 (9.0: 1.0 Benzene: Acetone); mp 242^0C; IR (KBr) vmax 3086, 2972, 1668, 1613, 1588, 1109, 712 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ = 2.58 (s, 3H, CH_3), 3.89 (s, 2H, CH-N), 6.98-8.72 (m, 13H, ArH); ^{13}CNMR ($CDCl_3$): δ = 19.8 (CH_3), 42.68 (CH_2-N), 118.9-149.2 (quinoline isoindoline and phenyl), 162.9 (N=C-O of oxadiazole), 166.7 (C=O); EIMS m/z 446(M+), 418, 355, 342, 260, 244, 231, 218; Anal. Calcd. for $C_{27}H_{18}N_4O_3$: C, 72.69; H, 4.03; N, 12.55. Found: C, 73.30; H, 4.07; N, 12.66.

Biological Activity

Antimicrobial Activity

All the test compounds were assayed in vitro for their antibacterial activity against ***Staphylococcus aureus*** (ATCC-9144), ***Bacillus subtilis*** (ATCC-6633) (representative for gram-positive bacteria), ***Escherichia coli*** (MTCC-739), ***Pseudomonas aeruginosa*** (ATCC-25615) and ***Klebsiella pneumoniae*** (MTCC-2405) (representative for Gram-negative bacteria), and for their antifungal activity against ***Candida albicans*** (ATCC-24433), ***Aspergillus niger*** (MTCC-1344), ***Aspergillus fumigatus*** (MTCC-2544) and ***Penicillium chrysogenum*** (MTCC-2725) using disc-diffusion method [46]. The MIC was determined by using two fold serial dilution method [47,48]. Gentamicin, Ampicillin and Fluconazole were used as reference standards to compare the antibacterial and antifungal activities, respectively. For determining both antibacterial and antifungal activities, the synthesized compounds were dissolved in chloroform (stock solution 5mg/mL). In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing broth supplemented with only chloroform at the same dilution used as in our experiment. The solvent used for evaluation of compounds exhibited no antimicrobial activity. This property represented a practical advantage for the antimicrobial evaluation of these water insoluble compounds. Further dilution was prepared at the required quantities of 100, 50, 25, 12.5, 6.25 and 3.125 µg /mL concentration. The MIC values were obtained from the lowest concentration of the test compound where the tubes remain clear, indicating that the bacterial growth was completely inhibited at this concentration. The Diameter of zone of inhibition is expressed in mm and, MIC values in µg/mL. The results are shown in **Table 1**. The graphical representations are shown in **Figure 1** and **2**.

Table 1. Antibacterial and Antifungal Activity of Newly Synthesized Compounds (4a-f and 5a-i) MIC Values (µg/mL) of Different Strains by Two Fold Serial Dilution Technique and Diameter of Zone of Inhibition (mm) of Various Bacterial and Fungal Strains (µg/disc) by Disc-Diffusion Assay

Compounds	*S.aureus*	*P.aeruginosa*	*B.subtilis*	*E.coli*	*K.pneumoniae*	*C.albicans*	*A.niger*	*A.fumigatus*	*P.chrysogenum*
4a	25.0	>100[a] (08)	25.0 (16)	50.0 (12)	>100[a] (08)	>100[a] (10)	>100 [a](08)	>100[a] (07)	>100[a] (08)
4b	**3.12[b] (38)**	>50.0 (14)	>100[a] (09)	>100[a] (10)	>100[a] (10)	**3.12[c] (40)**	50.0 (16)	50.0 (14)	>50.0 (10)
4c	**6.25 (32)**	>50.0 (16)	50.0 (13)	50.0 (14)	>50.0 (12)	**6.25 (27)**	>100 [a](12)	>100[a] (08)	>50.0 (13)
4d	25.0 (14)	>100[a] (09)	100[a] (08)	50.0 (15)	100[a](08)	50.0 (14)	100[a] (08)	>100[a] (10)	100[a] (09)
4e	>50.0 (11)	>100[a] (10)	**6.25 (23)**	>100[a](08)	>50.0 (13)	50.0 (15)	100[a] (12)	25.0 (14)	>50.0 (12)
4f	**12.5 (18)**	>100[a] (12)	>50.0 (10)	>100[a] (08)	>100[a] (08)	>100[a] (09)	50.0 (15)	>100[a](12)	>100[a] (08)

5a	>100[a] (08)	>100[a] (12)	100[a] (11)	50.0 (10)	>100[a] (08)	**6.25 (22)**	**6.25 (24)**	100[a] (08)	>100[a] (08)
5b	>50.0 (14)	25.0 (18)	>100[a] (08)	50.0 (15)	>50.0 (10)	100[a] (09)	>100 [a](10)	>100[a] (08)	>100[a] (08)
5c	100[a] (10)	>50.0 (13)	>100[a] (09)	>50.0 (14)	100[a](0 9)	**6.25 (24)**	>100[a](12)	>100[a] (08)	>100[a] (10)
5d	**3.12[b] (37)**	25.0 (19)	50.0 (10)	**12.5 (21)**	50.0 (11)	**3.12[c] (39)**	25.0 (14)	100[a] (10)	>50.0 (12)
5e	100[a] (08)	100[a] (09)	25.0 (16)	25.0 (19)	50.0 (13)	>50.0 (15)	>100 [a](12)	25.0 (18)	>100[a](08)
5f	100[a](09)	>100[a] (16)	25.0 (17)	>100[a] (08)	50.0 (15)	>100[a] (09)	>100 [a](10)	>100 (08)	>100[a] (10)
5g	50.0 (14)	>100[a] (10)	**6.25 (25)**	100[a] (09)	>100[a] (08)	>100[a] (10)	25.0 (16)	>50.0 (13)	25.0 (14)
5h	50.0 (12)	50.0 (12)	>100[a] (09)	50.0 (13)	>100[a] (09)	50.0 (14)	100[a] (12)	>100[a] (10)	100[a](08)
5i	**6.25 (35)**	>100[a] (08)	25.0 (15)	25.0 (16)	50.0 (11)	**6.25 (33)**	>100 [a](13)	>100[a] (12)	100[a] (09)
Gentamicin	6.25 (22)	12.5 (23)	-(22)	12.5 (20)	25 (-)	-	-	-	-
Ampicillin	6.25 (29)	25 (-)	-	6.25 (19)	25 (-)	-	-	-	-
Fluconazole	-	-	-	-	-	6.25 (21)	6.25 (18)	-	-

a No activity.

b Entries in bold font indicate better activity than reference drugs Gentamicin and Ampicillin (Bauer et al., 1966).

c Entries in bold font indicate better activity than reference drugs Fluconazole.

Entries in ( ) indicate zone of inhibition in mm.

ISSN 2321-807X

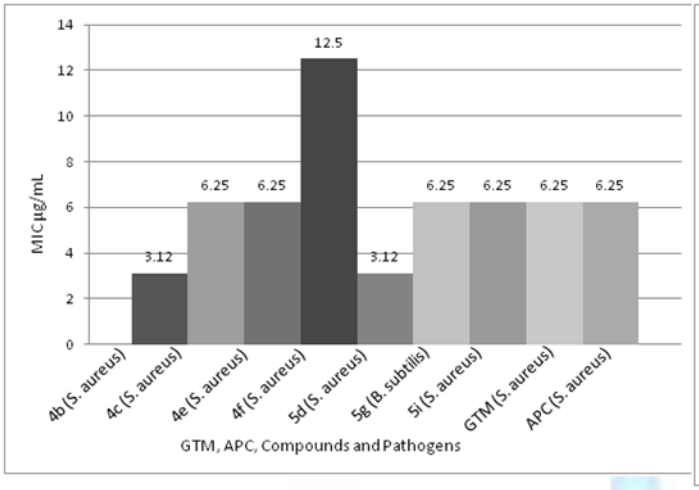

Fig 1: **Comparative antibacterial study plot with Genatmicin, Ampicillin compounds and Pathogens.**

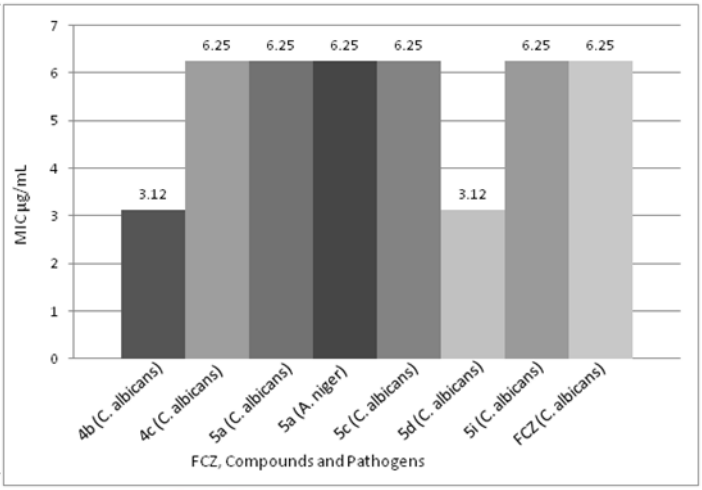

Fig 2: Comparative antifungal study plot Fluconazole compounds and Pathogens.

In vitro antibacterial Assay

The cultures obtained in Muller-Hinton broth for all the bacteria after 24 hr of incubation at 37^{0}C. Testing was carried out on Muller-Hinton broth at pH 7.4 using two fold serial dilution techniques. The final inoculum size was 106 CFU/mL. A set of tubes containing only inoculated broth was kept as control. After incubation for 24h at 37^{0}C, the last tube with no growth of microorganism was recorded to represent MIC expressed in µg/mL. Every experiment in the antibacterial assay was replicated twice in order to define the MIC values. Comparison of antibacterial activity of **4(a-f)** and **5(a-i)** with that of antibacterial drugs, Gentamicin and Ampicillin showed that compounds **4b** and **5d** had better activity while compounds **4c** and **5i** exhibited milder activity and compound **4f** showed poor activity against ***Staphylococcus aureus*** (ATCC-9144). Compound **5d** also exhibited milder activity against ***Escherichia coli*** (MTCC-739). Compound **4b** (MIC 3.12µg/mL) and **5d** (MIC 3.12µg/mL) had shown promising antibacterial profiles on comparison with antibacterial drugs, Gentamicin (MIC 6.25µg/mL) and Ampicillin (MIC 6.25µg/mL), against ***Staphylococcus aureus*** (ATCC-9144) (Table 1) as exhibited in **Fig 1**. Compounds **4(a-f)** and **5(a-i)** were also screened against ***Bacillus subtilis*** (ATCC-6633), ***Pseudomonas aeruginosa*** (ATCC-25615), ***Klebsiella pneumoniae*** (MTCC-2405), and ***Escherichia coli*** (MTCC-739) but did not exhibit significant antibacterial activity except **5d**, which exhibited milder activity against the mentioned strains.

In vitro antifungal Assay

The cultures were obtained in sabouraud dextrose broth after incubation for 24 hr at 35^{0}C. Testing was performed in sabouraud dextrose broth at pH 7.4 using two fold serial dilution techniques. The final inoculum size was 105 CFU/mL. A set of tubes containing only inoculated broth was kept as control. After incubation for 48hr at 35^{0}C, the last tube with no growth of microorganism was recorded to represent MIC expressed in µg/mL. Every experiment in the antifungal assay was replicated twice in order to define the MIC values. Comparison of antifungal activity of compounds **4(a-f)** and **5(a-i)** with that of antifungal drug, Fluconazole, showed that compound **4b** (MIC 3.12µg /mL)and **5d** (MIC 3.12µg/mL) had better antifungal activity against ***Candida albicans*** (ATCC-24433). **4c**, **5c**, **5i** and **5a** exhibited milder antifungal activity against ***Candida albicans*** (ATCC-24433) and ***Aspergillus niger*** (MTCC-1344) respectively. Compound **4b** and **5d** (MIC 3.12µg/mL) had shown promising antifungal profiles against ***Candida albicans*** (ATCC-24433) as exhibited in **Fig 2**.

Compounds **4(a-f)** and **5(a-i)** were also screened against ***Aspergillus niger*** (MTCC-872), ***Aspergillus fumigatus*** (MTCC-343) and ***Penicillium chrysogenum*** (MTCC-2725) but did not exhibit significant antifungal activity except **5a**, which showed some activity.

The compounds tested, exhibited specific antimicrobial activity against different bacterial and fungal strains with MIC values in a range of 3.12-100 µg/mL. Many of these compounds showed significant activity comparable to the standard drugs at the tested concentrations. The attachment of N-benzoxazine group to **1(a-c)** leading to **4(a-c)**, improved the antimicrobial activity, since **4b** and **4c** possess superior activity than other derivatives **4(d-f)** of quinazoline -4-carboxamide. The electronic property of para substituent of 2- phenyl ring of quinazoline seems to have slight effect on the antimicrobial activity. Both electron withdrawing (Cl) and electron donating (CH_3) groups afforded good antimicrobial activity. The result suggests that the volume of the substituents may play an important role for the activity as compounds with N-benzoxazine structural motif have a good activity than the compounds with bulky N-naphthoxazine group. The better activity of **5d** can be explained on the basis, that the presence of two quinazoline pharmacophores in a molecule reinforces its antimicrobial action. Consistent with these results, compounds **4b** and **5d** were found to be most potent among the tested compounds and exhibited better antimicrobial activity than the clinically prevalent antimicrobial drugs

such as Gentamicin, Ampicillin and Fluconazole. Interestingly, all the target compounds were found to be devoid of antimicrobial activity against ***P.aeruginosa***, ***K.pneumoniae***, ***A.fumigatus*** and ***P.chrysogenum***.

In general, compounds **4(b, c& e)**, **5(a, c, d, g &i)**, showed significant to moderate activity, whereas rest of the compounds are inactive against all tested bacterial as well as fungal strains.

Conclusion and Future Directions

A number of new N-(2H-benzo[e][1,3] oxazin-3(4H)-yl)-2-(4-substituted phenyl)quinoline-4-carboxamides or N-(2H-naphtho[2,3-e][1,3]oxazin-3-(4H)-yl)-2-(4-substitutedphenyl) quinoline-4-carboxamides **4(a-f)** and 2,5-bis(2-substituted phenyl quinolin-4-yl)-1,3,4-oxadiazoles or 2-(furan-2-yl)-5-(2-(4-substitutedphenyl)quinolin-4-yl)-1,3,4-oxadiazoles or 2-(5-(2-(4-substituted phenyl) quinolin-4-yl)-1,3,4-oxadiazol-2-yl)methyl)isoindole-1,3-diones **5(a-i)** have been prepared as a novel group of antimicrobials. In general, compounds **4 (b, c & e)**, **5 (a, c, d, g & i)** showed significant to moderate activity. The rest of the compounds showed no sensitivity at their highest tested concentrations against bacterial and fungal strains. Compound **4b** and **5d** were found to be the most active and they may lead to the discovery of potential antimicrobial agents and further work is being carried out at Central Drug Research Institute, Lucknow, India, concerning its toxicological evaluation. Efforts are paving ways to synthesize more potent biologically active derivatives bearing quinoline moiety in their molecular architecture. The mechanism of the antimicrobial activity and further structural modifications of the parent structures are presently under investigation to improve their potency as well as selectivity.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Department of Chemistry, University of Lucknow, Lucknow, for providing necessary Laboratory facilities and to the Director, Central Drug Research Institute (CDRI), Lucknow, India for providing spectral, elemental and biological activity data.

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