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Synthesis and Antimicrobial Evaluation of some novel 2, 3, 7-Trisubstituted Quinazolinones derivatives

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ABSTRACT

Reaction of 4-chloro anthranilic acid 1 with chloroacetyl chloride followed by 4-fluoro aniline provides 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one 3, which on treatment with 2-chloro benzimidazole in the presence of potassium carbonate yielded 7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluorophenyl)-3H-quinazolin-4-one 4. The latter on reaction with nitrogen nucleophiles in acetone containing K_2CO_3 and catalytic amount of KI gave novel 7-Chloro-3-(4-fluoro phenyl)-2-((substituted amino -1H-benz[d]imidazol-1-yl)methyl) quinazolin-4(3H)-one derivatives 5a-h. Furthermore all the compounds were also tested against Gram negative, Gram positive bacteria and fungi. Among the compound tested in this study, compounds 2-(2-pyrrolidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5b) and 2-(2-piperidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5c) found more potent against S. aureus and E. coli compare to reference standard ampicillin.

Keywords: Quinazolin-4(3H)-one, nucleophilic substitution, antibacterial activity, antifungal activity.

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INTRODUCTION

Infectious as well as highly contagious microbial diseases are increasing with course of time round the world due to the emergence of new multidrug resistant bacteria which are resistant to a number of antimicrobial agents due to the development of mutagenicity.[1] One way to battle with this challenge is the conscious usage of the currently marketed antibiotics and the other is to develop and screen new chemical entities for antimicrobial activities.[2] The exploration of new heterocycles that can accommodate potency to multiple biological targets remains an intriguing scientific endeavour. The quinazolinone moiety has been utilized extensively in medicinal chemistry and is considered to be a privileged structure that show various pharmacological activities, such as antiviral,[3] antibacterial,[4-5] antifungal,[6-7] antimalarial,[8] anticancer,[9-11] antihypertensive,[12] diuretic,[13-14] inhibitors of derived growth factor receptor phosphorylation,[15] antioxidant,[16] anticonvulsant, ghrelin receptor antagonists,[17] anti-inflammatory, analgesic and COX-II inhibitors.[18-20] It has been reported that substitution pattern by different aryl or heteroaryl moieties at 2/3 position of quinazoline nucleus markedly influences the anti-inflammatory activity.[21] Thus, due to the diverse range of the pharmacological activities of quinazolinone derivatives, there has been an enormous interest in the synthesis of quinazolinone derivatives.

On the other hand, despite a numerous attempts to develop new structural prototype in the search for more effective antimicrobials, the benzimidazoles still remain as one of the most versatile class of compounds against microbes and, therefore, are useful substructures for further molecular exploration. Recently, the chemistry and biological profiles of various pharmacophores of 1N-substituted and 2-substituted benzimidazoles derivatives have been worked out in detail.[22] The relevance of compounds composed from two or more heterocyclic rings for drug discovery, regardless of the target, can be best documented by the frequency with which bis-heterocyclic compounds were identified as the most potent ones.[23] This evidence boosted us to carry out synthetic work for the titled compound to evaluate their antimicrobial/antibacterial activity. Fluorine substitution may greatly increase a molecule's lipophilicity, an important consideration when making molecules that are designed to be active *in-vivo*. Incorporating fluorines increases fat solubility, improving its partitioning into membranes and hence increasing bioavailability. Fluorination can also aid hydrophobic interactions between the drug and binding sites on receptors or enzymes. Replacing hydrogen and other functional groups with fluorine can have a dramatic effect on biological activity.[24] Based on the above observations, it was of interest to synthesize a novel series of quinazolinone

derivatives with structure modifications involving incorporation of the fluorinated aromatic moiety at 3rd position and benzimidazole moiety at 2nd position of quinazolinone as a trial to obtain safer and potent anti-microbial agents.

MATERIAL AND METHODS

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB 104 with KBr pellets. The ^{1}H-NMR (400 MHz) spectra were recorded on a Bruker 400 NMR spectrometer (with TMS as internal references). Mass spectra were recorded on Shimadzu GC MS QP 5000. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF 254, 200 mesh) aluminum plates (E Merck).

Procedure for the synthesis of 4-chloro-2-[(chloroacetyl) amino] benzoic acid (2)

2-Amino-4-chloro benzoic acid (10.0 g, 1 mmol) was dissolved in dichloromethane. An equimolar amount of triethylamine (TEA) was added, and the reaction mass was cooled to 0 °C. Chloroacetyl chloride (5.14 ml, 1.1 mmol) was added over a period of 15 minutes, while maintaining the temperature at 0° C. The reaction mass was then stirred at room temperature for 4 h. A white solid precipitated, which was filtered and washed with water. Recrystallized from a mixture of ethyl acetate and hexane.

Procedure for the synthesis of 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one (3)

4-Chloro-2-[(chloroacetyl) amino] benzoic acid (10.0 g, 1 mmol) and an equimolar amount of p-fluoro aniline (3.89 ml, 1 mmol) were dissolved in tetrahydrofuran and stirred at room temperature for 30 minutes. Phosphorus trichloride (5.23 ml, 1.5 mmol) was then added with continued stirring. The temperature was raised, and the reaction mixture was stirred at 60-65 ^{o}C for 2 h. The reaction mixture was then cooled to room temperature, and the solvent was evaporated under vacuum. To the residue was added water, and the mixture was neutralized with sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed under vacuum, and the residue was slurred with hexane to produce 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one as a white solid.

Procedure for the synthesis of 7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluoro phenyl)-3H-quinazolin-4-one (4)

A mixture of 2-chloro benzimidazole (1 mmol), 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl)

quinazolin-4(3H)-one (1.02 mmol) and potassium carbonate (2 mmol) was stirred in dimethyl formamide (30 ml) at 25-30 ^{o}C for 1-2 h. The progress of reaction was monitored by thin layer chromatography. After the completion of reaction, reaction mass was poured in to ice cooled water, filtered and washed with water. The solid was dried to give 4 as an off white solid.

General experimental procedure for the preparation of 2-[(substituted amino-1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one (5a-h)

7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluorophenyl)-3H-quinazolin-4-one (1 mmol), amine (1 mmol), potassium carbonate (2 mmol) & potassium iodide (0.05 mmol) was heated to reflux in acetone for 4-5 h. The progress of reaction was monitored by thin layer chromatography. After the completion of reaction, reaction mass was poured in to ice cooled water, filtered and washed with water and then crude product was crystallized from ethanol-water.

Protocol for *In-vitro* antimicrobial evaluation

All the newly synthesized compounds 5a-h were screened for antibacterial and antifungal activity (MIC) *in-vitro* by broth dilution method[25] with two Gram positive bacteria (S. Aureus MTCC 96 and S. pyogenus MTCC 442), two Gram-negative bacteria (E. Coli MTCC 443 and P. aeruginosa MTCC 1688) and fungi A. niger MTCC 282. The solution of compounds at 250 µg/ml, 200 µg/ml, 125 µg/ml and 62.5 µg/ml concentrations, were compared with standard drug ampicillin, chloramphenicol, nystatin and griseofulvin. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

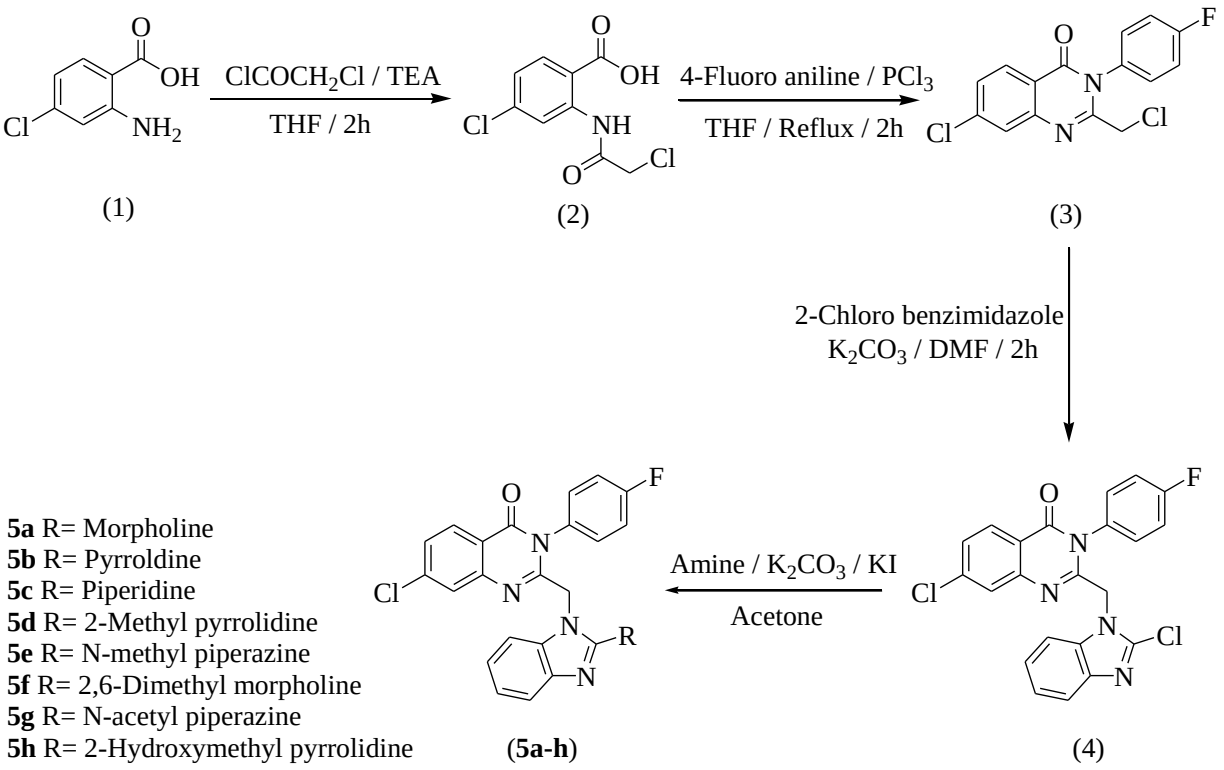
All the glass apparatus used were sterilized before use. The MICs of all the synthesized compounds was carried out by broth microdilution method.[25] Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to 10^{8} CFU [Colony Forming Unit] per milliliter by comparing the turbidity. Dimethyl sulfoxide (DMSO) was used as diluent to get desired concentration of drugs to test on standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{o}C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible

growth (in the same manner as control tube described above) was subcultured and incubated overnight at 37 ^{o}C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted to 2000 µg/ml concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200, 125, 100 and 62.5 µg/ml concentrations. The highest dilution showing at least 99% inhibition is taken as MIC. The activities of all the synthesized compounds are recorded in Table 2.

RESULTS AND DISCUSSION

The desired 7-chloro-3-(4-fluoro phenyl)-2-[(substituted amino -1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one **(5a-h)** were obtained by reacting 7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluorophenyl)-3H-quinazolin-4-one **4** with various amines (Scheme 1). Many synthetic methods have been reported for the synthesis of 4(3H)-quinazolinone. The simplest and most straightforward procedure was developed by Niementowski[26] in 1895 and improved by Grimmel et al.[27] in 1946 who reported that 4(3H)-quinazolinone could be synthesized from N-acetylanthranilic acids and anilines in toluene or xylene using phosphorous trichloride or phosphorous oxychloride as condensing agent.[28] Since his report, the method has frequently been used for the synthesis of diverse range of quinazolinones.[29]

The synthesis of 2-chloromethyl-3-aryl-3H-quinazolin-4-one **3** was carried out using a Grimmel's method, beginning with the chloro-acetylation of 4-chloro anthranilic acid to yield the corresponding 2-(2-chloro-acetyl amino) benzoic acid **2**, which was then treated with 4-fluoro aniline in tetrahydrofuran and trichlorophosphate (PCl_3) to generate cyclized product **3**.[30-31] Treatment of this 2-chloromethyl-3-aryl-3H-quinazolin-4-one **3** with 2-chloro benzimidazole in the presence of potassium carbonate provides 7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluorophenyl)-3H-quinazolin-4-one **4** which upon nucleophilic substitution with an amines provides 7-Chloro-3-(4-fluoro phenyl)-2-[(substituted amino -1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one **5a-h** (Scheme 1).



(1) (2) (3) (4) (**5a-h**)

Scheme 1. Synthesis of 7-chloro-3-(4-fluoro phenyl)-2-[(substituted amino -1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one (5a-h).

The synthesis of 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one 3 was carried out using a Grimmel's method. The reaction of 4-chloro 2-(2-chloro acetylamino) benzoic acid **2** and 4-fluoro aniline with trichlorophosphate (PCl_3) in toluene yielded cyclized product **3** in good yield.

Xue, S. et al.[32] have modified Grimmel's method for the synthesis of 4-quinzolinones by varying solvents (MeCN, MDC, THF) and amount of PCl_3 (2 equivalent) under mild reaction conditions (50 °C). In their paper, they suggested that lower solubility of intermediates in toluene might be contributed to the low yield (15%), when toluene was used as a solvent. When we applied the same protocol to N-acetyl anthranilic acid **2** and 4-fluoro aniline, 70% & 55% of quinazolinone product **3** was obtained in tetrahydrofuran and toluene respectively. This observation support the hypothesis suggested by Xue et al.[32] Furthermore, when N-acetyl anthranilic acid **2** and 4-fluoro aniline were reacted in acetonitrile at 60 ^{o}C for 2 h, 52% cyclized product **3** was obtained. When the same reaction was heated to 60 ^{o}C for 4 h, desired product **3** was isolated in 45% yield. Therefore, the reaction conditions were optimized by changing solvents, amount of PCl_3 and reaction time (Table 1). The best results were obtained when THF and 1.5 equivalent of PCl_3 were employed at 60 ^{o}C for 2 h (Table 1, Entry 9).

Table 1: Optimization of reaction conditions for the synthesis of 7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one 3.

Sr	Solvent	Mol. eq. of PCl_3	Reaction time(hour)	Reaction Temp.	Yield[a](%)
1	Toluene	1	2	60 °C	42
2	Toluene	1.5	2	60 °C	55
3	Toluene	1.5	4	60 °C	53
5	Acetonitrile	1	2	60 °C	37
6	Acetonitrile	1.5	2	60 °C	52
7	Acetonitrile	1.5	4	60 °C	45
8	THF	1	2	60 °C	58
9	THF	1.5	2	60 °C	**70**
10	THF	1.5	4	60 °C	65

Yield[a] refers to products obtained after purification.

The structures of all the compounds 5a-h were confirmed by various spectroscopic techniques, including IR, ^{1}H NMR and mass spectroscopy. The band at 1575-1600 cm^{-1} showed the confirmation of C=N bond of quinazolinone ring. Other characteristic band of all the compounds 5a-h appearing at 760-790 cm^{-1} is due to the C-Cl stretching of chloro group. The ^{1}H-NMR (400 MHz) spectra were recorded on a Bruker 400 NMR spectrometer (with TMS as internal references). Singlet at δ = 4.90-5.20 ppm, which can be attributed to the methylene group attached to benzimidazole nitrogen at 1-position and quinazolinone ring at 2-position. The aromatic protons were observed from 6.80 to 8.20 ppm in the ^{1}H NMR spectra.

Compounds 5a-h, apart from IR and ^{1}H-NMR, were well recognized by the help of mass spectroscopy. These mentioned compounds contained halogen in their structure and were confirmed with the help of characteristic isotopic peaks, in addition to molecular ion peak (MI). Compound 5a, 7-chloro-3-(4-fluorophenyl)-2-((morpholino-1H-benz[d]imidazol-1-yl)methyl) quinazolin-4(3H)-one, showed intense MI peak (due to the presence of ^{35}Cl) at 490 and isotopic peak (less intense as compared to MI) at m/z 492 (due to the presence of ^{37}Cl isotope). Thin layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion.

4-chloro-2-[(chloroacetyl) amino] benzoic acid (2)

Off white solid, Yield 75 %, 10.84 g, mp 193-195 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.25 (s, 2H, -CH2), 7.1 (d, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 8.52 (s, 1H, Ar-H), 11.52 (s, 1H, -NH), 13.45 (broad s, 1H, -COOH). MS m/z: 248 (M+1), IR (KBr, cm^{-1}): 1675 (C=O), Calculated: C (43.58 %), H (2.84 %), N (5.65 %), Found: C (43.54 %), H (2.81 %), N (5.62 %).

7-chloro-2-(chloromethyl)-3-(4-fluorophenyl) quinazolin-4(3H)-one (3)

White solid, Yield 70 %, 9.11 g, mp 146-148 ^{o}C, 1H NMR (400 MHz, DMSO-d6): δ 3.96 (s, 2H, -CH2), 6.87-7.02 (m, 3H, Ar-H), 7.49 (dd, 1H, Ar-H), 7.58 (d, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 8.27 (d, 1H, Ar-H). MS m/z: 323 (M+1), Elemental Analysis: Calculated: C (55.75 %), H (2.81 %), N (8.67 %), Found: C (55.72 %), H (2.80 %), N (8.64 %).

7-chloro-2-(2-chloro-benzoimidazol-1-ylmethyl)-3-(4-fluorophenyl)-3H-quinazolin-4-one(4)

Off white solid, Yield: 78 %, mp 152-154 ^{o}C, MS m/z 439 (M+1), Calculated: C (60.15 %), H (2.98 %), N (12.75 %), Found: C (60.11 %), H (2.93 %), N (12.72 %).

7-Chloro-3-(4-fluorophenyl)-2-(2-morpholin-4-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5a)

Yellow powder, Yield 54 %, mp 164-166 ^{o}C, MS m/z: 490 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 3.15 (t, 4H, -$(CH_2)_2$ morpholine), 3.68 (t, 4H, -$(CH_2)_2$ morpholine), 4.97 (s, 2H, -CH_2), 6.88 (m, 3H, Ar-H), 7.00 (m, 3H, Ar-H), 7.16 (t, 1H, Ar-H), 7.49 (dd, 1H, Ar-H), 7.58 (d, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 8.19 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 47.15, 50.24, 65.72, 109.90, 116.50, 117.17, 119.82, 120.75, 121.33, 126.28, 127.47, 128.42, 130.12, 135.13, 139.37, 148, 155, 158.5, 161.5; IR (KBr, cm^{-1}): 2839, 2943 (CH-aliphatic), 1639 (CO), 763 (C-Cl) and 1597 (C=N), Calculated: C (63.74 %), H (4.32 %), N (14.29 %), Found: C (63.71 %), H (4.29 %), N (14.25 %).

7-Chloro-3-(4-fluoro phenyl)-2-(2-pyrrolidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5b)

Light yellow powder, Yield 62 %, mp 123-125 ^{o}C, MS m/z: 474 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 1.82 (t, 4H, -$(CH_2)_2$ pyrrolidine), 3.12 (t, 4H, -$(CH_2)_2$ pyrrolidine), 4.95 (s, 2H, -CH_2), 6.93 (m, 3H, Ar-H), 7.14 (m, 3H, Ar-H), 7.27 (t, 1H, Ar-H), 7.53 (dd, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 8.18 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 25.72, 47.11, 49.59, 109.81, 116.21, 117.37, 119.87, 120.71, 121.33, 126.23, 127.43, 128.45, 130.17, 135.05, 139.32, 148.08, 155.11, 158.43, 161.67; IR (KBr, cm^{-1}): 2818, 2947 (CH-aliphatic), 1624 (CO), 769 (C-Cl) and 1587 (C=N), Calculated: C (65.89 %), H (4.47 %), N (14.78 %), Found: C (65.86 %), H (4.45 %), N (14.74 %).

7-Chloro-3-(4-fluoro phenyl)-2-(2-piperidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5c)

Yellow powder, Yield 45 %, mp 138-140 ^{o}C, MS m/z: 488 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 1.64-1.82 (m, 6H, -$(CH_2)_3$ piperidine), 2.87 (m, 4H, -$(CH_2)_2$ piperidine), 5.08 (s, 2H, -CH_2), 6.79-6.94 (m, 6H, Ar-H), 7.21 (t, 1H, Ar-H), 7.42 (dd, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 8.12 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 24.32, 25.46, 47.12, 49.53, 109.65,

116.25, 117.19, 119.26, 120.93, 121.27, 126.14, 127.25, 128.22, 130.14, 135.10, 139.24, 148.12, 155.21, 158.48, 162.05; IR (KBr, cm^{-1}): 2816, 2961 (CH-aliphatic), 1647 (CO), 782 (C-Cl) and 1583 (C=N), Calculated: C (66.46 %), H (4.75 %), N (14.35 %), Found: C (66.43 %), H (4.72 %), N (14.34 %).

7-Chloro-3-(4-fluoro phenyl)-2-(2-methyl pyrrolidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5d)

Light yellow powder, Yield 48 %, mp 114-116 ^{o}C, MS m/z: 488 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 1.32 (m, 3H, $-CH_3$), 1.79 (t, 4H, $-(CH_2)_2$ pyrrolidine), 3.18 (t, 2H, $-(CH_2)$ pyrrolidine), 5.08 (s, 2H, $-CH_2$), 6.82-6.89 (m, 6H, Ar-H), 7.19 (t, 1H, Ar-H), 7.41 (dd, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 8.20 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 18.12, 19.42, 27.76, 47.11, 49.52, 50.24, 109.84, 116.27, 117.32, 119.84, 120.69, 121.32, 126.24, 127.48, 128.51, 130.22, 135.15, 139.46, 148.11, 155.12, 158.46, 162.02; IR (KBr, cm^{-1}): 2812, 2956 (CH-aliphatic), 1658 (CO), 764 (C-Cl) and 1578 (C=N), Calculated: C (66.46 %), H (4.74 %), N (14.35 %), Found: C (66.43 %), H (4.71 %), N (14.33 %).

7-Chloro-3-(4-fluoro phenyl)-2-[2-(4-methyl piperazin-1-yl)-benzimidazol-1-ylmethyl]-3H-quinazolin-4-one (5e)

Brown powder, Yield 55 %, mp 172-174 ^{o}C, MS m/z: 503 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 2.37 (s, 3H, $-CH_3$), 2.89 (t, 4H, $-(CH_2)_2$ piperazine), 3.38 (t, 4H, $-(CH_2)_2$ piperazine), 4.93 (s, 2H, $-CH_2$), 6.89 (m, 3H, Ar-H), 7.02-7.12 (m, 4H, Ar-H), 7.45 (dd, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 8.21 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 45.2, 47.19, 53.2, 54.5, 109.82, 116.54, 117.12, 119.68, 120.71, 121.21, 126.23, 127.48, 128.46, 130.14, 135.12, 139.37, 148.08, 155.23, 158.39, 162.07; IR (KBr, cm^{-1}): 2856, 2932 (CH-aliphatic), 1656 (CO), 783 (C-Cl) and 1571 (C=N), Calculated: C (64.67 %), H (4.81%), N (16.71 %), Found: C (64.64 %), H (4.80 %), N (16.68 %).

7-Chloro-2-[2-(2,6-dimethylmorpholine-4-yl)-benzimidazol-1-ylmethyl]-3-(4-fluoro phenyl)-3H-quinazolin-4-one (5f)

Yellow powder, Yield 47 %, mp 132-134 ^{o}C, MS m/z: 518 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 1.23 (d, 6H, $-(CH_3)_2$), 2.93 (d, 4H, $-(CH_2)_2$ morpholine), 3.97 (m, 2H, $-(CH)_2$ morpholine), 4.93 (s, 2H, $-CH_2$), 6.84-7.13 (m, 7H, Ar-H), 7.48 (dd, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 8.19 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 18.6, 47.15, 50.24, 71.0, 109.82, 116.35, 117.26, 119.81, 120.67, 121.29, 126.29, 127.43, 128.41, 130.16, 135.11, 139.35, 148.11, 155.17, 158.52, 161.90; IR (KBr, cm^{-1}): 2825, 2947 (CH-aliphatic), 1632 (CO), 767 (C-Cl) and 1593 (C=N), Calculated: C (64.92 %), H (4.86 %), N (13.52 %), Found: C (64.89 %), H (4.84

%), N (13.48 %).

2-[2-(4-Acetyl piperazin-1-yl)-benzimidazol-1-ylmethyl]-7-Chloro-3-(4-fluoro phenyl)-3H-quinazolin-4-one (5g)

Yellow powder, Yield 52 %, mp 178-180 ^{o}C, MS m/z: 531 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 2.21 (s, 3H, -CH_3), 3.38 (t, 4H, -$(CH_2)_2$ piperazine), 3.48 (t, 4H, -$(CH_2)_2$ piperazine), 4.98 (s, 2H, -CH_2), 6.86 (m, 3H, Ar-H), 7.08-7.22 (m, 4H, Ar-H), 7.46 (dd, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 8.21 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 21.23, 47.14, 52.1, 53.48, 109.82, 116.54, 117.12, 119.68, 120.71, 121.21, 126.23, 127.48, 128.46, 130.14, 135.12, 139.37, 148.08, 155.23, 158.39, 162.07, 169.87; IR (KBr, cm^{-1}): 2820, 2958 (CH-aliphatic), 1661 (CO), 783 (C-Cl) and 1584 (C=N), Calculated: C (63.34 %), H (4.56 %), N (15.83 %), Found: C (63.30 %), H (4.53 %), N (15.81 %).

7-Chloro-3-(4-fluoro phenyl)-2-[2-(2-hydroxymethyl pyrrolidin-1-yl)-benzimidazol-1-ylmethyl]-3H-quinazolin-4-one (5h)

Yellow powder, Yield 64 %, mp 112-114 ^{o}C, MS m/z: 504 (M+1), ^{1}H NMR ($CDCl_3$, δ ppm): 1.58-1.62 (m, 4H, -$(CH_2)_2$ pyrrolidine), 2.87-2.91 (m, 3H, pyrrolidine), 3.64 (d, 2H, -CH_2), 4.98 (s, 2H, -CH_2), 6.91 (m, 3H, Ar-H), 7.08-7.23 (m, 4H, Ar-H), 7.47 (dd, 1H, Ar-H), 7.62 (d, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 8.20 (d, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO- d6) δ 18.12, 19.42, 49.52, 63.41, 61.58, 47.11, 109.84, 116.27, 117.32, 119.84, 120.69, 121.32, 126.24, 127.48, 128.51, 130.22, 135.15, 139.46, 148.11, 155.12, 158.46, 162.02; IR (KBr, cm^{-1}): 2856, 2908 (CH-aliphatic), 1645 (CO), 769 (C-Cl) and 1591 (C=N), Calculated: C (64.35 %), H (4.60 %), N (13.90 %), Found: C (64.32 %), H (4.58 %), N (13.87 %).

Antimicrobial evaluation

In the series 5a-h, compound 2-(2-piperidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5c) having piperidine substituent was found more potent against E. coli. with respect to ampicillin. Compound 2-[2-(2-hydroxymethyl pyrrolidin-1-yl)-benzimidazol-1-ylmethyl]-3H-quinazolin-4-one (5h) having 2-hydroxymethyl pyrrolidine substituent found equipotent antibacterial activity, while all other compounds in this series elicited relatively lesser activity against E. coli. with respect to ampicillin. Compound 5b found to be less active against P. auruginosa with respect to chloramphenicol. Compound 5d and 5h having substituent at 2-position of pyrrolidine ring found to be more active than 5b; however, chloramphenicol was still 2-fold more potent than 5d and 5h. These finding suggested that 2-methyl pyrrolidine and 2-hydroxymethyl pyrrolidine in 2-[(substituted amino-1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one were better substituent. Compound 5c having piperidine substituent was

found to possess comparative inhibitory activity against P. auruginosa with respect to chloramphenicol. Compound 5c having six membered piperidine substituent possessed equipotent inhibitory activity against S. aereus with respect to ampicillin, however, replacement of six membered piperidine substituent (compound 5c) with five membered pyrrolidine substituent (compound 5b) increases the inhibitory activity by more than two fold. After introducing methyl and hydroxymethyl group in the pyrrolidine ring in compound 5b, resulting compounds 5d and 5h found to possess same inhibitory activity as parent compound 5b against S. aereus. Compounds 5a, 5e, 5f and 5g having six membered ring substituent found to possess more inhibitory activity against S. aereus with respect to ampicillin, however, less potent than compounds having five membered ring substituent. Compounds 5b, 5c and 5e found to possess comparative inhibitory activity against S. pyogenes with respect to ampicillin. After introducing methyl group & hydroxymethyl group in pyrrolidine ring in compound 5b, resulting compounds 5d and 5h showed 2-fold less activity than parent compound 5b. Similarly, Replacement of methyl group in 5e with acetyl group, resulting compound 5g showed 2-fold less activity than parent compound 5e.

Table 2: Antibacterial and antifungal activity of the newly synthesized compounds.

Comp. No.	Minimal bactericidal concentration (µg/ml)				Minimal fungicidal concentration (µg/ml)
	Gram-negative		Gram-positive		
	E. coli	P. aeruginosa	S. aureus	S. pyogenes	A. niger
5a	250	250	200	200	>1000
5b	250	250	100	125	500
5c	62.5	100	250	125	250
5d	200	100	100	200	1000
5e	250	250	200	125	1000
5f	200	250	200	250	>1000
5g	200	200	200	200	500
5h	125	125	100	200	1000
Ampicillin	100	--	250	100	--
Chloramphenicol	50	50	50	50	--
Nystatin	--	--	--	--	100
Griseofulvin	--	--	--	--	100

For fungi, compounds 5c having unsubstituted pyrrolidine ring exhibited lesser antifungal activity against A. niger with respect to nystatin and griseofulvin but still found two fold more active than compounds 5d and 5h having substituted pyrrolidine ring. Compound 5c having pyrrolidine substituent found to be almost two fold less active against A. niger with respect to nystatin and griseofulvin, however, found most active in this series. The observed data on the anti-microbial activity of the synthesized compounds and standard drugs are given in Table 2.

CONCLUSION

We report a simple, efficient, economic and environment-friendly method for the synthesis of novel 2-[(substituted amino-1H-benz[d]imidazol-1-yl)methyl] quinazolin-4(3H)-one derivatives. The design and synthesis of the titled compounds were carried out with the objective of molecular hybridization of quinazolin-4(3H)-one nucleus and 2-amino substituted benzimidazole (Fig. 2). The objective was based upon the hypothesis that the both quinazolin-4(3H)-one nucleus and 2-amino substituted benzimidazole reported to possess promising antimicrobial activity. Compound 7-chloro-3-(4-fluoro phenyl)-2-(2-piperidin-1-yl-benzimidazol-1-ylmethyl)-3H-quinazolin-4-one (5c) having 2-substituted piperidine ring in the benzimidazole ring exhibited excellent activity against E. coli. with respect to ampicillin. The anti-microbial activity of the synthesized compounds may be due the presence of the versatile pharmacophores and fluorine which might increase the lipophilic character of the molecule, which facilitate the crossing through the biological membrane of the micro-organism and thereby inhibit their growth.

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