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RESEARCH ARTICLE

Synthesis and antileishmanial and antimicrobial activities of some 2,3-disubstituted 3*H*-quinazolin-4-ones

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Abstract

A series of 2,3-disubstituted 3*H*-quinazolin-4-ones was synthesized. Antimicrobial activities of the synthesized compounds were investigated against Gram (+ve) and Gram (–ve) bacteria, including *B. subtilis*, *S. aureus*, *S. flexneri*, *P. aeruginosa*, and *S. typhi*, and six fungi, namely *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glabrata* using the broth microdilution method. Compounds **9**, **11**, and **12** showed significant activities against the selected bacterial cultures, while **7–10**, **12**, **15**, and **16** showed good to moderate antifungal activities. Compound **11** exhibited strongest leishmanicidal activity against *Leishmania major* (MHOM/PK/88/DESTO) promastigotes, while other compounds showed weak to moderate leishmanicidal activities.

Keywords: *2,3-disubstituted quinazolin-4(3H)-ones; synthesis; antileishmanial activities; antimicrobial activities*

Introduction

The discovery of new antimicrobial and antileishmanial compounds from diversified sources such as microorganisms, animals, and plants and through synthetic means is an active area of scientific research around the world. The increasing prevalence of multi-drug resistance (MDR) pathogens with reduced susceptibility to the existing arsenal of antibiotics has increased the spectrum of non-treatable ailments, and has added urgency to the new drug discovery efforts[1]. Leishmaniasis is a tropical disease caused by protozoa of the *Leishmania* genus. These protozoa cause a disease with different clinical forms, namely, cutaneous, hyperergic, mucocutaneous, and anergic diffuse leishmaniasis[2]. Pentavalent antimonial (Sb[V]) compounds are the common drugs in practice for the treatment of leishmaniasis, but they cause renal and cardiac toxicity. This disease is also treated with various diamidines and pentamidine, formulated as a salt of pentamidine isethionate, which again has its serious side effects[3]. Alternative pharmaceutical formulations have been used in order to reduce the toxicity of these drugs[4]. The lack of an effective antileishmanial drug has necessitated the search for more effective, new chemotherapeutic agents with better activity and least toxicity. In this pursuit, we have synthesized some 2,3-disubstituted 3*H*-quinazolin-4-ones and have determined their leishmanicidal and antimicrobial activities. Quinazolin-4(3*H*)-ones are a large group of heterocyclic compounds possessing diverse and interesting biological activities[5,6].

3-Arylquinazolin-4(3*H*)-one in particular has been extensively utilized as a core structure in the field of medicinal chemistry[7–9]. The quinazolin-4(3*H*)-one nucleus is a basic unit found in various naturally occurring bioactive alkaloids such as febrifugine, isofebrifugine, vascinone, and others[10]. The chemistry of this group of alkaloids has found significant importance in medicinal chemistry and has been comprehensively reviewed[11–14]. However, antileishmanial activities of this group of alkaloids have not been explored. We therefore, in continuation of our synthetic work on quinazolin-4(3*H*)-one based bioactive compounds[15,16], report herein the

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synthesis and antimicrobial and antileishmanial activities of some 2,3-disubstituted quinazolin-4(3*H*)-ones.

Experimental

Chemistry

All the reagents and solvents were obtained from commercial suppliers and were used after preliminary purification wherever that was needed according to their standard methods of purification. Melting points were determined in open capillaries using Gallenkamp melting point apparatus and are uncorrected. All the reactions were monitored using aluminum-supported precoated thin layer chromatography (TLC; Merck 60 F$_{254}$) silica plates, and detection on TLC was accomplished with the help of an ultraviolet (UV) lamp (254 nm). [1]H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz or 300 MHz instrument in CDCl$_3$, CD$_3$OD, or dimethylsulfoxide (DMSO)-d$_6$ as solvent. Mass spectra were recorded on a Jeol MAT 312 instrument, while infrared (IR) spectra were recorded on Bio-Rad FTS-Win IR apparatus, using a KBr disk.

2-Phenyl-4H-3,1-benzoxazine-4-one (2)

To a well-stirred solution of anthranilic acid (2 g, 6 mmol), dissolved in dry pyridine (20 mL), was added, drop-wise, benzoyl chloride (3.5 mL) with continuous stirring for 30 min at room temperature. After completion of reaction, the mixture was poured into saturated NaHCO$_3$ solution. The product precipitated out was filtered, washed with water, and recrystallized from dilute ethanol. Yield: 95%; mp = 108–110°C; IR (KBr) ν$_{max}$ cm^{-1}: 2922 (C-H), 1759 (C=O), 1602 (C=N), 1557 (C=C), 1470 (C-H, bend), 1309 (C-H, bend), 1224 (C-O), 1172 (C-C); EI-MS *m/z* (%): 223 (M$^+$, 100), 146 (15), 120 (13), 105 (100), 77 (83), 51 (18); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.26 (d, 1H, *J* = 8), 7.82 (m, 4H), 7.56 (m, 4H).

General method for the preparation of compounds 3–6

Substituted benzoic acids in a slight excess of freshly distilled thionyl chloride (10 mL) catalyzed with 2–3 drops of dimethyl formamide (DMF) were boiled under reflux for 2–3 h. The mixture was allowed to cool below room temperature, and was then added drop-wise to a well-stirred solution of anthranilic acid in dry pyridine (15 mL). The stirring was continued for 30 min or till precipitate formation was complete. The mixture was then added to saturated NaHCO$_3$ solution and shaken well. The precipitated solid was filtered, washed with aqueous NaHCO$_3$ solution followed by distilled water, dried, and recrystallized from a suitable solvent to yield the corresponding 2-aryl-4*H*-3,1-benzoxazin-4-one (**3–6**) in 80–96% yield.

2-(4-Nitrophenyl)-4H-3,1-benzoxazin-4-one
(3) 4-Nitrobenzene (1.2 g, 0.007 mol), thionyl chloride (10 mL), anthranilic acid (0.5 g, 0.0036 mol), and pyridine (15 mL). Yield: 89%; mp = 202–204°C; IR (KBr) ν$_{max}$ cm^{-1}: 1709 (C=O, str.), 1600 (C=N, str.), 1471 (C=C-C, str.), 1344

(C-O, str.), 1233 (C-C, str.), 747, 690, 669 (C-H, bend, arom.); EI-MS *m/z* (%): 268 (M$^+$, 28), 253 (44), 236 (7), 235 (15), 207 (48), 179 (19), 178 (27), 167 (9), 105 (91), 104 (17), 102 (32), 92 (10), 77 (39), 76 (100), 63 (17), 51 (20); ^{1}H-NMR (400 MHz, CDCl$_3$), δ: 8.34 (d, 2H, *J* = 9, Ar-H's), 8.02 (d, 1H, *J* = 9, Ar-H's), 7.98 (m, 3H, Ar-H's), 7.81(t, 1H, *J* = 8.7), 7.39 (t, 1H, *J* = 8.5).

2-(4-Methylphenyl)-4H-3,1-benzoxazin-4-one
(4) 4-Methyl benzoic acid (3.9 g, 0.029 mol), thionyl chloride (10 mL), and anthranilic acid (2 g, 0.0155 mol). Yield: 92%; mp = 144°C; IR (KBr) ν$_{max}$ cm^{-1}: 3262 (C-H), 1725 (C=O), 1516 (C=N), 1464 (C=C-C), 1264 (C-O), 1198 (C-C): EI-MS *m/z* (%): 237 (M$^+$, 92), 146 (21), 120 (18), 119 (100), 91 (38), 76 (10), 65 (10); ^{1}H-NMR (300 MHz, CDCl$_3$), δ: 8.21 (d, 1H, *J* = 8.6), 8.11 (d, 1H, *J* = 8.7), 7.97 (d, 2H, *J* = 8), 7.82 (t, 1H, *J* = 8.4), 7.44 (t, 1H, *J* = 8.0), 7.18 (d, 2H, *J* = 8.4), 2.36 (s, 3H, CH$_3$).

2-(4-Chlorophenyl)-4H-3,1-benzoxazin-4-one
(5) 4-Chlorobenzoic acid (0.029 mol), thionyl chloride (10 mL), and anthranilic acid (2 g, 0.0155 mol). Yield: 89%; mp = 158–160°C; IR (KBr) ν$_{max}$ cm^{-1}: 3039 (C-H), 1716 (C=O), 1557 (C=N), 1470 (C=C), 1224 (C-O), 1173 (C-C), 765 (C-Cl); EI-MS *m/z* (%): 259 (M^{+2}, 3), 257 (M$^+$, 8), 243 (10), 241 (61), 215 (2), 213 (5), 146 (24), 139 (5), 113 (4), 111 (13), 76 (91), 50 (5); ^{1}H-NMR (300 MHz, CDCl$_3$), δ: 8.23 (d, 1H, *J* = 9), 8.15 (d, 1H, *J* = 9), 7.94 (d, 2H, *J* = 8), 7.8 (t, 1H, *J* = 8.5), 7.51–7.42 (m, 3H).

2-(4-Bromophenyl)-4H-3,   1-benzoxazin-4-one
(6) 4-Bromobenzoic acid (6 g, 0.029 mol), thionyl chloride (10 mL), and anthranilic acid (2 g, 0.0155 mol). Yield: 87%; mp = 184°C; IR (KBr) ν$_{max}$ cm^{-1}: 3021 (C-H), 1726 (C=O), 1602 (C=N), 1546 (C=C), 1415 (C-H), 1309 (C-O), 1172 (C-C); EI-MS *m/z* (%): 303 (M^{+2}, 48), 301 (M$^+$, 56), 287 (4), 285 (40), 259 (12), 257 (16), 182 (4), 181 (11), 180 (6), 168 (9), 166 (13), 146 (81), 120 (20), 118 (22), 105 (92), 92 (11), 76 (100), 63 (25), 57 (29), 51 (42); ^{1}H-NMR (300 MHz, CDCl$_3$), δ: 8.23 (d, 1H, *J* = 8.9), 8.12 (d, 1H, *J* = 8.7), 7.93 (dd, 2H, *J*$_{1,2}$ = 8.7, *J*$_{3,4}$ = 9.5), 7.81 (t, 1H, *J* = 9.2), 7.66 (m, 2H), 7.45 (d, 1H, *J* = 8.3).

General procedure for the synthesis of 2,3-disubstituted quinazolin-4(3H)-ones (7–17)

An appropriate 2-aryl-4*H*-3,1-benzoxazin-4-one (**2–6**) and primary aromatic/aliphatic amine were refluxed in a suitable high-boiling solvent for 3–7 h. After completion of the reaction, as monitored by TLC, excess of the solvent was evaporated under reduced pressure and the reaction mixture was then poured into ice-cold water. The precipitated product was filtered out and recrystallized from the appropriate solvent.

3-(2-Hydroxyethyl)-2-(4-nitrophenyl)   quinazolin-4(3H)-one (7) 2-(4-Nitrophenyl)-4*H*-3,1-benzoxazin-4-one (2 g, 0.0074 mol), monoethanolamine (2 mL, 0.0476 mol), and dry benzene (10 mL). Reflux time (3 h). Yield: 87%; mp = 228°C; IR (KBr) ν$_{max}$ cm^{-1}: 3450 (O-H), 2924 (C-H), 1676 (C=O), 1640 (C=N), 1582 (C=C), 1347 (C-N, C-O), 1256 (C-C), 853, 768, 698 (arom. =C-H, bend); EI-MS *m/z* (%): 311 (M$^+$, 23), 295 (53), 280 (35), 266 (100), 254 (7), 226 (29), 146 (12), 122 (6),

120 (44), 119 (13), 105 (5), 104 (41), 15 (15), 76 (19), 75 (7), 64 (5), 50 (4); ^{1}H-NMR (400 MHz, CDCl$_3$): δ: 8.68 (d, 1H, J = 8.28), 8.305 (t, 2H, J = 8.8), 8.1325 (t, 2H, 8.6), 7.632–7.495 (m, 3H), 3.724 (t, 2H, J = 5), 3.53 (t, 2H, J = 5.2), 3.31(b, s, 1H, OH).

3-Amino-2-(4-chlorophenyl)   quinazolin-4(3H)-one **(8)** 2-(4-Chlorophenyl)-4H-3,1-benzoxazin-4-one **(5)** (2 g, 0.0077 mol), hydrazine hydrate 80% (5 mL), and dry benzene (15 mL). Reflux time (2h). Yield: 90%; mp = 189–190°C; IR (KBr) ν$_{max}$: 3212 (N-H), 1769 (C=O), 1660 (C=N, C=C), 1245 (C-O, C-N), 1172 (C-C), 768 (C-Cl); EI-MS m/z (%): 273 (M^{+2}, 50), 272 (53), 271 (M$^+$, 100), 270 (86), 257 (4), 255 (13), 243 (100), 242 (61), 214 (4), 213 (5), 202 (24), 178 (73), 90 (3), 77 (5), 76 (8), 50 (4); ^{1}H-NMR (300 MHz, CDCl$_3$), δ: 8.30 (d, 1H, J = 6), 7.79–7.77 (m, 4H), 7.57–7.44 (m, 3H), 5.05 (b, s, 2H).

3-Amino-2-phenylquinazolin-4(3H)-one **(9)** 2-Phenyl-4H-3,1-benzoxazine-4-one (**2**) **(**2 g, 0.0089 mol), hydrazine hydrate 80% (5 mL), and dry benzene (15 mL). Reflux time (6 h). Yield: 86%, mp = 236–238°C; IR (KBr) ν$_{max}$ cm^{-1}: 3207 (N-H), 1768 (C=O), 1602 (C=N), 1484 (C=C), 1245 (C-O, C-C); EI-MS m/z (%): 237 (100), 236 (34), 222 (26), 221 (12), 208 (59), 180 (18), 179 (7), 152 (7), 119 (37), 105 (10), 103 (8), 77 (28), 63 (5), 50 (14); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.35 (d, 1H, J = 8), 7.82 (m, 4H), 7.56 (m, 4H), 4.43 (s, 2H).

3-(4-Iodophenyl)-2-phenylquinazolin-4(3H)-one **(10)** 2-Phenyl-4(H)-3,1-benzoxazine-4-one (**2**) **(**1 g, 0.00448 mol**)**, 4-iodoaniline (1 g, 0.00448 mol), and dry benzene (10 mL). Reflux time (2 h) Yield: 88%; mp = 233–235°C; IR (KBr) ν$_{max}$ cm^{-1}: 1664 (C=O), 1603 (C=N), 1534 (C=C, conjugated), 1257 (C-C), 565 (C-I); EI-MS m/z (%): 424 (12), 348 (3), 297 (3), 222 (96), 209 (10), 181 (5), 146 (11), 120 (11), 105 (100), 92 (14), 77 (24), 65 (5), 51 (7); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.4 (d, 1H, J = 7.8), 7.98 (d, 2H, J = 15), 7.85–7.81 (t, 1H, J = 8), 7.6–7.54 (m, 4H), 7.5–7.45 (m, 3H), 7.3–7.28 (d, 2H, J = 11.5).

3-Benzyl-2-phenylquinazolin-4(3H)-one **(11)** 2-Phenyl-4H-3,1-benzoxazine-4-one (**2**) (1 g, 0.00448 mol), freshly distilled benzyl amine (0.015 mL, 0.00896 mol), and dry benzene (10 mL). Yield: 89%; mp = 155–157°C; IR (KBr) ν$_{max}$ cm^{-1}: 2929 (C-H), 1668 (C=O), 1519 (C=C, conjugated), 1305 (C=C-C); FAB-MS (–ve): 311; EI-MS m/z (%): 312 (M$^+$), 252 (4), 223 (49), 195 (10), 139 (10), 120 (60), 105 (100), 92 (32); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.82 (d, 1H, J = 8), 8.042 (d, 2H, J = 8), 7.53–7.47 (m, 5H), 7.35–7.31 (m, 5H), 7.09 (t, 1H, J = 8), 4.63 (s, 2H, CH$_2$).

3-Mesityl-2-phenylquinazolin-4(3H)-one **(12)** 2-Phenyl-4H-3,1-benzoxazine-4-one **2** (1 g, 0.00448 mol), 2,4,6-tri-methylaniline, and dry benzene (10 mL). Reflux time (8h). Recrystallized from ethanol. Yield: 73%; mp = 210–213°C; IR (KBr) ν$_{max}$ cm^{-1}: 2934 (C-H), 1641 (C=O), 1496 (C=C), 1255 (C-C); EI-MS m/z (%): 340 (M$^+$, 12), 254 (10), 222 (60), 146 (5), 135 (100), 120 (18), 105 (58), 92 (5), 76 (20), 65 (4); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.7 (d, 1H, J = 8), 8.02 (d, 2H, J = 8), 7.95 (t, 1H, J = 8), 7.63–7.42 (m, 5H), 6.96 (s, 2H), 2.3 (s, 3H), 2.2 (s, 6H).

3-Amino-2-(4-methylphenyl)   quinazolin-4(3H)-one **(13)** 2-(4-Methylphenyl)-4H-3,1-benzoxazin-4-one **4** (2 g), hydrazine hydrate 80% (5 mL), and dry benzene (15 mL). Reflux time (8 h). Yield: 85%; mp = 165°C; IR (KBr) ν$_{max}$ cm^{-1}: 3308 (N-H), 1665 (C=O), 1530 (C=N), 1251 (C-O, C-N), 769, 695, 660 (arom, C-H, bend); EI-MS m/z (%): 251 (M$^+$, 4), 138 (100), 222 (2), 200 (5), 146 (19), 120 (96), 91 (38), 82 (10), 65 (18), ^{1}H-NMR (300 MHz, CDCl$_3$), δ: 8.35 (d, 1H, J = 8), 7.93 (d, 2H, J = 8), 7.64 (m, 3H), 7.45 (d, 1H, J = 8), 7.15 (q, 1H, J = 8), 4.08 (s, 2H), 2.348 (s, 3H, CH$_3$).

*2-(4-Nitrophenyl)-3-(1,3-thiazol-2-yl)quinazolin-4(3H)-one***(14)** 2-(4-Nitrophenyl)-4H-3,1-benzoxazine-4-one (0.2 g, 0.000813 mol), 2-aminothiazole (0.08 g), and dry pyridine (10 mL). Reflux time (5 h). Yield: 97%; mp = 231°C; IR (KBr) ν$_{max}$ cm^{-1}: 1645 (C=O), 1575 (C=N), 1520 (C=C), 1301 (C-H, bend), 1245 (C-O), 1234 (C-C), 834, 756, 681 (C-H, arom., bend): EI-MS m/z (%): 350 (M$^+$, 67), 319 (23), 294 (15), 281 (12), 267 (93), 253 (18), 229 (11), 145 (83), 132 (11), 120 (71), 105 (100); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.28 (d, 1H, J = 8.5), 8.127 (d, 2H, J = 8.5), 8.063 (d, 1H, J = 7.9), 7.632 (d, 2H, J = 7.8), 7.492 (t, 1H, J = 7.3), 7.13 (t, 1H, J = 7.7), 7.01 (d, 1H, J = 4), 6.70 (d, 1H, J = 3.5).

3-(2-Hydroxyethyl)-2-phenylquinazolin-4(3H)-one **(15)** 2-Phenyl-4H-3,1-benzoxazine-4-one (**2**), (2 g, 0.008968 mol), monoethanolamine (2 mL, 0.032 mol), and dry benzene (10 mL). Reflux time (6h). Recrystallized from ethanol. Yield: 85%; mp = 164–166°C; IR (KBr) ν$_{max}$: 3344 (O-H), 2280 (C-H), 1664 (C=O), 1496 (C=C), 1427 (C=C, conjugated), 1255 (C-C); EI-MS m/z (%): 266 (M$^+$, 25), 254 (11), 249 (7), 235 (10), 224 (40), 223 (43), 222 (14), 190 (6), 189 (39), 180 (4), 179 (22), 167 (3), 149 (6), 146 (28), 120 (16), 111 (7), 106 (12), 105 (100), 97 (11), 85 (11), 83 (10), 77 (81), 71 (16), 57 (25), 55 (13), 51 (14), 44 (100), 43 (15), 40 (5); ^{1}H-NMR (300 MHz, CD$_3$OD): δ: 8.622 (d, 1H, J = 8.3), 7.997 (d, 2H, J = 8.2), 7.795 (d, 1H, J = 7), 7.6–7.51 (m, 4H), 7.22–7.17 (t, 1H, J = 8.1), 3.74–3.70 (t, 2H, J = 5.7), 3.55–3.51 (t, 2H, J = 5.7), 3.29 (s, 1H).

3-(4-Bromophenyl)-2-phenylquinazolin-4(3H)-one **(16)** 2-Phenyl-4(H)-3,1-benzoxazine-4-one **(**1 g, 0.00448 mol**)**, 4-bromoaniline (0.78 g, 0.00448 mol), and dry pyridine (10 mL). Reflux time (6 h). Recrystallized from ethanol. Yield: 41%; mp = 228–230°C; IR (KBr) ν$_{max}$ cm^{-1}: 3066 (=C-H), 1603 (C=O), 1492 (C=C, conjugated), 1258 (C-C), 757 (C-Br); EI-MS m/z (%): 379 (M^{+2}, 14), 377 (M$^+$, 10), 301 (81), 221 (100), 208 (47), 180 (3), 157 (27), 105 (100), 92 (6), 77 (34), 65 (37), 57 (3); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.40 (d, 2H, J = 8.2), 8.01 (d, 1H, J = 13), 7.93–7.79 (m, 2H), 7.65 (t, 1H, J = 8), 7.58 (t, 1H, J = 8.7), 7.49–7.40 (m, 6H).

3-(4-Methylphenyl)-2-phenylquinazolin-4(3H)-one **(17)** 2-Phenyl-4(H)-3,1-benzoxazine-4-one **2 (**1 g, 4.48 mmol**)**, 4-methylaniline (0.015 mL, 8.96 mmol), and dry benzene (10 mL). Reflux time (30 min). Yield: 73%; mp =

186–188°C; IR (KBr) ν_{max} cm^{-1}: 2982 (C-H), 1664 (C=O), 1512 (C=C), 1448 (C=C-C), 1232 (C-C); EI-MS m/z (%): 312 (M$^+$, 3), 222 (18), 210 (4), 179 (8), 145 (18), 120 (36), 105 (100), 92 (19), 77 (73), 51 (13); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.70 (d, 1H, J = 8), 8.02 (d, 2H, J = 8), 7.57–7.45 (m, 6H), 7.21–7.02 (m, 4H), 2.34 (s, 3H, CH$_3$).

2-Methyl-3-(4-methylphenyl) quinazolin-4(3H)-one (18)
A mixture of methyl *N*-acetyl anthranilate (2 g, 0.0103 mol) and 4-methylaniline was boiled under reflux for 10 h in absolute ethanol (10 mL). After completion of reaction, monitored by TLC, ethanol was evaporated under reduced pressure and the mixture was allowed to cool by adding ice-cooled water. The precipitate formed upon cooling was filtered, dried, and recrystallized from ethanol. Yield: 67%; mp = 197–199°C; IR (KBr) ν_{max} cm^{-1}: 2935 (C-H), 1664 (C=O), 1534 (C=N), 1449 (C=C, conjugated), 1204 (C-C); EI-MS m/z (%): 250 (M$^+$, 5), 235 (34), 222 (19), 210 (2), 179 (10), 145 (18), 105 (64), 105 (100), 92 (19), 77 (67), 65 (13), 51 (12); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.27 (d, 1H, J = 9), 8.02 (t, 1H, J = 8), 7.69–7.59 (m, 2H), 7.21 (d, 2H, J = 9), 7.024 (d, 2H, J = 8.7), 2.53 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$).

3-(2-Hydroxyethyl)-2-methylquinazolin-4(3H)-one (19)
Anthranilic acid (0.14 g, 1 mmol), triethylorthoacetate (0.23 mL, 1.2 mmol), monoethanolamine (0.3 mL, 1.2 mmol), and *para*-toluenesulfonic acid (1 g, 5.0 mmol) were stirred at room temperature under an argon atmosphere. After completion of the reaction as indicated by TLC, the reaction contents were poured into ice-cold water. The solid precipitated was filtered out, washed with water, and recrystallized from ethanol. A second crop of the product was obtained by extracting the filtrate twice with dichloromethane, and subsequently the solvent was removed under reduced pressure. Yield: 89%; m.p 134°C IR (KBr) ν_{max} cm^{-1}: 3250 (O-H), 1664 (C=O), 1534 (C=N, C=C), 1449 (C=C, conjugated), 1304 (C-O), 1255 (C-C), 757, 700, 664 (arom. =C-H, bend); EI-MS m/z (%): 204 (M$^+$, 28), 185 (9), 173 (13), 161 (59), 169 (100), 159 (7), 146 (5), 145 (10), 143 (13), 119 (7), 118 (7), 116 (9), 92 (2), 90 (5), 77 (13), 76 (8), 50 (3); ^{1}H-NMR (300 MHz, CDCl$_3$): δ: 8.190 (d, 1H, J = 8), 7.38–7.682 (t, 1H, J = 7.9), 7.631 (d, 1H, J = 7.3), 7.45–7.39 (t, 1H, J = 7.7), 4.316–4.282 (t, 2H, J = 5.07), 4.02–3.99 (t, 2H, J = 5.3), 3.22 (b, s, 1H), 2.72 (s, 3H).

Antimicrobial activity
The synthesized compounds were assayed for their antimicrobial (antibacterial and antifungal) potential by the hole-diffusion method[17,18] followed by determination of their corresponding minimum inhibitory concentration (MIC) values against tested fungal and bacterial strains. The MICs of standard drugs and active test compounds were determined against test isolates by the broth microdilution technique.

Bactericidal bioassay (in vitro)
The synthesized compounds were screened for antibacterial activity against *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (clinical isolate), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhi* (ATCC 19430) by using literature protocols[19,20]. In the agar well diffusion method, wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm part. Then, 2–8 h old bacterial inocula containing approximately 104–106 colony forming units (CFU)/mL were spread on the surface of nutrient agar with the help of a sterile cotton swab. The cotton swab was rotated firmly against the upper inside well of the tube to express excess fluid. The entire agar surface of the plate was streaked with the swab three times, turning the plate 60° between each streaking. The recommended concentration of test sample (2 mg/mL of DMSO) was then added in the respective well. Other wells supplemented with DMSO and reference antibacterial drugs served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 14–19 h. Potency was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth inhibition and MIC values were calculated with reference to the positive control.

**Leishmanicidal bioassay (in vitro)**
Leishmania major (MHOM/PK/88/DESTO) promastigotes, cultivated in bulk, were aseptically sedimented down at 3000 rpm, counted with the help of an improved incubator chamber under the microscope, and diluted with fresh medium to a final concentration of 2.0×10^6 parasites mL^{-1}.

The compounds were dissolved in appropriate solvents and then diluted to a final concentration of 1.0 mg/mL of PBS (phosphate buffered saline; pH 7.4, containing 0.5% MeOH, 0.5% DMSO). The plates were then incubated at 21–22°C in the dark for 3–5 days, during which the control organism multiplied 3–6 times. Cultures were examined microscopically on an improved Neubauer chamber, and IC$_{50}$ values of the compounds exerting antileishmanial activity were calculated[21,22].

Results and discussion

Chemistry
2-Aryl-4*H*-3,1-benzoxazine-4-ones have been reported for their significant biological activities[23,24] as well as for being very good synthetic precursors in the synthesis of 2,3-disbstituted quianzolin-4(3H)-ones. The synthesis of 2-aryl-4*H*-3,1-benzoxazine-4-ones (**2–6**) was therefore accomplished using our recently developed three-component one-pot synthesis[15] starting from the easily available substituted benzoic acids (Scheme 1). The synthesized 2-aryl-4*H*-3,1-benzoxazine-4-ones were then refluxed with selected primary amines in appropriate solvents to accomplish the synthesis of 2,3-alkyl/aryl-quianzolin-4(3H)-ones (**7–19**). The synthesis of compounds **18** and **19** was accomplished by refluxing methyl *N*-acetyl anthranilate with toluene and using a solvent-free method recently developed by Narasimhulu *et al*.[25] (Scheme 1).
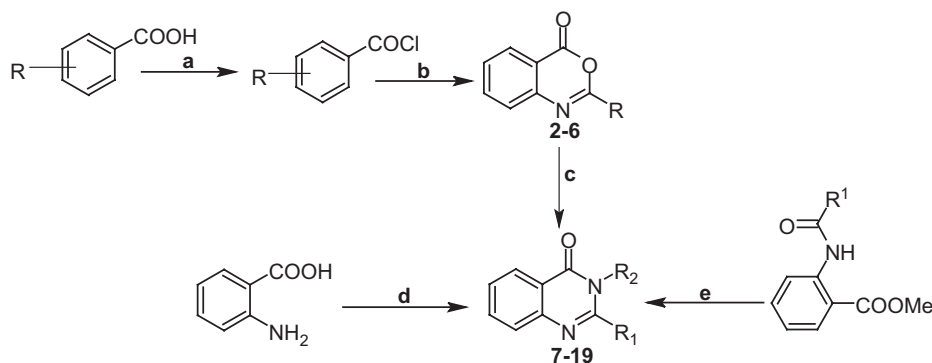
Scheme 1. General schematic synthesis of 2,3-disubstituted 3*H*-quinazolin-4-ones. Reagents and conditions: (a) thionyl chloride, DMF, reflux; (b) anthranilic acid, pyridine (dry), stir; (c) primary amine, pyridine/benzene, reflux 2–3 h; (d) triethylorthoacetate, *para*-toluenesulfonic acid (PTSA), primary amine, stir, r.t.; (e) primary amine, ethanol, reflux 2 h.
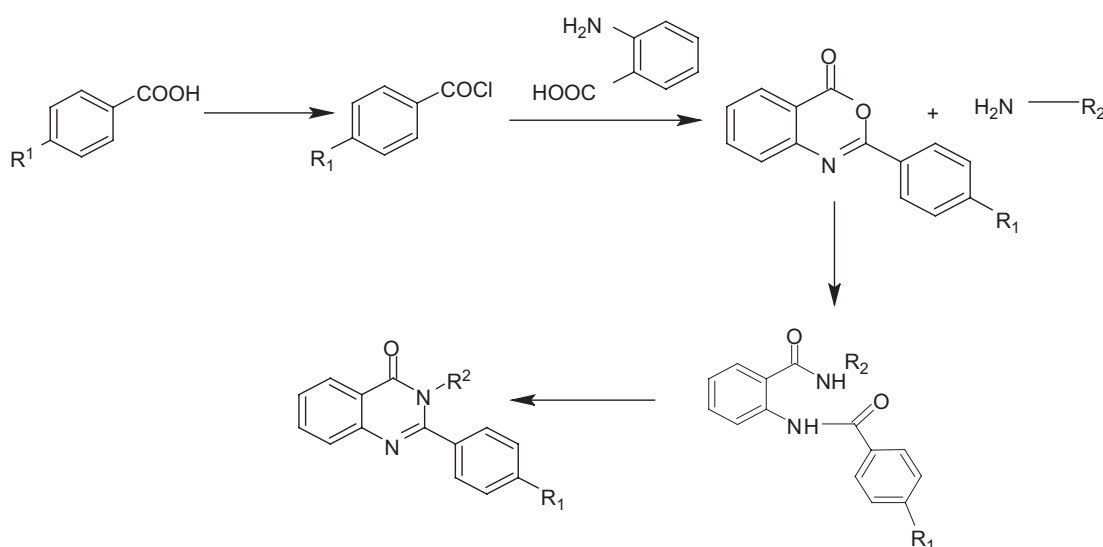
Scheme 2. Mechanism of formation of 2,3-disubstituted 3*H*-quinazolin-4-ones.

The mechanism of formation of 2,3-alkyl/aryl-quianzolin-4(3*H*)-ones (**7–19**) from 2-aryl-4*H*-3, 1-benzoxazine-4-ones (**2–6**) is believed to involve the initial formation of diamide intermediates, which upon subsequent cyclization and dehydration give the target 2,3-alkyl/aryl-quianzolin-4(3*H*)-ones (Scheme 2, Table 1).

Structures of the synthesized compounds were established on the basis of spectroscopic data. IR spectra of compounds **7**, **15**, and **19** showed absorption bands ranging 3250–3450 cm^{−1}, indicating the presence of the OH group in the compounds. Absorption bands showing the presence of the N-H group, ranging 3207–3308 cm[1], were found in the IR spectra of compounds **8**, **9**, and **13**. Molecular masses of all the compounds were confirmed from electron ionization-mass spectrometry (EI-MS); also, the presence of isotopic distributions of chloro and bromo substitutents were confirmed from the M[+2] peaks in the EI-MS spectra of the compounds. In the ^{1}H-NMR spectra, characteristic absorption bands were observed ranging from 6.7 to 9 ppm, with coupling constants (J = 7–9 Hz) for the aromatic protons. The

appearance of singlets at 3.31, 3.22, and 3.299 in the spectra of compounds **7**, **15**, and **19**, respectively, depicted O-H, while singlets at 5.05, 4.43, and 4.08 confirmed the presence of NH_2 in the spectra of compounds **8**, **9**, and **13**.

Antibacterial activity

The synthesized compounds were tested for their *in vitro* antibacterial activity against both Gram (+) and Gram (–) bacteria cultures including *B. subtilis*, *S. aureus*, *S. flexneri*, *P. aeruginosa*, and *S. typhi* using the agar well diffusion protocol[19,20]. Imipenem was used as the standard antibiotic; in a typical experimental procedure, 24-h old culture was spread over Müller–Hinton agar (MHA) plates. Plates were incubated for 24 h at 37°C. Zones of inhibition were measured and compared with the standard drug. Most of the compounds were found to be inactive against the tested bacteria; among the series tested, compounds **9**, **11**, **12**, and **19** showed significant activities against the tested bacterial culture (Table 2). Compound **9** was found to be more active against *Salmonella typhi* (ATCC 19430), MIC
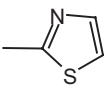
0.29 µg/mL, than the standard imipenem having MIC = 0.7 µg/mL (Table 3). The MIC values of active compound **12** against *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *S. typhi* (ATCC 19430) were found to be 0.15 µg/mL, 0.15 µg/mL, 0.13 µg/mL, and 0.11 µg/mL, respectively. Therefore, compound **12** was found to be more active against the selected strains than the standard

drug. Compound **19** was found to be more potent against *S. aureus* (ATCC 25923), MIC = 0.9 µg/mL. In conclusion, compounds **9**, **11**, **12**, and **19** were found to be more potent, and can therefore be used as a lead source for the development of better antibacterial agents.

Antifungal activity

Antifungal activities were determined using the agar tube dilution protocol[26] against some human pathogens: *Trichophyton longifusus* (clinical isolate), *Candida albicans* (ATCC 2091), *Aspergillus flavus* (ATCC 32611), *Mycosporum canis* (ATCC 11622), *Fusarium solani* (ATCC 11712), and *Candida glabrata* (ATCC 90030). Compounds **9** and **15** were found to be inactive against all fungi, while the rest of the tested compounds showed weak to moderate activities against some fungi (Table 4). The MIC values of the active compounds **7**, **8**, **10**, **12**, and **16** are given in Table 5. The determined MIC value of compound **8** was 5.8 µg/mL against *C. albicans* (ATCC 2091), while the MIC value of the standard miconazole against *Candida albicans* (ATCC 2091) was 4.8 µg/mL. Compound **8** can therefore be used as a good starting point for the development of better antifungal agents against *Candida albicans* (ATCC 2091).

Table 1. Synthesized 2,3-disubstituted quianzolin-4(3*H*)-ones **7–19** (see Scheme 2).

Compound	R_1	R_2
7	4-NO$_2$-C$_6$H$_4$	CH$_2$CH$_2$OH
8	4-Cl-C$_6$H$_4$	NH$_2$
9	C$_6$H$_5$	NH$_2$
10	C$_6$H$_5$	4-I-C$_6$H$_4$
11	C$_6$H$_5$	CH$_2$C$_6$H$_5$
12	C$_6$H$_5$	2,3,4-Trimethylphenyl
13	4-CH$_3$-C$_6$H$_4$	NH$_2$
14	4-NO$_2$-C$_6$H$_4$	(2-methyl-1,3-thiazol-2-yl)
15	C$_6$H$_5$	CH$_2$CH$_2$OH
16	C$_6$H$_5$	4-Br-C$_6$H$_4$
17	C$_6$H$_5$	4-CH$_3$-C$_6$H$_4$
18	CH$_3$	4-CH$_3$-C$_6$H$_4$
19	CH$_3$	CH$_2$CH$_2$OH

Table 2. Antibacterial activities (mm) at 1 mg/mL.

Compound	*B. subtilis*	*S. aureus*	*E. coli*	*S. flexneri*	*P. aeruginosa*	*S. tyhphi*
7	—	—	—	—	—	—
8	—	—	—	—	—	—
9	12	16	15	—	—	23
10	—	—	—	—	—	—
11	—	13	—	—	—	—
12	40	40	44	—	—	46
13	—	—	—	—	—	—
14	—	—	—	—	—	—
15	—	—	—	—	—	—
16	—	—	—	—	—	—
17	—	—	—	—	—	—
18	—	—	—	—	—	—
19	16	20	16	13	—	16
SD	26	17	25	24	17	21

Note. SD (imipenem) = 100 µg/mL; —, inactive.

Table 3. Antibacterial activities (MIC) of active compounds **9**, **11**, **12**, and **13**.

Compound	i		ii		iii		iv		v		vi	
	a	b	a	b	a	b	a	b	a	b	a	b
9	12	3.7	16	3.0	15	3.0	—	—	—	—	23	0.29
11	—	—	13	3.7	—	—	—	—	—	—	—	—
12	40	0.15	40	0.15	44	0.13	—	—	—	—	46	0.11
19	16	3.0	20	0.9	16	3.0	13	—	—	—	16	3.0
Imi	26	0.28	17	2.9	25	0.26	24	0.34	17	2.9	21	0.7

Note. i, *Bacillus subtilus* (ATCC 6633); ii; *Staphylococcus aureus* (ATCC 25923); iii, *Escherichia coli* (ATCC 25922); iv, *Shigella flexneri* (clinical isolate); v, *Pseudomonas aeruginosa* (ATCC 27853); vi, *Salmonella typhi* (ATCC 19430); a, zone of inhibition (mm); b, MIC (µg/mL); Imi, imipenem; —, not determined/inactive.

Table 4. Antifungal activities (% inhibition) at 200 μg/mL.

Compound	*T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
7	20	00	00	50	50	00
8	50	18	00	20	30	00
9	00	00	00	00	00	00
10	40	00	00	50	00	00
12	40	00	00	50	00	00
15	00	00	00	00	00	00
16	50	00	00	20	00	00
SD[1,2]	70[1]	110[1]	20[2]	98.4[1]	73[1]	110[1]

Note. SD[1], miconazole; SD[2], amphotericin B.

Table 5. Antifungal activities (MIC) of compounds **7**, **8**, **10**, **12**, and **18**.

	i		ii		iii		iv		v		vi	
Compound	a	b	a	b	a	b	a	b	a	b	a	b
7	20	5.7	00	—	—	—	50	3.1	50	3.0	—	—
8	50	3.0	18	5.8	—	—	20	5.7	30	5.0	—	—
10	40	3.4	—	—	—	—	50	3.0	—	—	—	—
12	40	3.4	—	—	—	—	50	3.0	—	—	—	—
16	50	3.0	—	—	—	—	20	5.6	—	—	—	—
Mic	70	1.4	29	4.8	ND	ND	98	0.4	73	1.6	99	0.4
Amp	ND	ND	ND	ND	73	1.6	ND	ND	ND	ND	ND	ND

Note. i, *Trichophyton longifusus* (clinical isolate); ii, *Candida albicans* (ATCC 2091); iii, *Aspergillus flavus* (ATCC 32611); iv, *Mycosporum canis* (ATCC 11622); v, *Fusarium solani* (ATCC 11712); vi, *Candida glabrata* (ATCC 90030); a, % inhibition; b, MIC (μg/mL); Mic, miconazole; Amp, amphotericin B; ND, not done.

Table 6. Leishmanicidal activities (IC$_{50}$ values) of compounds **7–19**.

Compound	IC$_{50}$ (μg/mL)
7	71.00
8	96.20 (±0.78)
9	—
10	—
11	48.19 (±0.57)
12	98.021 (±0.71)
13	—
14	—
15	71.95
16	—
17	—
18	—
19	95.6 (±1.0)
Amphotericin B	0.50

Leishmanicidal activity

Leishmanicidal activity was determined at 100 μg/mL levels. Amphotericin B was used as the standard drug, with IC$_{50}$ = 0.50 μg/mL. Among the tested compounds, **7**, **8**, and **15** showed moderate activity while most others either showed weak activity or were inactive (Table 6). The compound 3-benzyl-2-phenylquinazolin-4(3*H*)-one (**11**) was found to be more potent (IC$_{50}$ 48 μg/mL), and is therefore anticipated to be a better new leishmanicidal candidate. The structure–activity relationship of the substituents is important. The chloro susbtituent on the phenyl group at position 2 enhances the activity; similarly, the alkyl group at the C-3 position increases the activity, as observed in compounds **8** and **7**. A profound effect in compound **11** was observed due to the presence of the benzylic moiety at the C-3 position. In conclusion, we report here some new potent antileishmanial compounds and further work in this area is in progress.

Declaration of interest

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