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Original Article

Quinazolino-thiadiazoles as antimicrobial agents

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ABSTRACT

In the present research, we report the synthesis and *in vitro* antimicrobial activity of a new series of novel quinazolino-thiadiazoles as fused pharmacophore (3–20). In general, the results of the *in vitro* antibacterial activity are encouraging, as out of 18 compounds tested, Compounds 3 and 8 with a 4-chlorophenyl and 4-nitro phenyl at C-2 of thiadiazole and chloromethyl substituent at C-2 of quinazolinone displayed a broad spectrum antimicrobial activity against all the strains, while compounds 13 and 16 again with a 4-chlorophenyl and 4-nitrophenyl at C-2 of thiadiazole and ethyl substituent at C-2 of quinazolinone showed the same potency but with a narrower spectrum (Bacterial and Fungal strains) with MIC values of 62.5 µg/ml. The structures of the compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass analysis. It is worth to mention that the combination of two biologically active moieties quinazoline and thiadiazole profoundly influences the biological activity.

1. Introduction

Quinazolines are a class of fused heterocycles that are of considerable interest because of their diverse pharmacological profile [1]. Quinazolines attracted the scientist since 1888, with the discovery of the first natural representative of them –(+)-preganine (vasicine) [2]. Quinazolinones and quinazolines are very interesting molecules and their pharmacological activities are well documented. It has been reported as antihypertensive [3] antimicrobial [4–8], antiviral [9,10], anti-HIV [11] anticonvulsant [12,13], anti-inflammatory [14] and anticancer [15–18] activity, *etc.* The rapid rise in bacterial resistance to the traditional antibiotics has encouraged a continuing search for new classes of compounds with novel modes of antimicrobial activity. To overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents [19].

Quinazolinones show a wide spectrum of chemotherapeutic activity and a considerable amount of work has been done on the synthesis of new potent antibacterial and antifungal quinazolinones. Albaconazole (UR-9825) chemically known as 7-chloro-3-[(2R,3R)-3-(2,4-difluoro phenyl)-3-hydroxy-4-(1,2,4-triazol-1-yl)butan-2-yl]quinazolin-4-one, is a triazole antifungal. It has potential broad-spectrum antibacterial activity [2].

Among the heterocyclic systems, thiadiazole template represents one of the privileged structure fragments in the modern medicinal chemistry. 1,3,4-thiadiazoles are associated with diverse biocidal activities probably by the virtue of a toxophoric –N=C-S- grouping. Abdel-Wahab et al. reported the synthesis of new 1,3,4-thiadiazole derivatives of 5-(benzofuran-2-yl)-1-phenylpyrazole moiety [7]. All the synthesized compounds were screened against bacterial strains and found to possess significant activity against *E. coli* and *C. albicans* (Fig. 1). Kadi *et al* synthesized of a new series of 5-(1-adamantyl)-1,3,4-thiadiazole derivatives and evaluated them for their antimicrobial activity which revealed that all the synthesized compounds exhibited better activity than reference drugs (gentamicin and ampicillin) on *E. coli* and *Pseudomonas aeuroginosa*. SAR studies have shown that introduction of a benzyl- or 4-substituted benzyl and adamantly moiety on C-5 of thiadiazole nucleus enhances the antibacterial and antifungal activity respectively, as shown in Fig. 1 [8].

Based on the above facts and in continuation to our research for new antimicrobial agents [20–24], in the present study we planned to synthesize novel compounds that are hybrids of the 2 biologically active ring systems: quinazoline and 1,3,4 thiadiazole. This combination aims to get antimicrobial agents with better activity than those agents containing only one ring of them.

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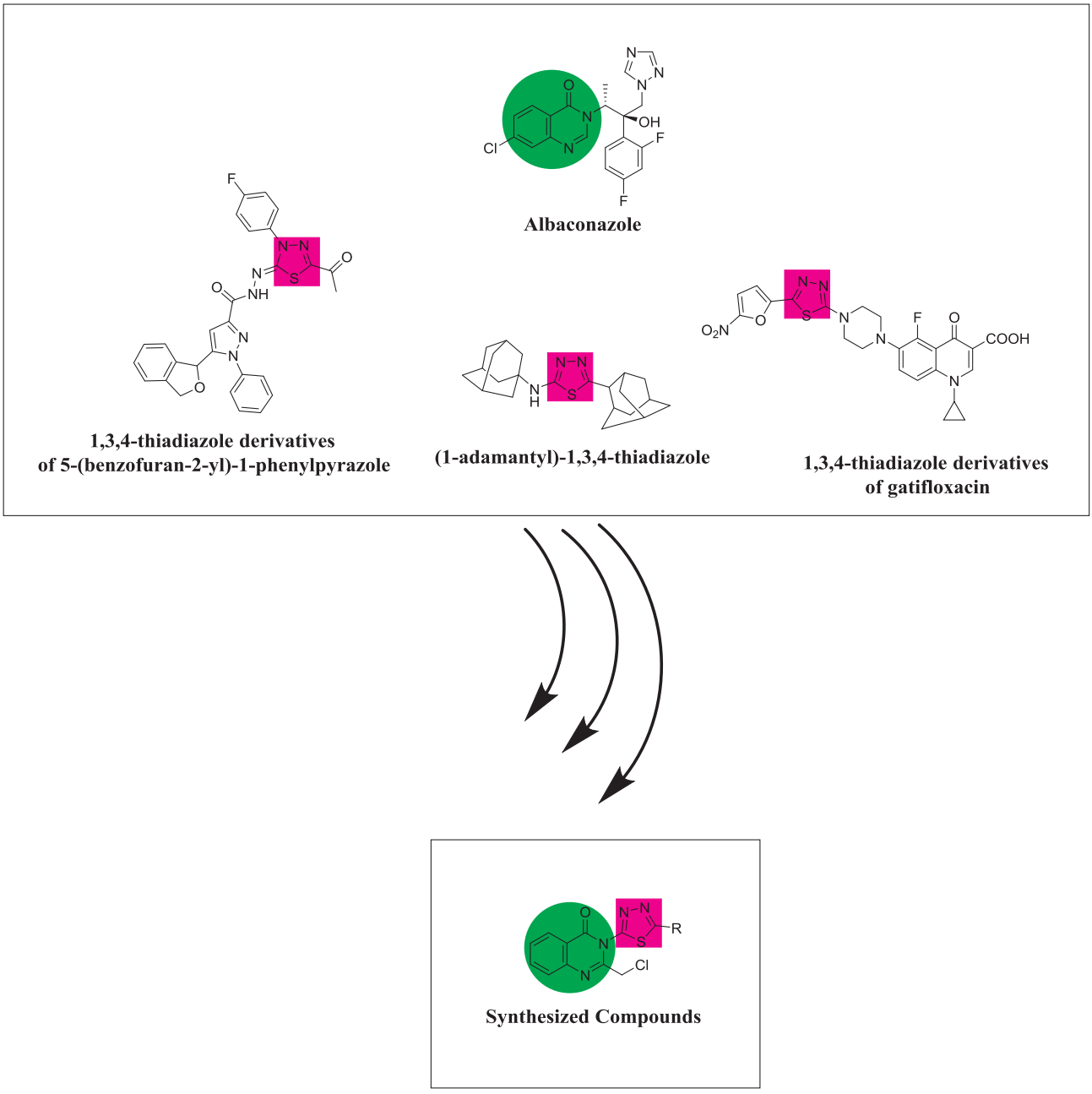


Fig. 1. Rationale for the synthesis of compounds.

2. Materials and methods

2.1. Experimental

All the chemicals and solvents were supplied by Loba, S. D. Fine, E-Merck, and Rankem chemicals, Sigma-Aldrich and Spectrochem Pvt. Ltd. Solvents were distilled and dried before use as required. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF_{254} silica gel, 0.2 mm layer thickness (Merck) by using solvent systems benzene : acetone (7:3 and 9:1), toluene: ethyl acetate: formic acid (5:4:1) and chloroform: methanol (9:1). The spots were visualized under UV lamp. Melting points of the synthesized compounds were determined and are uncorrected using one end open capillary tubes on a scientific melting point apparatus Analab Scientific Instruments. FTIR spectrum was recorded using KBr on FTIR-8400S Shimadzu spectrometer. Both ^{1}H NMR (DMSO) and ^{13}C NMR spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR spectrometer operating at 400 MHz in SAIF, Punjab University, Chandigarh. Chemical shifts were measured relative to internal standard TMS and are reported in (δ ppm). Mass spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University.

2.1.1. Synthesis of 2-(chloromethyl)-4H-benzo[d][1,3]oxazin-4-one (2a)

It is prepared as the reported procedure [25].

2.1.2. Synthesis of 2-ethyl-4H-benzo[d][1,3]oxazin-4-one (2b)

Equimolar quantities of anthranilic acid and propionic anhydride were refluxed for 3 h, after cooling solid was obtained. The obtained product was filtered and washed with methanol. Recrystallization of final the product was done from ethanol to get pure compound [26].

2.1.3. General procedure for the synthesis of 2-ethyl-3-(5-substituted phenyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (3-12) and 2-(chloromethyl)-3-(5-substituted phenyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (13-20)

Equimolar quantities of 2a or 2b and the corresponding 5-substituted-2-amino 1,3,4 thiadiazole were refluxed in dry pyridine for 24 h. The mixture so obtained was added to crush ice and the separated precipitate was filtered off. It was washed with dilute HCl and purified by column chromatography using mobile phase Benzene: Acetone (7:3).

2.1.3.1. 2-(chloromethyl)-3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (3). IR [KBr] *ν*max : 3085.24 (Ar C—H stretch), 2928.04 (Ali C—H stretch), 1691.63 (C═O), 736.83 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.17–8.59 (m, 8H, Ar-H) and 4.36 (s, 2H, CH_2) ppm; ^{13}C NMR ($CDCl_3$) δ:167.80, 164.35, 160.62, 155.44, 148.23, 139.40, 136.61, 132.56, 130.32, 128.16, 126.56, 125.80, 122.46, 119.06, 49.07 ppm; MS (TOF MS ES+) *m/z*: 387 $[M]^+$ 389[M + 2].

2.1.3.2. 2-(chloromethyl)-3-(5-(2-methoxyphenyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (4). IR [KBr] *ν*max : 2940.58 (Ar C—H stretch), 2840.28 (Ali C—H stretch), 1685.84 (C═O), 754.19 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.15–8.40 (m, 8H, Ar-H), 4.21 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.23, 163.78, 161.32, 157.34, 156.34, 145.19, 134.44, 129.67, 128.25, 127.43, 126.57, 126.65, 124.13, 122.54, 120.85, 118.61, 56.31, 49.56 ppm; MS (TOF MS ES+) *m/z*: 384 $[M]^+$, 385 [M + 1].

2.1.3.3. 2-(chloromethyl)-3-(5-m-tolyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (5). IR [KBr] *ν*max : 2921.29 (Ar C—H stretch), 2857.64 (Ali C—H stretch),1691.63 (C═O), 758.05 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.12–8.26 (m, 8H, Ar-H), 4.10 (s, 2H, CH_2, 2.34 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.45, 165.45, 162.61, 158.24, 146.93, 139.29, 136.34, 132.84, 130.31, 129.21, 128.92, 127.43, 126.17, 126.16, 120.58, 48.52, 20.16 ppm; MS (TOF MS ES+) *m/z*: 368 $[M]^+$, 369 [M + 1].

2.1.3.4. 2-(chloromethyl)-3-(5-cyclopropyl-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (6). IR [KBr] *ν*max : 2924.18 (Ar C—H stretch), 2855.71 (Ali C—H stretch), 1690.66 (C═O), 758.05 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.82–7.91 (m, 4H, Ar-H),4.10 (s, 2H, CH_2), 0.91–1.54 (m, 5H, cyclopropyl) ppm; ^{13}C NMR ($CDCl_3$) δ: 167.34, 164.23, 162.63, 158.24, 148.92, 134.42, 128.32, 126.37, 125.63, 120.48, 49.52, 9.22, 8.22 ppm; MS (TOF MS ES+) *m/z*: 318 $[M]^+$, 319 [M + 1].

2.1.3.5. 2-(chloromethyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (7). IR [KBr] *ν*max : 2868.24 (Ar C—H stretch), 2846.87 (Ali C—H stretch), 1596.15 (C═O), 752.26 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.54–8.12 (m, 9H, Ar-H), 3.92 (s, 2H, CH_2) ppm; ^{13}C NMR ($CDCl_3$) δ:168.12, 164.35, 161.64, 158.43, 148.94, 136.56, 133.44, 130.93, 129.42, 128.67, 127.63, 126.67, 126.16, 120.48, 49.15 ppm; MS (TOF MS ES+) *m/z*: 354 $[M]^+$, 355 [M + 1].

2.1.3.6. 2-(chloromethyl)-3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (8). IR [KBr] *ν*max : 2840.58 (Ar C—H stretch), 1686.81 (C═O), 760.94 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.12–8.32 (m, 8H, Ar-H), 4.01(s, 2H, CH_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.12, 164.35, 160.12, 158.34, 148.93, 146.49, 140.36, 134.44, 130.24, 128.33, 126.37, 126.16, 124.34, 120.28, 49.23 ppm; MS (TOF MS ES+) *m/z*: 399 $[M]^+$, 400 [M + 1].

2.1.3.7. 2-(chloromethyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (9). IR [KBr] *ν*max : 2868.24 (Ar C—H stretch), 1696.15 (C═O), 752.26 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.45–7.89 (m, 4H, Ar-H), 4.01 (s, 2H, CH_2), 2.45 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.23, 162.63, 156.34, 148.29, 142.37, 134.44, 128.23, 126.47, 125.16, 120.84, 48.53, 20.12 ppm; MS (TOF MS ES+) *m/z*: 292 $[M]^+$, 293 [M + 1].

2.1.3.8. 2-(chloromethyl)-3-(5-o-tolyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (10). IR [KBr] *ν*max 3056.31 (Ar C—H stretch), 2923.22 (Ali C—H stretch), 1689.70 (C═O), 751.30 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.45–8.12 (m, 8H, Ar-H), 3.82 (s, 2H, CH_2), 2.23 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 169.11, 165.34, 160.62, 158.34, 148.93, 138.32, 136.49, 133.44, 129.45, 128.46, 127.6, 127.13, 126.47, 126.16, 125.42, 120.84, 49.53, 19.72 ppm; MS (TOF MS ES+) *m/z*: 368 $[M]^+$ 369 [M + 1].

2.1.3.9. 2-(chloromethyl)-3-(5-p-tolyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (11). IR [KBr] *ν*max 2922.25 (Ar C—H stretch), 2855.71 (Ali C—H stretch), 1690.66 (C═O), 758.05 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.15–8.04 (m, 8H, Ar-H), 3.92 (s, 2H, CH_2), 2.43 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.13, 164.34, 162.63, 158.43, 146.94, 134.43, 133.74, 132.53, 130.45, 128.43, 127.33, 126.37, 125.65, 120.58, 48.35, 20.23 ppm; MS (TOF MS ES+) *m/z*: 368 $[M]^+$, 369 [M + 1].

2.1.3.10. 2-(chloromethyl)-3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (12). IR [KBr] *ν*max 2934.79 (Ar C—H stretch), 2837.38 (Ali C—H stretch), 1695.49 (C═O), 757.09 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.17–8.12 (m, 8H, Ar-H), 4.01 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3) ppm; ^{13}C NMR($CDCl_3$)δ: 168.12, 164.34, 160.36, 158.42, 148.39, 138.34, 128.25, 127.43, 126.47, 125.63, 125.18, 122.18, 114.28, 56.18, 48.51 ppm; MS (TOF MS ES+) *m/z*: 384 $[M]^+$, 385 [M + 1].

2.1.3.11. 3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-ethylquinazolin-4(3H)-one (13). IR [KBr] *ν*max 2938.65 (C—H stretch), 1690.66 (C═O), 751.30 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.09–7.59 (m, 8H, Ar-H), 2.42 (q, 2H, CH_2, J = 8 Hz), 1.18 (t, 3H, CH_3, J = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 169.13, 165.45, 160.6, 156.14, 146.49, 134.53, 133.44, 131.36, 129.43, 128.59, 127.35, 126.57, 126.56, 120.38, 21.93, 10.17 ppm; MS (TOF MS ES+) *m/z*: 368 $[M]^+$, 369 [M + 1].

2.1.3.12. 3-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-ethylquinazolin-4(3H)-one (14). IR [KBr] *ν*max 2979.16 (C—H stretch), 1658.84 (C═O), 746.48 (C—Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.10–7.68 (m, 8H, Ar-H), 2.40 (q, 2H, CH_2, J = 8 Hz), 1.17 (t, 3H, CH_3, J = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.12, 164.22, 160.46, 156.44, 146.29, 134.48, 134.29, 133.43, 129.55, 129.04, 128.58, 127.54, 127.53, 126.57, 126.16, 120.28, 21.92, 10.37 ppm; MS (TOF MS ES+) *m/z*: 368 $[M]^+$,369 [M + 1].

2.1.3.13. 3-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)-2-ethylquinazolin-4(3H)-one (15). IR [KBr] *ν*max 2979.16 (C—H stretch), 1685.84 (C═O), 689.57 (C-Br) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.19–7.98 (m, 8H, Ar-H), 2.36 (q, 2H, CH_2, J = 8 Hz), 1.23 (t, 3H, CH_3, J = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.12, 164.34, 160.63, 156.44, 146.39, 132.12, 132.25, 133.43, 129.47, 127.32, 126.27, 126.26, 123.14, 120.28, 21.19, 10.12 ppm; MS (TOF MS ES+) *m/z*: 411 $[M]^+$, 413 [M + 2].

2.1.3.14. 2-ethyl-3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (16). IR [KBr] *ν*max 2957.94 (C—H stretch), 1672.34 (C═O), 1348.29 and 1526.71 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.16–7.58 (m, 8H, Ar-H), 2.28 (q, 2H, CH_2, J = 8 Hz), 1.24 (t, 3H, CH_3, J = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.12, 164.23, 160.62, 156.43, 147.39, 146.39, 140.63, 134.44, 128.24, 127.13, 126.37, 126.16, 124.34, 120.48, 21.92, 10.37 ppm; MS (TOF MS ES+) *m/z*: 379 $[M]^+$.

2.1.3.15. 2-ethyl-3-(5-o-tolyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (17). IR [KBr] *ν*max 2940.58 (C—H stretch), 1658.49 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.45–7.78 (m, 8H, Ar-H), 2.24 (s, 3H, CH_3), 2.18 (q, 2H, CH_2, J = 8 Hz), 1.16 (t, 3H, CH_3, J = 7.8 Hz) ppm; ^{13}C NMR

($CDCl_3$) δ: 168.12, 164.34, 160.65, 156.54, 146.79, 137.32, 136.29, 133.45, 129.45, 128.36, 127.34, 127.13, 126.27, 126.61, 125.23, 120.38, 21.92, 16.23, 10.23 ppm; MS (TOF MS ES+) *m/z*: 348 $[M]^+$.

2.1.3.16. 2-ethyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (18). IR [KBr] *ν*max 2924.36 (C−H stretch), 1677.82 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.09–7.89 (m, 9H, Ar-H), 2.18 (q, 2H, CH_2, *J* = 8 Hz), 1.18 (t, 3H, CH_3, *J* = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.12, 164.23, 160.62, 156.43, 148.92, 134.52, 133.42, 130.39, 130.22, 128.437, 127.35, 126.17, 126.16, 120.18, 21.92, 10.12 ppm; MS (TOF MS ES+) *m/z*: 334 $[M]^+$.

2.1.3.17. 2-ethyl-3-(5-(2-methoxyphenyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one (19). IR [KBr] *ν*max 2938.56 (C−H stretch), 1685.84 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.09–7.68 (m, 8H, Ar-H), 3.91(s, 3H, OCH_3), 2.34 (q, 2H, CH_2, *J* = 8 Hz), 1.16 (t, 3H, CH_3, *J* = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ:169.11, 164.23, 160.62, 157.23, 156.14, 146.29, 133.14, 129.37, 128.15, 127.33,126.77, 125.63, 122.31, 121.45, 120.48, 116.46, 56.23, 21.93, 10.37 ppm; MS (TOF MS ES+) *m/z*: 364 $[M]^+$.

2.1.3.18. 2-ethyl-3-(5-methyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (20). IR [KBr] *ν*max 2924.18 (C−H stretch), 1690.66 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.29–7.45 (m, 4H, Ar-H), 2.52 (s, 3H, CH_3), 2.20 (q, 2H, CH_2, *J* = 8 Hz), 1.16 (t, 3H, CH_3, *J* = 7.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 169 .23, 165.23, 160.36, 156.44, 146.59, 142.75, 134.45, 128.43, 126.57, 125.64, 21.93, 18.2, 10.17 ppm; MS (TOF MS ES+) *m/z*: 272 $[M]^+$.

2.2. Determination of the in vitro antimicrobial activity

All the test compounds were evaluated for the antibacterial activity against *Bacillus subtillis NCIM 2250, Staphylococcus aureus NCIM 2079* (gram-positive), *Escherichia coli NCIM 2109, Psuedomonas vulgaris NCIM 2813* (gram-negative) and for antifungal activity against pathogenic fungi viz. *Aspergillus niger NCIM 545* and *Candida albicans NCIM 3471*. Test solution of the compounds was prepared by dissolving 10 mg of compound in 10 mL of DMSO giving solution with concentration of 1000 μg/mL. Standard solution of antibiotics and antifungal agents were prepared by dissolving 10 mg of compound in 10 mL of DMSO. For each tested strain, the growth conditions and the sterility of the medium were checked in two negative controls.

2.2.1. Culture medium

Nutrient broth was used for the preparation of inoculums of the bacteria and Mueller Hinton agar was used for the screening method. The test bacteria and fungi were subcultured using nutrient agar medium and potato dextrose agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37 ± 1 °C (bacteria) and 25 ± 1 °C (fungi) for 24 h, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculums were prepared by transferring a loop full of stock culture to nutrient broth (100 mL) in conical flask (250 mL) the flasks were incubated 37 ± 1 °C (bacteria) and 25 ± 1 °C (fungi) for 24 h before the experimentation.

2.2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the standardized disk diffusion and the agar dilution methods of the National Committee for Clinical Laboratory Standards. Inhibitory zone diameters were measured on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi, with conventional metrical filter paper discs (6 mm in diameter) containing specified doses of compounds. The inhibitory zone diameters were read with a calliper, and all results were rounded up to the nearest whole numbers (millimetre) for analysis.

2.2.3. Antibacterial testing

The Mueller Hinton Agar medium, the Petri-plates, filter paper discs and flask plugged with cotton was sterilized by autoclaving at 121 °C (151 lb/sq. inch). In each sterilized Petri plate (10 cm in diameter) about 30 mL of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred aseptically. The plates were left at room temperature to allow the solidification. In each plate, four discs of 6 mm diameter were placed on the medium which were previously dipped into the solution of test compounds which were prepared and labelled accordingly. The plates were kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 37 ± 1°C for 24 h.

2.2.4. Antifungal testing

The Potato Dextrose Agar medium, the Petri-plates, filter paper discs and flask plugged with cotton were sterilized by autoclaving at 121 °C (151 lb/sq. inch). In each sterilized Petri plate (10 cm in diameter) about 30 mL of molten nutrient agar medium inoculated with respective strains of fungi was transferred aseptically. The plates were left at room temperature to allow the solidification. In each plate, four discs of 6 mm diameter were placed on the medium which were previously dipped into the solution of test compounds which were prepared and labelled accordingly. The plates were kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 25 ± 1 °C for 24 h.

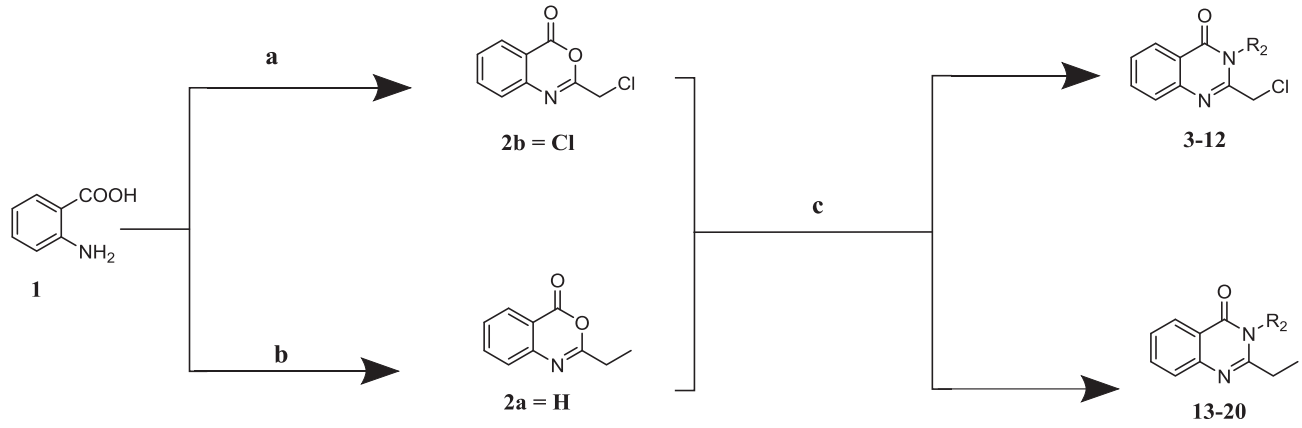
2.2.5. Minimum inhibitory concentration determination

The solution of the newly synthesized compounds and standard drugs were prepared at 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.98, 0.48, 0.24, 0.12 mg/ml concentrations in the wells of microplates by diluting in the liquid double stranded Nutrient Broth. The bacterial suspensions used for inoculation were prepared of 10^5 cfu/mL by diluting fresh cultures at MacFarland 0.5 density (10^7 cfu/mL). Suspensions of the bacteria at 10^5 cfu/ml concentration were inoculated to the twofold diluted solution of the compounds. There were 10^4 cfu/mL bacteria in the wells after inoculations. Nutrient Broth was used for diluting the bacterial suspension and for twofold dilution of the compound. DMSO, pure microorganisms and pure media were used as control wells. A 10 μL bacteria inoculum was added to each well of the micro dilution trays. The trays were incubated at 37 °C in a humid chamber and MIC endpoints were read after 24 h of incubation. For antifungal activity, same procedure was used. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were calculated using the reported procedure [27–29].

3. Results and discussion

3.1. Chemistry

Anthranilic acid 1 was treated with chloroacetyl chloride in pyridine to obtain 2-(chloromethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 2a. Similarly Anthranilic acid 1 was treated with propanoic anhydride to obtain 2-(ethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 2b. Equimolar quantities of 2a or 2b and the appropriate 5-substituted-2-amino 1,3,4 thiadiazoles were refluxed in dry pyridine for 24 h. The mixture so obtained was added to crushed ice and the separated precipitate was filtered off to obtain final compounds 3–20 as shown in Scheme 1 and Table 1. The desired quinazolino-thiadiazoles (3–20) were prepared from 2-chloromethyl-4*H*-benzo[*d*][1,3]oxazin-4-one 2a and 2-(ethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 2b by conventional method. The structures of the synthesized quinazolino-thiadiazoles (3–20) were confirmed by the absence of the N−H band in the IR spectra ~3200 and the presence of amidal carbonyl stretching band around ~1650. ^1H



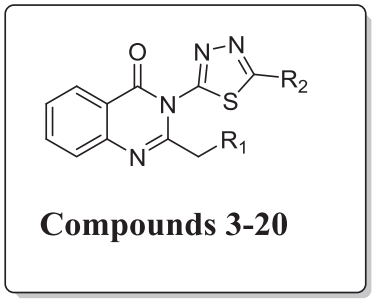
Scheme 1. Reagents and Conditions: a) chloroacetyl chloride, pyridine, stir for 3 h; b) propionic anhydride, reflux for 3 h; c) different substituted 2-amino thiadiazoles in dry pyridine, reflux for 24 h.

NMR and ^{13}C NMR spectra of 3–12 shows characteristic CH_2 singlet at ~4.30 δ ppm and ~49 δ ppm respectively. In the same way compound 13–20 showed characteristic CH2 quartet at ~2.30 δ ppm and triplet of CH3 at ~1.30 δ ppm in 1H NMR; additionally 2 bands appeared at ~10 δ ppm and ~21 δ ppm in 13C NMR. The assigned structures of these compounds were further confirmed by Mass spectra.

3.2. Anti-microbial activity

Newly prepared compounds (3–20) were screened for their antibacterial and antifungal activity. MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. The MIC values are generally within the range of 62.5–250 μg/ml against all evaluated strains. The results of the *in vitro* antibacterial activity screening of the novel series of quinazolino-thiadiazoles (3–20) are summarized in Table 2, against *Bacillus subtillis NCIM 2250, Staphylococcus aureus NCIM 2079* (gram-positive), *Escherichia coli NCIM 2109, Psuedomonas vulgaris NCIM 2813* (gram-negative), pathogenic fungi viz. *Aspergillus niger NCIM 545* and *Candida albicans NCIM 3471*. Anti-microbial tests for the 18 compounds have established some interesting structure-activity relationships among the tested compounds; it was found that the presence of aromatic ring carrying an electron withdrawing group at the para position is beneficial for the activity. Compound 3 and 8 with a 4-chloro phenyl and 4-nitro phenyl at C-2 of thiadiazolyl of quinazolino-thiadiazoles, displayed the excellent antibacterial and antifungal activities against all the tested

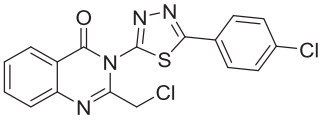
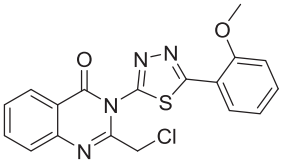
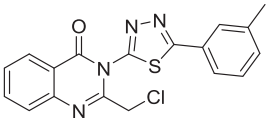
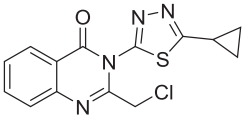
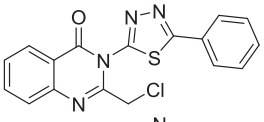
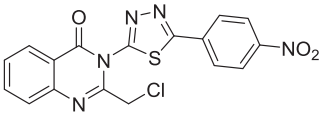
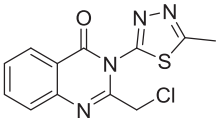
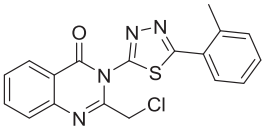
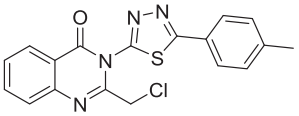
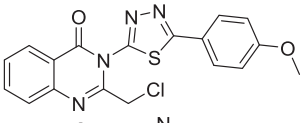
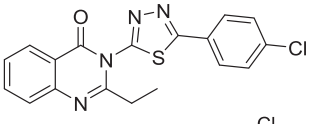
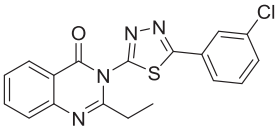
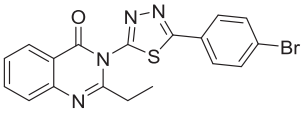
Table 1
Physical data of synthesized compounds (**3–20**).



Comp. No.	R_1	R_2	Molecular formula	Molecular weight	Melting Point (°C)	%Yield	Comp. No.	R_1	R_2	Molecular formula	Molecular weight	Melting point (°C)	%Yield
3	Cl	4-Cl-C_6H_4	$C_{17}H_{10}Cl_2N_4OS$	389.26	223–227	48	**12**	Cl	4-OCH_3-C_6H_4	$C_{18}H_{13}ClN_4O_2S$	384.84	255–258	66
4	Cl	2-OCH_3-C_6H_4	$C_{18}H_{13}ClN_4O_2S$	384.84	225–228	56	**13**	H	4-Cl-C_6H_4	$C_{18}H_{13}ClN_4OS$	368.8400	244–246	52
5	Cl	3-CH_3-C_6H_4	$C_{18}H_{13}ClN_4OS$	368.84	199–203	58	**14**	H	3-Cl-C_6H_4	$C_{18}H_{13}ClN_4OS$	368.8400	252–254	48
6	Cl	C_3H_5	$C_{14}H_{11}ClN_4OS$	318.78	235–238	53	**15**	H	4-Br-C_6H_4	$C_{18}H_{13}BrN_4OS$	413.2910	238–242	44
7	Cl	C_6H_5	$C_{17}H_{11}ClN_4OS$	354.81	235–239	48	**16**	H	4-NO_2-C_6H_4	$C_{18}H_{13}N_5O_3S$	379.3925	230–232	62
8	Cl	4-NO_2-C_6H_4	$C_{17}H_{10}ClN_5O_3S$	399.81	263–267	46	**17**	H	2-CH_3-C_6H_4	$C_{19}H_{16}N_4OS$	348.4215	226–228	58
9	Cl	CH_3	$C_{12}H_9ClN_4OS$	292.74	242–246	48	**18**	H	C_6H_5	$C_{18}H_{14}N_4OS$	334.3950	215–218	62
10	Cl	2-CH_3-C_6H_4	$C_{18}H_{13}ClN_4OS$	368.84	218–222	49	**19**	H	4-OCH_3-C_6H_4	$C_{19}H_{16}N_4O_2S$	364.4209	198–202	42
11	Cl	4-CH_3-C_6H_4	$C_{18}H_{13}ClN_4OS$	368.84	289–293	62	**20**	H	CH_3	$C_{13}H_{12}N_4OS$	272.3256	235–238	68

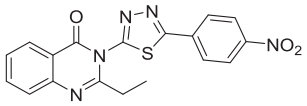
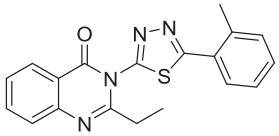
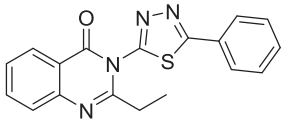
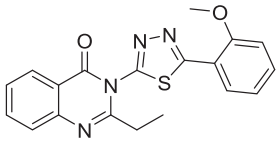
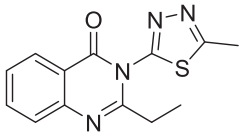
Elemental analysis for C, H, N were within 0.4% of the theoretical values.

Table 2
Minimum Inhibitory Concentration (MIC) of compounds (**3–20**).

Comp. code	Structure	Antibacterial activity MIC (μg/ml)				Antifungal activity MIC (μg/ml)	
		S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*C. albicans*	*A. niger*
3		**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**
4		**62.5**	**62.5**	125	250	125	125
5		125	250	125	250	250	250
6		250	250	250	250	250	250
7		250	250	250	250	250	250
8		**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**
9		250	250	250	250	250	250
10		250	250	250	250	250	250
11		250	250	250	250	250	250
12		125	125	125	125	250	250
13		125	**62.5**	**62.5**	125	250	**62.5**
14		125	125	125	125	250	125
15		125	**62.5**	**62.5**	125	250	**62.5**

(*continued on next page*)

Table 2 (*continued*)

Comp. code	Structure	Antibacterial activity MIC (μg/ml)				Antifungal activity MIC (μg/ml)	
		S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*C. albicans*	*A. niger*
16		125	**62.5**	**62.5**	125	250	**62.5**
17		250	250	250	250	250	250
18		250	250	250	250	250	250
19		125	125	125	125	250	250
20		250	250	250	250	250	250
Chloramphenicol		**0.48**	**0.48**	**3.9**	**3.9**	NT	NT
Amphoterecin B		NT	NT	NT	NT	**0.98**	**1.80**

Bold values indicate Significant inhibition.

micro-organisms with MIC values of 62.5 μg/mL as compared to the positive control drugs. Compound 4 having o-methoxy substitution also displayed significant activity against gram positive bacterial strain, *S. aureus* and *B.* Subtilis, with MIC value of 62.5 μg/mL. In another series of compounds where 2-ethyl group is present on the quinazoline ring, compounds 13,15 and 16 all bearing electron withdrawing groups, Cl, Br and NO_2 respectively- displayed the significant MIC value of 62.5 μg/mL against *S. aureus*, *B. Subtilis* and *A. Niger*. The results showed that the rest of the compounds were moderately active against the bacterial and fungal strain with MIC value of 125–250 μg/mL.

4. Conclusion

In conclusion, eighteen quinazolino-thiadiazoles (3–20) have been synthesized and evaluated for their antibacterial and antifungal activity. These synthesized hybrids namely compound 3 and 8 with a 4-chloro phenyl and 4-nitro phenyl at C-2 of thiadiazolyl of quinazolino-thiadiazoles, displayed promising antibacterial and antifungal activities against all the tested micro-organisms (Bacterial and Fungal strain) with MIC values of 62.5 μg/mL. The encouraging antimicrobial activity of the synthesized quinazolino-thiadiazoles derivatives through modification of ring substituents and/or additional functionalization indicate that further derivatization of such compounds will be of interest with hope to get more potent agents.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

Acknowledgements

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