Research Article

Synthesis and Antimicrobial Evaluation of Some Novel Trisubstituted s-Triazine Derivatives Based on Isatinimino, Sulphonamido, and Azacarbazole

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A study directed towards exploring the temperature-dependent reactivity of the chlorine atoms of 2,4,6-trichloro-s-triazine (TCT) in the nucleophilic displacement reaction, allowed a facile replacement of its chlorine atoms in succession with (i) N-amino methyl substituted isatin-3-hydrazones, (ii) N$_1$-substituted-4-amino benzene sulphonamides, and (iii) 8-amino-4-oxo-N-benzyl-azacarbazole to produce the corresponding 2,4,6-trisubstituted-s-triazine derivatives in acceptable yields. The compounds prepared were further evaluated for their antibacterial activity against *E. coli* and *B. subtilis* and antifungal activities against *A. niger* and *A. flavus*, and some of them showed promising activity profile.

1. Introduction

Due to rapid development in drug resistance, tolerance, and side effects, there is a critical need for the development of a new generation of antimicrobial agents that exhibit improved pharmacological properties and drug-resistance profiles. In this aspect substituted s-triazine derivatives have been receiving central attention due to their significant antimicrobial [1], antibacterial [2], antifungal [3], anti-HIV [4], anticaner [5], and a wide array of other biological activities [6, 7]. Our hypothesis is to incorporate the certain pharmacophoric features and scaffolds on the templates of s-triazine molecule in order to design as a novel antimicrobial agents.

Mannich bases of isatin derivatives have been reported to form an integral part of the bioactive molecules exhibiting wide range of activities such as cytotoxic [8], anticonvulsant [9], antidepressant [10], and anti-inflammatory [11] agents. Sulphonamides have been widely known in the literature to exhibit a broad range of biological activities [12, 13]. The SO$_2$–NH group of sulphonamide constitutes a key structural motif shared by a large number of bioactive compounds spanning a variety of effects such as the microbial activities [14], specific enzyme inhibition [15] and hormonal regulation [16].

The ubiquitous presence of azacarbazoles and pyridocarbazoles in a vast array of biologically active molecules (such as ellipticine, olivacine and carbaquinacine [17], to name a few) have stimulated as intense research efforts to the synthesis of their structural analogues where different constitution and biological activity of new materials could allow them to be used as novel chemotherapeutic agents.

Greatly encouraged by the biological profiles of these heterocyclic scaffolds, we aimed in the present work to synthesize 2,4,6-trisubstituted-s-triazine molecules incorporating in them the structural features of (i) N-Mannich’s bases of isatin-3-hydrazones, 2(a–f), (ii) established sulpha drugs, 4(a,b), (iii) N-benzyl-4-oxo azacarbazole derivatives (6), on the premise that their presence in tandem in a single molecular framework in 7(a–l) should contribute significantly to the biological activity in the resulting molecules and should allow them to be used as novel chemotherapeutic agents.
2. Experimental Section

2.1. General Remarks. Melting points were determined in open glass capillaries and are uncorrected. The reactions were monitored by the TLC on silica gel "G" plates and were visualized with UV light and by exposing to iodine. The purity of the compounds was checked by spectral data, namely, IR, NMR, mass and elemental analysis. IR spectra were recorded on CE (SIMADZU) FTIR-8400S. 1H NMR spectra were recorded on model Avance 300 (Bruker 300 MHz) using DMSO-d6 as solvent and TMS as an internal reference.

3. Chemical Synthesis

3.1. General Methods for the Preparation of 3(a–f), 5(a–l), 7(a–l)

3.1.1. Preparation of 3-(2-(4,6-dichloro-1,3,5-triazin-2-yl)hydrazono)-1-(morpholinomethyl)indolin-2-one (3a). Cyanuric chloride (1) (0.5 g, 0.002 mol) was taken in dry THF containing K2CO3 (0.1 g). 1-(morpholinomethyl)indolin-2-one-3-hydrazide (2a) (0.7 g, 0.002 mol) was added to it. Reaction was kept on stirring at 0–5°C for 2-3 hr. Reaction mixture was poured in crushed ice and neutralized with dil. HCl. The resulting solid was filtered, washed with dil. Ethanol. The resultant solid was dried and recrystallized from ethanol: water (1:9) mixture to give 3a, 0.48 g (yield 68.8%) m.p. 180-181°C. Anal. Calcd. C, 47.07; H, 3.70; N, 24.02; Cl, 17.37. Found: C, 47.01; H, 3.75; N, 23.98; Cl, 17.31 IR (KBr) cm⁻¹ 1340 (NH), 3100 (amom. Str.), 1711 (C=O), 1H-NMR (DMSO-d6)–δ 10.7 (1H, s, NH), 7.77–8.61 (4H, m, Ar–H), 4.03 (2H, s, CH2), 3.65 (4H, t, CH2, J = 1.1 Hz), 2.47 (4H, t, CH2, J = 1.8 Hz).

Compounds 3(b–f) were prepared by a similar method using appropriate reactants with required change in stirring time.

3.1.2. Preparation of 3-(2-(4-chloro-6-(2-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonylamide 5(a). Compound 3a (0.3 g, 0.0073 moles) was taken in dry THF and K2CO3 (0.1 g). Sulphathiazole 4a (0.187 g, 0.0073 moles) was added to this. Reaction mixture was kept on stirring at 30–35°C for 2-3 hr and was then poured in to crushed ice and neutralized with dil. HCl. The resulting solid was filtered, washed with dil. Ethanol. The resultant solid was dried and recrystallized from ethanol: water (1:9) mixture to give 5a, 0.182 g (yield 58.8%) m.p. 200-201°C. Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32. 1H-NMR (DMSO-d6)–δ 10.67 (1H, s, NH), 9.44 (1H, s, NH), 7.61–7.18 (9H, m, Ar–H), 3.87 (1H, t, CH, J = 0.9 Hz), 3.65 (4H, q, CH2), 3.11 (2H, s, CH2), 2.67 (4H, q, CH2).

Compounds 5(b–l) were prepared by a similar method using appropriate reactants with required change in temperature and stirring time.

4. (4-chloro-6-(2-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(methylpyrimidin-2-yl)benzenesulfonylamide (5b). Yield 67%; m.p. 221-222°C. Anal. Calcd. C, 63.41; H, 4.75; N, 21.55; S, 3.32. 1H-NMR (DMSO-d6)–δ 12.64 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 7.61–7.18 (9H, m, Ar–H), 3.87 (1H, t, CH, J = 0.9 Hz), 3.65 (4H, q, CH2), 3.11 (2H, s, CH2), 2.67 (4H, q, CH2).

225-226°C; Anal. Calcd. C, 48.47; H, 4.31; N, 26.60; Cl, 16.83. 1H-NMR (DMSO-d6)–δ 10.81 (1H, s, NH), 7.86–6.45 (4H, m, Ar–H), 4.03 (2H, s, CH2), 2.35 (8H, t, CH2, J = 2.1 Hz), 2.26 (3H, s, CH3).

3.1.3. Preparation of 3-(2-(4,6-dichloro-1,3,5-triazin-2-yl)hydrazono)-1-((4-methylpiperazin-1-yl)methyl)indolin-2-one (3f). Yield 72%; m.p. 228-229°C. Anal. Calcd. C, 54.67; H, 4.17; N, 23.18; Cl, 14.67. 1H-NMR (DMSO-d6)–δ 10.17 (1H, s, NH), 3.65 (4H, t, CH2, J = 1.1 Hz), 2.47 (4H, t, CH2, J = 1.8 Hz).
s, NH), 7.35–6.93 (10H, m, Ar–H), 3.65 (4H, t, CH₂, J = 1.3 Hz), 2.67 (4H, q, CH₂), 1.53 (3H, s, CH₃).

4-(4-chloro-6-(2-oxo-1-piperedin-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (5c). Yield 68%; m.p. 299-300°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3180 (NH), 3020 (aromatic str.), 1712 (C=O), 1590 (C=N), 1480 (C=C str.), 1357, 1134 (S=O), 1H-NMR (DMSO-d₆)—12.64 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 8.11 (1H, s, Ar–H), 7.86–7.24 (9H, m, Ar–H), 4.03 (2H, s, CH₂), 2.45 (4H, t, CH₂, J = 0.9 Hz), 1.59 (2H, q, CH₂), 1.53 (4H, m, CH₂).

4-(4-chloro-6-(2-oxo-1-pyrrolidin-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (5d). Yield 69%; m.p. 291-292°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3180 (NH), 3025 (aromatic str.), 1712 (C=O), 1595 (C=N), 1490 (C=C str.), 1357, 1134 (S=O), 1H-NMR (DMSO-d₆)—11.27 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 8.11 (1H, s, Ar–H), 7.86–7.24 (9H, m, Ar–H), 4.03 (2H, s, CH₂), 2.45 (4H, t, CH₂, J = 0.78 Hz), 2.33 (3H, s, CH₃), 1.59 (2H, q, CH₂), 1.53 (4H, m, CH₂).

4-(4-chloro-6-(2-oxo-1-(pyrrolidin-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (5e). Yield 70%; m.p. 240-241°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3180 (NH), 3125 (aromatic str.), 1712 (C=O), 1595 (C=N), 1490 (C=C str.), 1357, 1134 (S=O), 1H-NMR (DMSO-d₆)—12.64 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 7.86–6.75 (10H, m, Ar–H), 4.03 (2H, s, CH₂), 2.51 (4H, t, CH₂, J = 5.6 Hz), 1.68 (4H, m, CH₂).

4-(4-chloro-6-(2-oxo-1-(pyrrolidin-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (5f). Yield 65%; m.p. 250-251°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3225 (NH), 3120 (aromatic str.), 1715 (C=O), 1485 (C=C str.), 1510 (C=C str.), 1357, 1134 (S=O), 1H-NMR (DMSO-d₆)—11.27 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 8.11 (1H, s, Ar–H), 7.86–7.24 (9H, m, Ar–H), 4.03 (2H, s, CH₂), 2.51 (4H, t, CH₂, J = 0.9 Hz), 2.33 (3H, s, CH₃), 1.68 (4H, m, CH₂).

4-(4-chloro-6-(2-oxo-1-(4-methylpiperazin-1-yl)methyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (5h). Yield 62%; m.p. 245-246°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3210 (NH), 3235 (aromatic str.), 1712 (C=O), 1500 (C=N str.), 1520 (C=C str.), 1357, 1134 (S=O), 1H-NMR (DMSO-d₆)—11.27 (1H, s, NH), 10.67 (1H, s, NH), 9.44 (1H, s, NH), 8.11 (1H, s, Ar–H), 7.86–7.24 (9H, m, Ar–H), 4.03 (2H, s, CH₂), 2.35 (8H, t, CH₂, J = 0.85 Hz), 2.33 (3H, s, CH₃), 2.26 (3H, s, CH₃).

4-(4-chloro-6-(2-oxo-1-(4-phenylpiperazin-1-yl)methyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (5i). Yield 65%; m.p. 234-235°C; Anal. Calcd. C, 63.41; H, 4.90; N, 21.75; S, 3.32 (C₅₁H₄₇N₁₅O₄S). Found: C 63.01; H, 4.75; N, 21.55; S, 3.02; IR (KBr) cm⁻¹ 3180 (NH), 3235 (aromatic str.), 1712.
3.1.3. Preparation of N-benzyl-8-amino-azacarbazole-4-one (6). A solution of p-aminocetanilide (0.76 g, 0.05 mol) in aqueous HCl (2 mL conc. HCl in 5 mL water) was treated with a cold saturated solution of sodium nitrite (0.7 gm in 5 mL aqueous HCl (2 mL conc. HCl in 5 mL water) to yield 6. M.P. 305 °C. 3.1.4. Preparation of 4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-7-ylamino)-6-(2-oxo-1-(piperidine-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-methyl-indol-7-ylamino)-6-(2-(2-oxo-1-(pyrrolidine-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-methyl-indol-9-ylamino)-6-(2-(2-oxo-1-(piperidine-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-methylindol-2-yl)benzenesulfonamide (7). Yield 61%; m.p. 365 °C; Anal. Calcd. C, 62.15; H, 4.99; N, 22.06; S, 3.61 (C_{45}H_{44}N_{14}O_{8}S). Found: C, 62.10; H, 4.45; N, 22.16; S, 3.55; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3050 (aromatic str.), 1700 (C=O), 1600 (C=N str.), 1 H-NMR (DMSO-d₆) δ 11.53 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.66-318 (18H, m, Ar-H), 4.51 (2H, s, CH₂), 4.03 (2H, s, CH₂), 3.39 (2H, t, CH₂, J = 7.2 Hz), 2.63 (2H, t, CH₂, J = 7.7 Hz), 2.50 (4H, t, CH₂, J = 5.1 Hz), 2.33 (3H, s, CH₃), 1.59 (2H, m, CH₂), 1.53 (2H, m, CH₂).

4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-7-ylamino)-6-(2-oxo-1-(piperidine-1-ylmethyl)indolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-methylindol-2-yl)benzenesulfonamide (7). Yield 60%; m.p. 313 °C; Anal. Calcd. C, 59.64; H, 4.84; N, 21.03; S, 7.41 (C_{45}H_{44}N_{14}O_{8}S). Found: C, 59.34; H, 4.34; N, 20.97; S, 7.22; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3050 (aromatic str.), 1700 (C=O), 1600 (C=N str.), 1 H-NMR (DMSO-d₆) δ 11.63 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.76-318 (18H, m, Ar-H), 4.51 (2H, s, CH₂), 4.03 (2H, s, CH₂), 3.39 (2H, t, CH₂, J = 7.2 Hz), 2.63 (2H, t, CH₂, J = 7.7 Hz), 2.50 (4H, t, CH₂, J = 5.1 Hz), 2.33 (3H, s, CH₃), 1.59 (2H, m, CH₂), 1.53 (2H, m, CH₂).

Compound (b) were prepared by a similar method using appropriate reagents.
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1690 (C=O), 1625 (C=N str.), 1H-NMR (DMSO-d6)—δ, 11.53 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.6–6.75 (18H, m, Ar–H), 4.51 (2H, s, CH2), 4.03 (2H, s, CH2), 3.39 (2H, t, CH2, J = 3.6 Hz), 2.63 (2H, t, CH2, J = 2.6 Hz), 2.51 (4H, t, CH2, J = 2.2 Hz), 2.33 (3H, s, CH3), 1.66 (4H, m, CH2).

4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-9-yamino)-6-(2-(1-((4-phenylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (7g). Yield 64%; m.p. 138°C; Anal. Calcd. C, 59.46; H, 5.16; N, 21.57; S, 7.05 (C45H44N14O15S2). Found: C, 58.45; H, 4.95; N, 22.86; S, 7.07; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3000 (aromatic str.), 1675 (C=O), 1590 (C=N str.), 1H-NMR (DMSO-d6)—δ, 11.63 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.6–6.38 (17H, m, Ar–H), 4.51 (2H, s, CH2), 4.03 (2H, s, CH2), 3.39 (2H, t, CH2, J = 3.3 Hz), 2.53 (2H, t, CH2, J = 1.4 Hz), 1.20 (8H, t, CH2, J = 2.2 Hz), 2.26 (3H, s, CH3).

Figure 1: Antibacterial activity against B. subtilis.

Figure 2: Antibacterial activity against E. coli.

4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-9-yamino)-6-(2-(1-((4-ethylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(thiazol-2-yl)benzenesulfonamide (7h). Yield 58%; m.p. 314°C; Anal. Calcd. C, 61.12; H, 4.88; N, 23.24; S, 3.55 (C46H42N15O8S). Found: C, 61.52; H, 4.77; N, 23.04; S, 3.51; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3005 (aromatic str.), 1710 (C=O), 1600 (C=N str.), 1H-NMR (DMSO-d6)—δ, 11.53 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.6 (1H, s, Ar–H), 8.4 (1H, s, Ar–H), 7.5–6.2 (17H, m, Ar–H), 4.51 (2H, s, CH2), 4.03 (2H, s, CH2), 3.39 (2H, t, CH2, J = 2.2 Hz), 2.63 (2H, t, CH2, J = 2.6 Hz), 2.35 (8H, t, CH2, J = 3.6 Hz), 2.33 (3H, s, CH3), 2.26 (3H, q, CH2).

4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-9-yamino)-6-(2-(1-((4-ethylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-methylpyrazin-2-yl)benzenesulfonamide (7i). Yield 62%; m.p. 345°C; Anal. Calcd. C, 61.49; H, 4.63; N, 23.24; S, 3.49 (C45H42N14O8S). Found: C, 61.19; H, 4.13; N, 22.84; S, 3.43; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3005 (aromatic str.), 1690 (C=O), 1692 (C=N str.), 1H-NMR (DMSO-d6)—δ, 11.53 (1H, s, NH), 10.67 (1H, s, NH), 10.04 (1H, s, NH), 9.44 (1H, s, NH), 8.91 (1H, s, NH), 8.6–6.38 (17H, m, Ar–H), 4.51 (2H, s, CH2), 4.03 (2H, s, CH2), 3.39 (2H, t, CH2, J = 4.2 Hz), 2.53 (2H, t, CH2, J = 3.8 Hz), 2.35 (8H, t, CH2, J = 2.5 Hz), 2.33 (2H, q, CH2).

4-(4-(1-benzyl-4-oxo-2,3,4,5-tetrahydro-1H-pyrido [3,2-b] indol-9-yamino)-6-(2-(1-((4-phenylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)hydrazinyl)-1,3,5-triazin-2-ylamino)-N-(6-thiazol-2-yl)benzenesulfonamide (7k). Yield 64%; m.p. 345°C; Anal. Calcd. C, 61.49; H, 4.54; N, 20.49; S, 6.70 (C46H42N15O8S). Found: C, 61.09; H, 4.34; N, 20.29; S, 7.56; IR (KBr) cm⁻¹ 3300 (NH symm. str.), 3000 (aromatic str.), 1690 (C=O), 1595 (C=N str.), 1H-NMR

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**Figure 1**: Antibacterial activity against *B. subtilis*.

**Figure 2**: Antibacterial activity against *E. coli*.
Among all of the synthesized compounds, compounds 7(a–l) were screened for antimicrobial activity by disc diffusion method [18]. The test compounds 7(a–l), in measured quantities, were dissolved in dimethyl formamide (DMF) to get the final concentrations 400 mg/L, 200 mg/L, 100 mg/L respectively. The bacterial (24 h) and fungal (48 h) cultures from the slants were diluted with sterile water and mixed thoroughly to prepare a clear homogeneous suspension. These suspensions were spread on solidified agar (NA—nutrient agar—for bacteria and PDA—potato dextrose agar for fungi) medium. The filter paper disks [18] prepared by only DMF (as a negative control) and with solutions of different concentrations of test compounds 7(a–l) as well as standard compounds (ciprofloxacin and fluconazole as positive control) were carefully placed over the spread cultures and incubated at 37 °C for 24 hr for bacteria and at 28–30 °C for 48 hr for fungi. After the incubation period, the plates were examined for the zone of inhibition. The diameter for the zones of inhibition were measured including the diameter of disk also. All determinations were made in triplicate for each of the compound and the average value was taken. The antibacterial and antifungal activity was evaluated against E. coli, B. subtilis, A. niger, and A. flavus against the standard drugs ciprofloxacin for bacteria and fluconazole for fungi. The outcome of this study is presented in Table 1 and in Figures 1–4.

5. Results and Discussion

The synthetic approach which allowed the incorporation of the aforementioned bioactive heterocyclic systems on to the 2, 4, and 6 positions of s-triazine molecule was confined to three series outlined in Schemes 1, 2, and 3. The first series was derived from isatin which afforded its hydrazone from the reaction with hydrazine hydrate. Scheme 1 shows the synthetic strategy applied to obtain 3(a–f) by replacing one of the chlorine atoms of s-triazine (1) with isatinyl-3-hydrazone Mannich’s bases 2(a–f), by conducting the reaction in Dry THF/K2CO3 at 0–5 °C for 2–3 hrs. Replacement of chlorine atoms of s-triazine is a temperature dependent nucleophilic substitution reaction [19]. While the second series was derived by the replacement of one of the chlorine atoms of 3(a–f) by replacing one of the chlorine atoms of s-triazine (1) with isatinyl-3-hydrazone Mannich’s bases 2(a–f), by conducting the reaction in Dry THF/K2CO3 at 0–5 °C for 2–3 hrs.
Scheme 1: Monosubstitution reaction (i) Dry THF, 0–5°C, K₂CO₃. 3a, R = morpholinyl, 3b, R = piperidinyl, 3c, R = pyrrolidinyl, 3d, R = N-methyl piperazinyl, 3e, R = N-ethyl piperazinyl, 3f, R = N-phenyl piperazinyl.

Scheme 2: Disubstitution (ii) dry THF, 30–35°C, K₂CO₃. 5a, R = morpholinyl, R’ = thiazolyl, 5b, R = morpholinyl, R’ = methyl pyrimidinyl, 5c, R = piperidinyl, R’ = thiazolyl, 5d, R = piperidinyl, R’ = methyl pyrimidinyl, 5e, R = pyrrolidinyl, R’ = thiazolyl, 5f, R = pyrrolidinyl, R’ = methyl pyrimidinyl, 5g, R = N-methyl piperazinyl, R’ = thiazolyl, 5h, R = N-methyl piperazinyl, R’ = methyl pyrimidinyl, 5i, R = N-ethyl piperazinyl, R’ = thiazolyl, 5j, R = N-ethyl piperazinyl, R’ = methyl pyrimidinyl, 5k, R = N-phenyl piperazinyl, R’ = thiazolyl, 5l, R = N-phenyl piperazinyl, R’ = methyl pyrimidinyl.

Scheme 3: Trisubstitution (iii) dry THF, reflux (60–65°C), K₂CO₃.

Scheme 4: Synthesis of N-benzyl-8-amino-azacarbazole-4-one (4) (a) Japp-Klingemann-sodium acetate trihydrate, methanol, water (b) Fischer-indolization-acetic acid, HCl (5) hydrolysis, Dil. HCl.
The third series which consisted of the target compounds 7(a–l), was derived by replacement of the last chlorine atom of 5(a–l) with N-benzyl-8-amino-azacarbazole-4-one derivative (6). The reaction was carried out in THF/K₂CO₃ medium at 60–65°C for 6 hrs. as shown in Scheme 3.

Compound, N-benzyl-8-amino-azacarbazole-4-one (6) required in the preparation of the target compounds, 7(a–l), was synthesized from p-acetamidobenzenediazonium chloride (8) following the strategy shown in Scheme 4. The reaction of 8 when allowed to take place with 3-hydroxy methylidene-N-benzyl-4-piperidone (9) under the conditions of Japp-Klingeman [20] reaction afforded the corresponding hydrazone 10, from which 6 resulted on Fischer indolization with Kent's reagent [AcOH:HCl, 4:1, v/v] followed by subsequent hydrolysis with acid.

The antibacterial and antifungal activity was evaluated against *E. coli*, *B. subtilis*, *A. niger*, and *A. flavus* against the standard drugs ciprofloxacin for bacteria and fluconazole for fungi. The zone of inhibition was measured in mm, on three different concentrations 400 mg/L, 200 mg/L, 100 mg/L. Maximum inhibitory activity was observed for 400 mg/L and compounds showed their effect in a dose-dependent manner. The compound 7f was found to be most potent against *B. subtilis*, while the compound 7a and 7i these were found to be equipotent remaining compounds also showed the promising antibacterial activity. The compounds 7a, 7i, 7h these were most potent against *E. coli* while the compound 7e and 7h these were equipotent against the same strain as shown in Figures 1 and 2. The compounds 7c and 7d, these showed promising activity against *A. niger* and *A. flavus* respectively, while the remaining compounds also showed promising inhibitory activity as shown in Figures 3 and 4. The morpholine, pyrrole ring, and thiazole ring were found more potent in their inhibitory effect on growth of both bacterial and fungal strains which indicates the positive modulator effect of electronegativity on activity in these molecules.

6. Conclusion

We developed novel trisubstituted triazine derivatives containing, N-Mannich bases of isatin-3-hydrazones, established sulpha drugs and N-benzyl-4-oxo azacarbazole derivatives using nucleophilic substitution reaction, Japp-Klingmann, Fischer-indolization, and other reactions. Furthermore, it was found that the morpholine, pyrrole ring, and thiazole ring derivatives 7a, 7i, and 7h were more potent in their inhibitory effect on growth of both bacterial and fungal strains which indicates the positive modulator effect of electronegativity on activity in these molecules. Their further quantitative structure relationship (QSAR) studies are currently under investigation.

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