American Journal of Clinical Microbiology and Antimicrobials

Review Article
Published: 25 Sep, 2018

Synthesis, Characterization and Structure Activity Relationship (SAR) Studies of Differently Substituted Naphthalene and Triazine Incorporated Heterocyclic Molecule as Possible Anti-Bacterial and Anti-Fungal Agents

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Abstract

Nitrogen heterocyclic compound play an important role in medicinal chemistry due to their biological activities towards microorganisms. Differently substituted Naphthalene and Triazine incorporated heterocyclic compounds were synthesized and screened for antibacterial activity against Gram-positive and Gram-negative bacteria and anti-fungal activity. These aromatic nitrogen heterocyclic derivatives displayed remarkable *in vitro* antimicrobial activities against *E. coli* AT CC25922, *S.aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Streptococcus mutans* (MTCC 890), *K. pneumoniae* and *Bacillus subtili* re-cultured bacterial strain and *A. flavus*, *A. fumigatus*, *P. marneffei*, *T. mentagrophytes*, *C. Albicans*.

Keywords: Naphthalene; Triazine; $Pd(dppf)_2Cl_2$; Antibacterial activity; Antifungal activity; SAR

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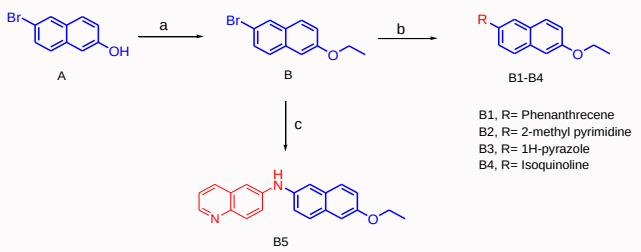
Received Date: *14 Aug 2018*
Accepted Date: *18 Sep 2018*
Published Date: *25 Sep 2018*

Citation:
Pakkath R, karuvalam RP, Eeda KR, Sajith AM, Haridas KR. Synthesis, Characterization and Structure Activity Relationship (SAR) Studies of Differently Substituted Naphthalene and Triazine Incorporated Heterocyclic Molecule as Possible Anti-Bacterial and Anti-Fungal Agents. Am J Clin Microbiol Antimicrob. 2018; 1(5): 1024.

Introduction

The nature has been blessed us by providing numerous chemical molecules, especially nitrogenous chemical molecules. These nitrogenous molecules are of paramount importance both structurally as well as biologically [1]. Basically nitrogen is the building block of plant protein and also plays vital importance in the growth and development of various tissues and cells. Over the past five decades several progresses can be seen after the discovery and development of antimicrobial agents. It is also equipped with many classes of natural products and semi-synthetic or synthetic compounds [2,3]. Today due to the affirmative pharmacological and biological properties of these heterocyclic compounds and their derivatives had become a staunch cerebration in the field of medicinal research. Since their structural subunits are itself entrenched in various natural products such as antibiotics, vitamins, and hormones, these heterocyclic compounds have gained affixed importance and also these compounds are prospering in nature. Heterocyclic compounds like the isoquinoline [4], pyrimidine [5], pyrazole [6], quinazoline [7] and triazine [8] exhibits prominent biological activity on various therapeutic targets [9]. The therapeutic target may be a biological target or may be a protein or nucleic acid whose activity can be changed by an external stimulus. Out of which quinoline, triazine and isoquinoline derivatives are very significant classes which are therapeutically useful antibacterial drugs. These compounds are present in various natural products. It is very interesting to note that many of them possess impressive physiological and biological properties [10,11]. In the fields of pharmaceuticals, agro chemistry [12], and industry these compounds show diverge applications. These compounds are also present in natural sources that show insecticidal, plant growth regulation, pigment functions and antibacterial properties. Heterocyclic compounds have antibacterial activity, which has had a growing interest because in appropriated use of antibiotic has increased the resistance of bacterial to the commercial antibiotics, even the appearance of bacterial strain with no treatment knows. Another prominent study in the fields of science including organic chemistry has been emerged with the rise of antibiotic resistance in certain bacterial multitude. In a wide range of natural and synthetic products these compounds act as substructures and hence gain importance.



Scheme 1: Synthesis of compounds B1-B5.
Reagents & conditions: (a) NaH, EtI, THF; (b) Cs_2CO_3, tetrakis, 1,4-dioxane: water (8:2), 110ºC, 3h, (c) KOt-Bu, Pd(dppf)$2Cl_2$, t-butylxphos, DMF, 150ºC, 3h

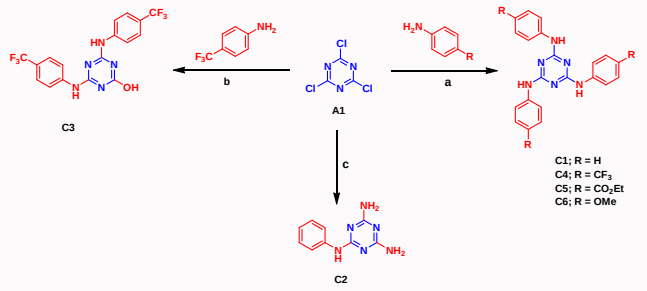
In order to develop a new derivative of heterocyclic [13-15] system with more biological properties [16-18], significant attempts is being continually inculcated [19]. Today almost every harmful bacteria and fungi have acquired resistance against the antimicrobial agents which is currently available. And hence it has become a cardinal way to design various frames of reference and to orchestrate new antimicrobial agents. The medicinal chemist also should make sure that the developed antimicrobial agent should be having vigorous effects in a very short time and also should be with less toxicity. As an extension to this, chemists have flourishingly developed very effective antimicrobial agents based on heterocyclic compounds. Here we mainly dealt with the antibacterial and antifungal activity of the derivatives of various nitrogen heterocyclic compounds using standard anti-bacterial and antifungal strains.

As a part of our research work on the development of new anti-microbial agents [20,21] it is intended to introduce nitrogen hetro cyclic like pyrazole, pyrimidine, isoquinoline and quinoline into a 6-bromo 2 napthyl ethyl ether substrate. In order to compare the better activity of nitrogen heterocyclic to other normal poly nuclear hydro carbon, we planned to introduce phenathracene in to the same substrate. It has been observed that the nitrogen heterocyclic substituted napthylethyl ether shows the better activity of compound B1, among these B2 and B3 shows good activity. In addition to the above synthesized compound, we also tried to compare the antimicrobial activity of substituted 1, 3, 5triazine. Result shows that suitably substituted (electron withdrawing) triazine show good anti-microbial activity. The resulted compounds were separated and purified by HPLC. It has been hoped that the synthesis of these active molecules in the new molecular design would lead to better antimicrobial agents. In this communication, we here in report the synthesis, characterization, antimicrobial and antifungal activities of differently substituted naphthalene and triazine incorporated heterocyclic compounds (Scheme 1). In addition, we report the antibacterial and antifungal activity of some substituted triazine (Scheme 2).

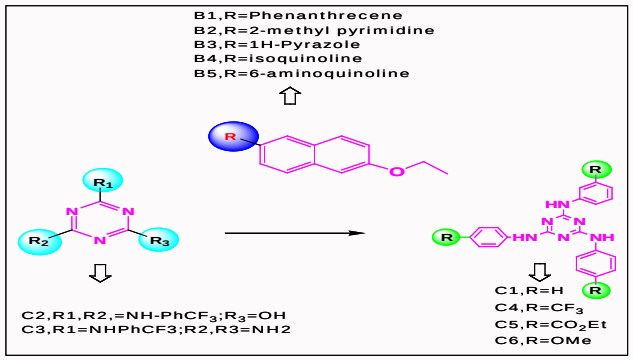
The compounds were synthesized by a series of reaction as shown in the Scheme 1 and Scheme 2. The intermediate 2-bromo 6-ethoxy naphthalene in Scheme 1 was synthesized by condensing 6-bromo 2-napthol and ethyl iodide in dry THF using NaH as base at ambient temperature. The intermediate 2-bromo 6-ethoxy naphthalene was converted into different substitution (B1 to B4) by Suzuki coupling using different aryl boronic acids, cesium carbonate as base and tetrakis as catalyst in 1-4dioxane –water (8:2) as solvent. Whereas B5 is synthesized from same intermediate by Buchwald coupling using 6 amino quinoline, Pd(dppf)$_2Cl_2$, t-ButylXphos and potassium tertiary butoxide in DMF at 150ºC. The compounds C1 to C6 are synthesized from cyanuric chloride. Compound C1, C4, C5 & C6 were synthesized by mixing the cyanuric chloride (A1) with respective aniline in methanol/ ethanol and the reaction heated to 170ºC- 200ºC for 12 to 24 hours in seal tube (Scheme 2). The compound C3 was prepared by taking compound A1 in methanolic ammonia and heated to 50ºC for 4 hours. Then followed by the addition of aniline in methanol and heated to 150ºC for 12 hours. The compound C2 was synthesized from A1 with 4-amino phenol followed by the addition of water. The newly synthesized compounds were characterized by 1H-NMR and LCMS analysis. The detailed experimental procedures and spectral data for all the synthesized compounds are given as follows.

As a part of developing active biological molecule we have synthesize two series of molecule as per the Scheme 1 and Scheme 2. Interestingly both the series molecules show moderate to good activity against tested organisms. Antimicrobial data of targeted compounds in Table 1 and Table 2 has clearly shown that they have broad spectrum activity. There for keeping B and A1 as starting point, developed some of the derivatives. At the region 1 (Scheme 1) substituted with differently active heterocyclic molecule and from the antimicrobial evaluation it is evident that compound B2 and B3 show good activity, Based on the interesting results we have synthesized more derivatives (electron withdrawing and electron donating) of B2 and B3 at region (R) of the molecule is under progress in our laboratory in order to get the highly active antimicrobial molecule which will be communicated. At the same time we have developed amine substituted isoquinoline derivative at the region (R) of the molecule (B5) and were showing moderate activity towards the tested organism. Based on the interesting result we have substituted some electron withdrawing and electron donation group at the different region of the triazine molecule to know the effect of substitution on the biological activity of the molecule. Surprisingly compounds C3, C4and C5 were showing enhanced activity and compound C1,C2 and C6 shows very less activity against tested microorganisms, this might be due to the presence of phenyl and amino group attached to the triazine ring decreases the lipophilic nature of the compound, thereby making the molecule impermeable to the cell membrane. From the above results it has been very clear that electron withdrawing group at the selected region of the triazine molecule increase the activity and electron donating group in the triazine molecule lower the activity. Work aimed at investigating further the scope of the active molecule is currently being pursued. As per the literature we know that the presence of the some heterocyclic ring is essential for a broad spectrum antimicrobial activity. The substitution on the napthyl ether with phenanthrene, pyrimidine, pyrazole, isoquinoline and quinoline group was carefully selected for considering electronic environments of the structures.

All reagents were purchased from Aldrich. Solvents used were extra dried. Final purifications were carried out using Quad biotage Flash purifier (A Dyax crop. Company). TLC experiments were performed on alumina backed silicagel 40 F254 plates (Merck, Darmstadt, Germany).The plates were illuminated under UV(254 nm) and molesybidinic acid. All ^{1}H NMR spectra were recorded on a Bruker AM-300 (300.12 MHz) and AM-400 (400.13), Bruker Biospin Corp., Germany. Molecular weights of unknown compounds were checked by LCMS 6200series Agilent Technology. Chemical shifts are reported in ppm (δ) with reference to internal standard TMS. The signals are designated as follows: S, singlet; d, doublet; t, triplet; m, multiplet.



Scheme 2: Synthesis of compounds C1-C6.
The compound **C1, C4, C5 & C6** were synthesized by mixing the compound **A1** with respective aniline in methanol/ ethanol and the reaction heated to 170ºC - 200ºC for 12–24 h in seal tube **(scheme 2).** The compound **C3** was prepared by taking compound **A1** in methanolic ammonia and heated to 50ºC for 4h. Then followed by the addition of aniline in methanol and heated to 150ºC for 12 h. The compound C2 was synthesized from A1 with 4-amino phenol followed by the addition of water **(scheme 2).**



Reagents & conditions: (a) Ethanol/ methanol, 170ºC - 200ºC, 12 - 24 h (b) i) Methanol, 150ºC, 4 h ii) H_2O, 150 ºC, 12 h (c) methanolic ammonia, 50ºC, 4 h ii) aniline, methanol, 150ºC, 12 h.
2. Graphical abstract.

Synthesis of 2-bromo-6-ethoxynaphthalene (B)

An oven dried round bottom flask a solution of 6-bromo-2-napthol (5.0 g, 0.022 mol) in dry THF solution (50 mL) was added cooled the mixture using an ice bath, added sodium hydride (1.08 g, 0.045 mol) followed by the addition of ethyl iodide (2.2 mL 0.0275 mol) at 0ºC and the reaction mixture was stirred for 3h at ambient temperature. The reaction was monitored by Thin Layer Chromatography (TLC). After completion of the reaction, the reaction mixture was quenched with ice and diluted with 100 mL of water. The aqueous layer was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography using 5% ethyl acetate in hexane as eluent gave 2-bromo-6-ethoxynaphthalene B (5.0 g, 91.23% yield).

Synthesis of 9-(6-ethoxynaphthalen-2-yl) phenanthrene (B1)

To a solution of 2-bromo-6-ethoxynaphthalene B (0.5 g, 0.002 mol) in dry 1,4-dioxane and water (8:2) solution (10 mL) was added phenanthrecene-9-boronic acid (0.44g, 0.0022 mol) followed by the addition of cesium carbonate solution (0.97 g, 0.003 mol). Before the addition of tetrakis (0.23 g, 0.0002 mol), the reaction mixture was degassed for 10 minutes and the reaction mixture was heated in sealed tube at 110ºC for 3 h. Reaction was monitored by TLC and showed the completion of starting material. The reaction mixture filtered through celite and thoroughly washed with ethyl acetate (50 mL). The reaction mixture was washed with water (50 mL) and the organic layer was dried over sodium sulphate and concentrated under vacuum. The crude product was purified by column chromatography using 3% ethyl acetate in hexane as eluent gave 9-(6-ethoxynaphthalen-2-yl) phenanthrene B1 (0.4 g, 57% yield). White solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.90 (m, 2H), 7.90 (m, 3H), 7.84 (m, 3H), 7.62 (m, 4H), 7.56 (m, 1H), 7.22 (m, 2H), 4.22 (q, J = 13.2, 6.4 Hz), 1.54 (t, J = 6.5 Hz, 3H); LCMS: m/z calculated for $C_{26}H_{20}O$: 348.4; Observed mass: 349.2(M + H); Anal. Calculated for $C_{26}H_{20}O$: C, 89.62; H, 5.79; Found: C, 89.82; H, 5.78.

Synthesis of 5-(6-ethoxynaphthalen-2-yl)-2-methylpyrimidine (B2)

To a solution of 2-bromo-6-ethoxynaphthalene B (0.5 g, 0.002 mol) in dry 1,4-dioxane and water (8:2) solution (10 mL) was added 2-methyl-pyrimidine-5-boronic ester (0.484g, 0.0022 mol) followed by the addition of cesium carbonate solution (0.97 g, 0.003 mol). Before the addition of tetrakis (0.23 g, 0.0002 mol), the reaction mixture was degassed for 10 min and the reaction mixture was heated in seal tube at 110ºC for 3 h. Reaction was monitored by TLC and showed the completion of starting material. The reaction mixture filtered through celite and thoroughly washed with ethyl acetate (50 mL). The reaction mixture was with water (50 mL) and the organic layer was dried over sodium sulphate and concentrated over vacuum. The crude product was purified by column chromatography using 3% ethyl acetate in hexane as eluent gave 5-(6-ethoxynaphthalen-2-yl)-2-methylpyrimidine B2 (0.3 g, 56.8% yield). White solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (s, 2H), 7.94 (s, 1H), 7.84 (m, 2H), 7.63 (dd, J = 4.4 Hz 1H), 7.23 (m, 1H), 7.21 (m, 1H), 4.17 (q, J = 14.0, 6.8 Hz), 2.82 (s, 3H), 1.51 (t, J = 6.8 Hz, 3H); LCMS: m/z calculated for $C_{17}H_{16}N_2O$: 264.3; Observed mass: 265.2 (M+H); Anal. Calculated for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60; Found: C, 77.27; H, 6.10; N, 10.59.

Synthesis of 4-(6-ethoxynaphthalen-2-yl)-1H-pyrazole (B3)

To a solution of 2-bromo-6-ethoxynaphthalene B (0.5 g, 0.002 mol) in dry 1,4-dioxane and water (8:2) solution (10 mL) was added 1H-pyrazole-4-boronic ester (0.426 g, 0.0022 mol) followed by the addition of cesium carbonate solution (0.97 g, 0.003 mol). Before the addition of tetrakis (0.23 g, 0.0002 mol), the reaction mixture was degassed for 10 min and the reaction mixture was heated in seal tube at 110ºC for 3 hours. Reaction was monitored by TLC and showed the completion of starting material. The reaction mixture filtered through celite and thoroughly washed with ethyl acetate (50 mL). The reaction mixture was with water (50 mL) and the organic layer was dried over sodium sulphate and concentrated over vacuum. The crude product was purified by column chromatography using 3% ethyl acetate in hexane as eluent gave 4-(6-ethoxynaphthalen-2-yl)-1H-pyrazole B3 (0.25 g, 52% yield). White solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.88 (m, 1H), 7.79 (m, 1H), 7.68 (m, 2H), 7.17 (m, 1H), 6.73 (s, 1H), 6.39 (s, 1H), 4.19 (q, J = 14.0, 7.2 Hz, 2H), 1.51 (t, J = 7.2 Hz, 3H); LCMS: m/z calculated for $C_{15}H_{14}N_2O$: 238.2; Observed mass: 239.2 (M+H); Anal. Calculated for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; Found: C, 75.60; H, 5.90; N, 14.76.

Synthesis of 6-(6-ethoxynaphthalen-2-yl) isoquinoline (B4)

To a solution of 2-bromo-6-ethoxynaphthalene B (0.5 g, 0.002 mol) in dry 1,4-dioxane and water (8:2) solution (10 mL) was added isoquinolin-6-ylboronic acid (0.346 g, 0.0022 mol) followed by the addition of cesium carbonate solution (0.97 g, 0.003 mol). Before the addition of tetrakis (0.23 g, 0.0002 mol), the reaction mixture was degassed for 10 min and the reaction mixture was heated in seal tube at 110°C for 3 hours. Reaction was monitored by TLC and showed the completion of starting material. The reaction mixture filtered through celite and thoroughly washed with ethyl acetate (50 mL). The reaction mixture was with water (50 mL) and the organic layer was dried over sodium sulphate and concentrated over vacuum. The crude product was purified by column chromatography using 3% ethyl acetate in hexane as eluent gave 4-(6-ethoxynaphthalen-2-yl) isoquinoline B4 (0.3 g, 50% yield). White solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (s, 1H), 8.31 (m, 1H), 8.18 (m, 1H), 7.82 (m, 4H), 7.58 (m, 3H), 7.38 (m, 1H), 7.30 (m, 1H), 4.22 (q, J = 13.5, 6.6 Hz, 2H), 1.53 (t, J = 6.9 Hz, 3H); LCMS: m/z calculated for $C_{21}H_{17}NO$: 299.37; Observed mass: 300.2 (M+H); Anal. Calculated for $C_{21}H_{17}NO$: C, 84.25; H, 5.72; N, 4.68; Found: C, 84.26; H, 5.74; N, 4.68.

Synthesis of N-(6-ethoxynaphthalen-2-yl) quinolin-6-amine (B5)

To a solution of 2-bromo-6-ethoxynaphthalene B (0.5 g, 0.002 mol) in dry DMF solution (10 mL) was added quinolin-6-amine (0.316 g, 0.0022 mol) followed by the addition of potassium tert - Butoxide (0.336 g, 0.003 mol). Before the addition of Pd (dppf) 2Cl2 (0.146 g, 0.0002 mol) and t-ButylXphos (0.084 g, 0.0002 mol), the reaction mixture was degassed for 10 min and the reaction mixture was heated in seal tube at 150°C for 3 hours. Reaction was monitored by TLC and showed the completion of starting material. The reaction mixture filtered through celite and thoroughly washed with ethyl acetate (50 mL). The reaction mixture was with water (50 mL) and the organic layer was dried over sodium sulphate and concentrated over vacuum. The crude product was purified by column chromatography using 3% ethyl acetate in hexane as eluent gave N-(6-ethoxynaphthalen-2-yl) quinolin-6-amine B5 (0.3 g, 47% yield). White solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (d, J = 4.0 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 8.65 (d, J = 8.8 Hz, 1H), 7.57 (s, 1H), 7.47 (dd, J = 9.2, 2.8 Hz, 1H), 7.35 (m, 3H), 7.17 (m, 1H), 7.14 (s, 1H), 6.08 (s, 1H), 4.17 (q, J = 14.0, 6.8 Hz, 2H), 1.51 (t, J = 6.8 Hz, 3H); LCMS: m/z calculated for $C_{21}H_{18}N_2O$: 314.38; Observed mass: 315.2 (M+H); Anal. Calculated for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91; Found: C, 80.24; H, 5.76; N, 8.90.

Synthesis of N2, N4, N6-triphenyl-1, 3, 5-triazine-2, 4, 6-triamine (C1)

To a solution of 2, 4, 6-trichloro-1, 3, 5-triazine (0.5 g, 0.0027 mol) in methanol (20 mL) was added Aniline (5 mL) at room temperature and the reaction mixture was heated to 150°C in seal tube for 24 hours. A white precipitate obtained was filtered and dried over vacuum for 4 h gave N2,N4,N6-triphenyl-1,3,5-triazine-2,4,6-triamine C1 (0.6 g, 63 % yield). White solid; 1H NMR (400 MHz, DMSO-d6): δ 9.24 (s, 3H), 7.81 (d, J = 8.0 Hz, 6H), 7.29 (t, J = 7.6 Hz, 6H), 7.00 (d, J = 7.2 Hz, 3H); LCMS: m/z calculated for $C_{21}H_{18}N_6$: 354.4; Observed mass: 355.2 (M+H); Anal. Calculated for $C_{21}H_{18}N_6$: C, 71.17; H, 5.12; N, 23.71; Found: C, 71.18; H, 5.13; N, 23.69.

Synthesis of N2-phenyl-1,3,5-triazine-2,4,6-triamine (C2)

To a solution of 2,4,6-trichloro-1,3,5-triazine (1.0 g, 0.0054 mol) in methanol (20 mL) was added methanolic ammonia (5 mL) at room temperature and the reaction mixture was heated to 150°C in seal tube. A white precipitate formed was filtered and dried over vacuum for 4 h gave 6-chloro-1,3,5-triazine-2,4-diamine (0.75 g). To the above compound in methanol (10 mL) was added Aniline (2 mL) in seal tube and the reaction was heated to 160°C for 24 h gave N2-phenyl-1,3,5-triazine-2,4,6-triamine C2 (0.5 g, 50% yield). White solid;1H NMR (400 MHz, DMSO-d6): δ 9.702 (s, 4H), 9.22 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.02 (d, J = 7.2 Hz, 1H); LCMS: m/z calculated for C9H10N6: 202.2; Observed mass: 203.2 (M+H); Anal. Calculated for $C_{21}H_{18}N_6$: C, 53.46; H, 4.98; N, 41.56; Found: C, 53.49; H, 4.96; N, 41.57.

Synthesis of 4,6-bis((4-(trifluoromethyl) phenyl)amino)-1,3,5-triazin-2-ol (C3)

To a solution of 2, 4, 6-trichloro-1, 3, 5-triazine (0.5 g, 0.0027 mol) in ethanol (20 mL) was added 4-(trifluoromethyl) aniline (1 mL) at room temperature and the reaction mixture was heated to 100°C in seal tube for 12 h. A white precipitate formed was filtered and dried over vacuum for 4 h gave compound (6-Chloro-N,N'-bis-(4-trifluoromethyl-phenyl)-[1,3,5]triazine-2,4-diamine) (0.6 g). To the compound in ethanol (10 mL) was added water (2 mL) in seal tube and the reaction was heated to 200° C for 4hour gave 4,6-bis((4-(trifluoromethyl)phenyl)amino)-1,3,5-triazin-2-ol C3 (0.5 g, 44% yield).1H NMR (500 MHz, $CDCl_3$): δ 8.70 (s, 2H), 8.03 (s, 2H), 7.90 (s, 1H), 7.53 (m, 1H), 7.36 (d, 1H), 6.11(s, 1H), 4.17(m, 2H); LCMS: m/z calculated for $C_{17}H_{11}F_6N_5O$: 415.29; Observed mass: 415.41 (M+H); Anal. Calculated for $C_{17}H_{11}F_6N_5O$: C, 49.17; H, 2.67; N, 16.86; Found: C, 50.18; H, 2.93; N, 16.79.

Synthesis of N2,N4,N6-tris(4-(trifluoromethyl)phenyl)-1,3,5-triazine-2,4,6-triamine (C4)

To a solution of 2,4,6-trichloro-1,3,5-triazine (0.5 g, 0.0027 mol) in ethanol (20 mL) was added 4-(trifluoromethyl) aniline (2 mL) at room temperature and the reaction mixture was heated to 100°C in seal tube for 24 h. A white precipitate formed was filtered and dried over vacuum for 4 h gave N2,N4,N6-tris(4-(trifluoromethyl)phenyl)-1,3,5-triazine-2,4,6-triamine C4 (0.5 g, 33% yield). White solid; 1H NMR (300 MHz, DMSO-d6): δ 9.69 (s, 3H), 8.20 (bs, 3H), 7.98 (s, 3H), 7.53 (t J = 7.5 Hz, 3H), 7.36 (d, J = 7.2 Hz, 3H); LCMS: m/z calculated for $C_{24}H_{15}F_9N_6$: 558.4; Observed mass: 559.0 (M+H); Anal. Calculated for $C_{24}H_{15}F_9N_6$: C, 51.62; H, 2.71; F, 30.62; N, 15.05; Found: C, 51.64; H, 2.70; F, 30.61; N, 15.05.

Synthesis of triethyl 4,4',4''-((1,3,5-triazine-2,4,6-triyl)tris(azanediyl))tribenzoate (C5)

To a solution of 2,4,6-trichloro-1,3,5-triazine (0.5 g, 0.0027 mol) in ethanol (10 mL) was added 4-methoxy aniline (2 mL) at room temperature and the reaction mixture was heated to 200°C in seal tube for 24 h. A white precipitate formed was filtered and dried over vacuum for 4 h gave triethyl 4,4',4''-((1,3,5-triazine-2,4,6-triyl) tris(azanediyl))tribenzoate C5 (0.5 g, 26 % yield). White solid; 1H NMR (400 MHz, DMSO-d6): δ 9.70 (s, 3H), 8.19 (s, 6H), 7.59 (d, J = 8.0 Hz, 3H), 7.41 (t, J = 8.0 Hz, 3H), 4.25 (q, J = 14.0, 7.2 Hz, 6H), 1.24 (t, J = 7.2 Hz, 9H); LCMS: m/z calculated for $C_{30}H_{30}N_6O_6$: 570.6;

Observed mass: 571.0 (M+H); Anal. Calculated for $C_{30}H_{30}N_6O_6$: C, 63.15; H, 5.30; N, 14.73; Found: C, 63.14; H, 5.32; N, 14.73.

Synthesis of N2,N4,N6-tris(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine (C6)

To a solution of 2,4,6-trichloro-1,3,5-triazine (0.5 g, 0.0027 mol) in methanol (10 mL) was added 4-methoxy aniline (2 mL) at room temperature and the reaction mixture was heated to 170°C in seal tube for 24 h. A white precipitate formed was filtered and dried over vacuum for 4 h gave N2,N4,N6-tris(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine C6 (0.4 g, 33% yield). White solid; 1H NMR (400 MHz, DMSO-d6): δ 8.95 (s, 3H), 7.64 (bs, 6H), 6.86 (d, J = 8.8 Hz, 6H), 3.73 (s, 9H); LCMS: m/z calculated for $C_{24}H_{24}N_6O_3$: 444.4; Observed mass: 445.2 (M+H); Anal. Calculated for $C_{24}H_{24}N_6O_3$: C, 64.85; H, 5.44; N, 18.91; Found: C, 64.86; H, 5.45; N, 18.90.

The synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) *Bacillus subtili and Klebsiella pneumonia* (recultured) bacterial strains by serial plate dilution method [22,23]. Serial dilutions of the drug in Mueller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16 hours to 18 hours at 37°C. The Minimum Inhibitory Concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zone of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentration of the test compounds in Dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3 days to 4 days. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [24,25].

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigarus* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) and *Candida albicans* in DMSO by serial plate dilution method [26,27]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal stain for lawning. A loopfull of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar medis was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37°C for 1 hour. Using a punch, wells were made on these seeded agar plates minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3 days to 4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Ciclopiroxolamine as standard.

Acknowledgments

We thankful to the Head of the Department of Biochemistry, Biogenix Research Center, Thiruvananthapuram to carryout biological studies. We are also gratified to the Head, Chemistry Department, and School of Chemical Sciences for the kind support and providing necessary laboratory facilities.

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