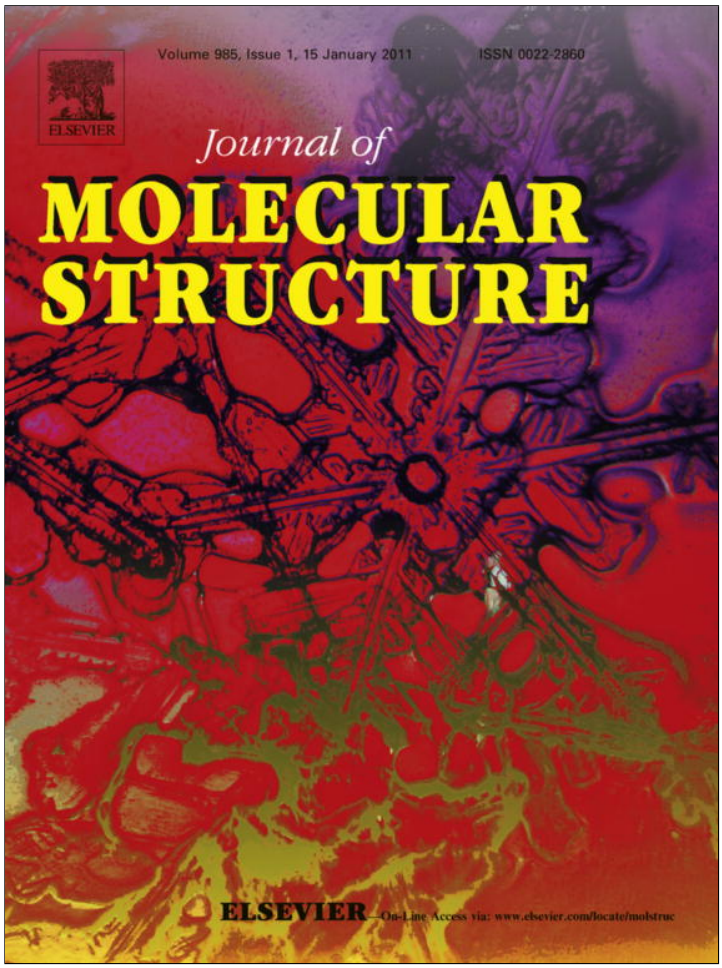

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Synthesis, characterization and anticancer evaluation of novel tri-arm star shaped 1,3,5-triazine hydrazones

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ABSTRACT

A series of novel trisubstituted triazine hydrazones [N_3C_3(—OC_6H_4-*p*-CH=N—NH—C(O)—C_6H_4-*p*-X)$_3$] (X = H, Br, Cl, F, OH, OCH_3, CH_3, NO_2, NH_2) were prepared by a three-fold condensation reaction of 2,4,6-tris (4-formylphenoxy)-1,3,5-triazine with *p*-substituted benzoic acid hydrazides [NH_2—NH—C(O)—C_6H_4-*p*-X] with excellent yields. The structures were confirmed by elemental analysis, FT-IR, ^{1}H, ^{13}C, 2D-HSQC NMR and mass spectrometry (MALDI-TOF). These derivatives bearing hydrolysable hydrazone linkages were evaluated for their *in vitro* antiproliferative activity against the human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa).

1. Introduction

The synthetic utility and much of the industrial importance of triazine heterocycle arises from the ease by which nucleophilic aromatic substitutions occur on halogenated 1,3,5-triazines [1,2]. In the synthesis of numerous compounds bearing the *s*-triazine core, 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) has been largely used as a starting material due to the ease of displacement of chlorine atoms by various nucleophiles. The systematic replacement of chlorine atoms under controlled temperature enhances the utility of this low-cost and readily available reagent for the preparation of mono-, di- and trisubstituted *s*-triazines [2,3] and offers access to a multitude of useful molecules, from medicinal chemistry to materials [4,5]. 1,3,5-Triazine-containing compounds are powerful chelating agents and have been used for the preparation of various metal complexes [6] and liquid crystals [7]. *s*-Triazine derivatives have proven their great potential in supramolecular chemistry [8]. Recently triazines have found extensive use as reagents in the conversion of functional groups [9]. Stepwise selective substitution pattern of cyanuric chloride is successfully utilized in the preparation of triazine based dendrimers [10].

In addition to the selective reactivity of three chlorine atoms of cyanuric chloride towards a variety of nucleophiles, the reaction of functional groups on the substituents attached to the 1,3,5-triazine ring system have drawn considerable interest. Recently hyperbranched polymers have been synthesized using the reactivity of peripheral functional groups on the aryl substituents appended on *s*-triazine AB_2 type monomer structural units [11,12]. 2,4,6-Tris(4-formylphenoxy)-1,3,5-triazine (Tripod) possessing three reactive peripheral aldehyde groups is used as a template for molecular imprinting of solid surfaces [13] and the synthesis of a three-helix bundle protein [14]. It is also used for immobilization of proteases [15]. Recently Tripod has been utilized for the preparation of Schiff bases [16], tripodal-benzimidazole [17] and star-shaped small monomer containing terminal cyanovinylene 4-nitrophenyls arms [18]. Although some examples of formation of thiosemicarbazones by the condensation of Tripod with thiosemicarbazides is described [19], to our knowledge formation of hydrazones by the reaction of aromatic hydrazides with Tripod has not been reported and deserves further exploration.

Hydrazones containing an azomethine (—C(O)NHN=CH—) group are important synthons for several transformations and have gained importance due to their broad spectrum of biological activities [20,21]. Aryl hydrazones and their metal complexes have attracted attention due to their therapeutic activity and application in materials research [22,23]. Hydrazone linkage provides a suitable system for pH-dependent release of drugs from drug-conjugates [24]. Several hydrazide–hydrazone derivatives have been shown to exhibit antiproliferative activities and act as cytotoxic agents

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with the ability to prevent cell progression in cancerous cells through different mechanisms [25] and also exhibited antimicrobial [26,27] and antitumoral [28] activities.

The triazine scaffold has provided basis for the design of biologically relevant molecules with broad biomedical value as therapeutics. It was reported that some of the triazine derivatives possess potent biological activity [29–31]. In continuation of our work on star shaped hydrazones [32], in this article we present the synthesis, characterization and *in vitro* anticancer efficacy of a series of *s*-1,3,5-triazine hydrazones prepared by the three-fold condensation of Tripod with *p*-substituted benzoic hydrazides against human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa).

2. Experimental

2.1. Materials and measurements

IR spectra were recorded in KBr matrix using an Impact-410 Nicolet (USA) FT-IR spectrometer in 4000–400 cm^{-1} range. The ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on BRUKER AV-400 MHz High Resolution Multinuclear FT-NMR Spectrometer at room temperature. The ^1H and ^{13}C NMR chemical shifts were measured using $SiMe_4$ as an internal standard at $\delta = 0$ ppm. In ^1H NMR spectrum, DMSO-d_6 solvent residual peak was observed at 2.49 ppm and water peak at 3.30 ppm. In ^{13}C NMR spectrum DMSO-d_6 solvent residual peak was observed at 39.50 ppm. Elemental analyses (C, H, N) were carried using the Leco Model Truespec CHNS Analyser. The GCMS spectrum was measured with SHIMADZU GCMS-QP2010S spectrometer. The MALDI-TOF mass spectrum was measured with a Voyager-DE STR spectrometer with α-cyano-4-hydroxycinnamic acid as the matrix. Melting points were determined in an open capillary on a Gallenkamp melting point apparatus and are uncorrected.

Solvents were purified by standard methods [33]. Cyanuric chloride was procured from Sigma Aldrich and used as received. The purity of compounds were checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates.

2.2. Synthesis

2.2.1. 2,4,6-tris(4-formylphenoxy)-1,3,5-triazine **(1)** [$N_3C_3(-OC_6H_4$-*p*-$CHO)_3$]

Tripod (**1**) was synthesized according to reported method [34]. Yield: 61%, m.p. 174–176 °C. IR (KBr, cm^{-1}): 1702 (C=O), 1569 (C=$N_{Triazine}$). ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.31 (d, 6H, C^2H), 7.93 (d, 6H, C^3H), 10.00 (s, 3H, CHO). ^{13}C NMR ($CDCl_3$, δ ppm): 122.59 (C^2), 131.69 (C^3), 134.85 (C^4), 156.07 (C^1), 173.62 (C^T), 190.99 (CHO). MS m/z: 441 (M^+). Anal. Calc. For $C_{24}H_{15}N_3O_6$: C, 65.31; H, 3.43; N, 9.52%. Found: C, 65.23, H, 3.38; N, 9.46%.

2.2.2. Synthesis of *p*-substituted benzoic hydrazides ***2a–i***

Methyl benzoates were synthesized from their respective *p*-substituted benzoic acids, using excess of dry methanol in presence of H_2SO_4. *p*-Substituted benzoic acid hydrazides **(2a–i)** were prepared by reaction of the corresponding methyl benzoates (10 mmol) with hydrazine hydrate 99% (50 mmol) in methanol under reflux for 4–6 h. The excess solvent was removed under vacuum and the residue was filtered under suction, washed with water and dried. The spectral and analytical data of benzoic hydrazide (**2a**) [35], 4-bromobenzoic hydrazide (**2b**) [36], 4-chlorobenzoic hydrazide (**2c**) [37], 4-fluorobenzoic hydrazide (**2d**) [35], 4-hydroxybenzoic hydrazide (**2e**) [38], 4-methoxybenzoic hydrazide (**2f**) [39], 4-methylbenzoic hydrazide (**2g**) [37], 4-nitrobenzoic hydrazide (**2h**) [37] and 4-aminobenzoic hydrazide (**2i**) [38] are in good agreement with literature values.

2.2.3. General procedure for the preparation of triazine hydrazone compounds ***3a–i***

Tripod **(1)** (0.106 g, 0.24 mmol) was added to a solution of benzoic hydrazide (**2a**) (0.109 g, 0.8 mmol) in tetrahydrofuran (75 mL) and the reaction mixture was stirred under reflux for 15–18 h. The solvent was removed by evaporation under reduced pressure. The residue was filtered under suction and washed several times with hot tetrahydrofuran. The resulting solid (**3a**) was dried in vacuo at 40 °C for 4 h. The same general procedure was followed for the compounds **3b–i**.

2.2.4. Experimental details of ***3a-i***

2.2.4.1. 2,4,6-tris[4-{(E)-[2′(phenylcarbonyl)hydrazinylidene]methyl} phenoxy]-1,3,5-triazine (3a). Yield: 96.8%. m.p. 255–258 °C. IR (KBr) cm^{-1}: 3239 (N—H), 1655 (C=O), 1607 (C=N), 1573 (C=$N_{Triazine}$), 1367 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 7.34 (d, 6H, *J* = 7.2 Hz, C^2H), 7.50 (d, 6H, *J* = 7.2 Hz, C^3H), 7.59 (d, 3H, *J* = 7.6 Hz, $C^{10}H$), 7.77 (t, 6H, *J* = 7.6 Hz, *J* = 8.0 Hz, C^9H), 7.88 (d, 6H, *J* = 8.0 Hz, C^8H), 8.48 (s, 3H, C^5H), 11.84 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 121.95 (C^2), 124.85 (C^8), 127.46 (C^9), 128.40 (C^3), 131.70 (C^4), 132.25 (C^{10}), 133.33 (C^7), 146.82 (C^5), 152.44 (C^1), 163.09 (C^6), 172.92 (C^T). MS (MALDI-TOF) m/z: 818.50 $(M + Na)^+$. Anal. Calcd. For $C_{45}H_{33}N_9O_6$: C, 67.92; H, 4.18; N, 15.84%. Found: C, 67.83; H, 4.12; N, 15.76%.

2.2.4.2. 2,4,6-tris[4-{(E)-[2′(4-bromophenylcarbonyl)hydrazinylidene] methyl}phenoxy]-1,3,5-triazine (3b). Yield: 98.0%. m.p. > 300 °C. IR (KBr) cm^{-1}: 3232 (N—H), 1656 (C=O), 1614 (C=N), 1567 (C=$N_{Triazine}$), 1364 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 7.33 (d, 6H, *J* = 7.1 Hz, C^2H), 7.70 (d, 6H, *J* = 7.1 Hz, C^3H), 7.76 (d, 6H, *J* = 7.6 Hz, C^9H), 7.83 (d, 6H, *J* = 7.6 Hz, C^8H), 8.46 (s, 3H, C^5H), 11.89 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 121.98 (C^2), 125.50 (C^{10}), 128.31 (C^8), 129.66 (C^3), 131.43 (C^4), 132.14 (C^9), 132.35 (C^7), 147.17 (C^5), 152.52 (C^1), 162.11 (C^6), 172.93 (C^T). MS (MALDI-TOF) m/z: 1052.23 $(M + Na)^+$. Anal. Calcd. For $C_{45}H_{30}Br_3N_9O_6$: C, 52.35; H, 2.93; N, 12.21%. Found: C, 52.31; H, 2.89; N, 12.26%.

2.2.4.3. 2,4,6-tris[4-{(E)-[2′(4-chlorophenylcarbonyl)hydrazinylidene] methyl} phenoxy]-1,3,5-triazine (3c). Yield: 98.0%. m.p. 289–293 °C. IR (KBr) cm^{-1}: 3237 (N—H), 1655 (C=O), 1601 (C=N), 1569 (C=$N_{Triazine}$), 1363 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 7.36 (d, 6H, *J* = 7.1 Hz, C^2H), 7.58 (d, 6H, *J* = 7.6 Hz, C^9H), 7.81 (d, 6H, *J* = 7.1 Hz, C^3H), 7.92 (d, 6H, *J* = 7.6 Hz, C^8H), 8.47 (s, 3H, C^5H), 11.95 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 121.94 (C^2), 126.27 (C^9), 126.45 (C^8), 129.44 (C^3), 131.09 (C^4), 132.07 (C^7), 136.49 (C^{10}), 147.04 (C^5), 152.43 (C^1), 161.92 (C^6), 172.96 (C^T). MS (MALDI-TOF) m/z: 920.38 $(M + Na)^+$. Anal. Calcd. For $C_{45}H_{30}Cl_3N_9O_6$: C, 60.11; H, 3.36; N, 15.84%. Found: C, 60.04; H, 3.29; N, 15.90%.

2.2.4.4. 2,4,6-tris[4-{(E)-[2′(4-florophenylcarbonyl)hydrazinylidene] methyl} phenoxy]-1,3,5-triazine (3d). Yield: 89.7%. m.p. 212–216 °C. IR (KBr) cm^{-1}: 3236 (N—H), 1655 (C=O), 1605 (C=N), 1569 (C=$N_{Triazine}$), 1366 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 7.34 (d, 6H, *J* = 8.0 Hz, C^2H), 7.79 (d, 6H, *J* = 8.0 Hz, C^9H), 7.97 (d, 6H, *J* = 8.0 Hz, C^3H), 7.99 (d, 6H, *J* = 8.0 Hz, C^8H), 8.46 (s, 3H, C^5H), 11.91 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 115.26 (C^9), 121.93 (C^2), 128.14 (C^8), 129.65 (C^3), 130.20 (C^7), 132.12 (C^4), 146.80 (C^5), 152.39 (C^1), 161.93 (C^6), 165.03 (C^{10}), 172.86 (C^T). MS (MALDI-TOF) m/z: 872.47 $(M + Na)^+$. Anal. Calcd. For $C_{45}H_{30}F_3N_9O_6$: C, 63.60; H, 3.56; N, 14.83%. Found: C, 63.66; H, 3.62; N, 14.76%.

2.2.4.5. 2,4,6-tris[4-{(E)-[2′(4-hydroxyphenylcarbonyl)hydrazinylidene]methyl} phenoxy]-1,3,5-triazine (3e). Yield: 98.6%. m.p. 276–281 °C. IR (KBr) cm^{-1}: 3410 (O—H), 3251 (N—H), 1641 (C=O), 1608 (C=N), 1571 (C=$N_{Triazine}$), 1374 (C—O—Ar). ^1H NMR

Table 1
Effect of test compounds on growth of HepG2 and HeLa cell lines incubated for 24 h *in vitro*.

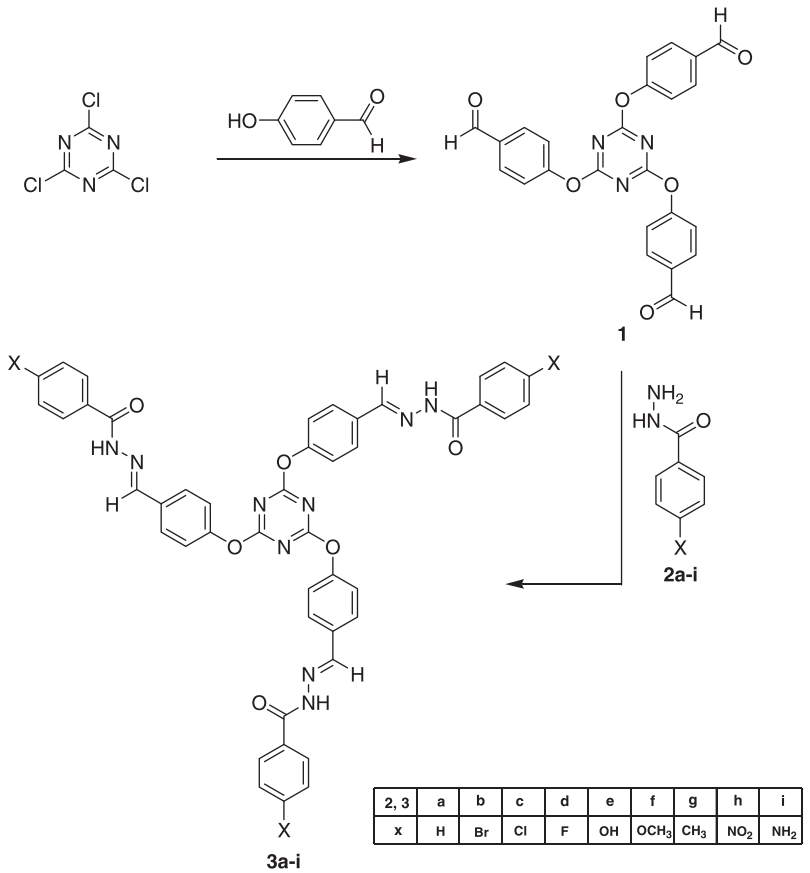
Test compounds	% Inhibition	
	HepG2	HeLa
3a	26.31	13.23
3b	8.42	19.46
3c	6.41	28.36
3d	4.32	18.19
3e	36.24	16.45
3f	34.69	9.87
3g	52.34	21.39
3h	58.91	8.36
3i	30.77	23.35

(400 MHz, DMSO-d_6, δ ppm): 6.83 (d, 6H, *J* = 7.1 Hz, C^2H), 7.32 (d, 6H, *J* = 7.6 Hz, C^9H), 7.74 (d, 6H, *J* = 7.6 Hz, C^8H), 7.78 (d, 6H, *J* = 7.1 Hz, C^3H), 8.45 (s, 3H, C^5H), 10.07 (s, 3H, OH), 11.63 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 114.94 (C^9), 121.92 (C^2), 128.09 (C^7), 128.62 (C^8), 129.64 (C^4), 132.46 (C^3), 145.80 (C^5), 152.27 (C^1), 159.18 (C^{10}), 161.96 (C^6), 172.93 (C^T). MS (MALDI-TOF) m/z: 866.48 $(M + Na)^+$. Calcd. For $C_{45}H_{33}N_9O_9$: C, 64.05; H, 3.94; N, 14.94%. Found: C, 64.13; H, 3.85; N, 14.84%.

2.2.4.6. 2,4,6-tris[4-{(E)-[2′(4-methoxyphenylcarbonyl)hydrazinylidene]methyl}phenoxy]-1,3,5-triazine (3f). Yield: 97.8%. m.p. 258–262 °C. IR (KBr) cm^{-1}: 3238 (N—H), 1649 (C=O), 1607 (C=N), 1570 ($C=N_{Triazine}$), 1365 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.82 (s, 9H, OCH_3), 7.01 (d, 6H, *J* = 8.0 Hz, C^2H), 7.32 (d, 6H, *J* = 8.4 Hz, C^9H), 7.75 (d, 6H, *J* = 8.0 Hz, C^3H), 7.88 (d, 6H, *J* = 8.4 Hz, C^8H), 8.46 (s, 3H, C^5H), 11.71 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 55.36 (OCH_3), 113.64 (C^9), 121.95 (C^2), 125.34 (C^7), 128.13 (C^8), 129.49 (C^3), 132.38 (C^4), 146.21 (C^5), 152.33 (C^1), 161.96 (C^6), 162.56 (C^{10}), 172.93 (C^T). MS (MALDI-TOF) m/z: 908.53 $(M + Na)^+$. Anal. Calcd. For $C_{48}H_{39}N_9O_9$: C, 65.08; H, 4.44; N, 14.23%. Found: C, 65.16; H, 4.54; N, 14.32%.

2.2.4.7. 2,4,6-tris[4-{(E)-[2′(4-methylphenylcarbonyl)hydrazinylidene]methyl}phenoxy]-1,3,5-triazine (3g). Yield: 97.6%. m.p. 266–270 °C. IR (KBr) cm^{-1}: 3237 (N—H), 1653 (C=O), 1610 (C=N), 1569 ($C=N_{Triazine}$), 1365 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.36 (s, 9H, CH_3), 7.28 (d, 6H, *J* = 7.1 Hz, C^9H), 7.33 (d, 6H, *J* = 7.4 Hz, C^2H), 7.76 (d, 6H, *J* = 7.4 Hz, C^8H), 7.80 (d, 6H, *J* = 7.4 Hz, C^3H), 8.47 (s, 3H, C^5H), 11.77 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 20.98 (CH_3), 121.95 (C^2), 127.59 (C^8), 128.20 (C^9), 128.92 (C^7), 130.43 (C^3), 132.32 (C^4), 141.74 (C^{10}), 146.55 (C^5), 152.39 (C^1), 162.92 (C^6), 172.93 (C^T). MS (MALDI-TOF) m/z: 860.54 $(M + Na)^+$. Anal.Calcd. For $C_{48}H_{39}N_9O_6$: C, 68.81; H, 4.69; N, 15.05%. Found: C, 68.73; H, 4.72; N, 15.13%.

2.2.4.8. 2,4,6-tris[4-{(E)-[2′(4-nitrophenylcarbonyl)hydrazinylidene]methyl} phenoxy]-1,3,5-triazine (3h). Yield: 98.6%. m.p. 225–229 °C. IR (KBr) cm^{-1}: 3214 (N—H), 1662 (C=O), 1604 (C=N), 1568 ($C=N_{Triazine}$), 1366 (C—O—Ar). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 7.34 (d, 6H, *J* = 7.1 Hz, C^2H), 7.79 (d, 6H, *J* = 7.1 Hz, C^3H), 8.10 (d, 6H, *J* = 7.4 Hz, C^8H), 8.30 (d, 6H, *J* = 7.4 Hz, C^9H), 8.49 (s, 3H, C^5H), 12.10 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 122.54 (C^2), 124.03 (C^9), 128.96 (C^8), 129.61 (C^3), 132.48 (C^4), 139.44 (C^7), 148.47 (C^5), 149.69 (C^{10}), 153.19 (C^1), 161.91 (C^6), 173.45 (C^T). MS (MALDI-TOF) m/z: 953.45 $(M + Na)^+$. Anal. Calcd. For



Scheme 1. Synthetic pathway for tri-arm 1,3,5-triazine hydrazones (**3a–i**).

$C_{45}H_{30}N_{12}O_{12}$: C, 58.07; H, 3.25; N, 18.06%. Found: C, 58.02; H, 3.32; N, 18.13%.

2.2.4.9. 2,4,6-tris[4-{(E)-[2′(4-aminophenylcarbonyl)hydrazinylidene] methyl} phenoxy]-1,3,5-triazine (3i). Yield: 97.3%. m.p. 267–270 °C. IR (KBr) cm^{-1}: 3219 (N—H), 1656 (C=O), 1605 (C=N), 1558 ($C{=}N_{Triazine}$), 1360 (C—O—Ar). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.46 (s, 6H, NH_2), 7.13 (d, 6H, J = 8.4 Hz, C^9H), 7.30 (d, 6H, J = 7.6 Hz, C^2H), 7.76 (d, 6H, J = 7.6 Hz, C^3H), 7.81 (d, 6H, J = 8.4 Hz, C^8H), 8.48 (s, 3H, C^5H), 11.86 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 115.04 (C^9), 122.48 (C^2), 123.62 (C^7), 128.59 (C^8), 130.03 (C^3), 131.54 (C^4), 147.36 (C^5), 152.45 (C^{10}), 152.92 (C^1), 162.71 (C^6), 172.91 (C^T). MS (MALDI-TOF) m/z: 863.53 $(M + Na)^+$. Anal. Calcd. For $C_{45}H_{36}N_{12}O_6$: C, 64.28; H, 4.32; N, 19.99%. Found: C, 64.27; H, 4.35; N, 19.90%.

2.3. Protocols for anticancer activity

Under a sterile condition, human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa) were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). Cytotoxicity of compounds **3a–i** at 100 μM concentration was tested against HepG2 and HeLa cell lines using MTT assay [40,41]. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO.

The cells at approximately 80% confluence were selected for trypsinization. The cells were harvested by removing the medium and then 1 mL of trypsin-EDTA (200 mg/L for EDTA, 500 mg/L for trypsin in a ratio (1:250) is added and incubated at 37 °C for about 5 min. The cells were detached from the plate and collected in a centrifuge tube and centrifuged at 1000 rpm for 5 minutes. Supernatant solution was removed and the cells were resuspended in 10 mL RPMI-1640 culture medium. Cell number was determined using hemocytometer. The cell suspension was diluted to required concentration of 5×10^4 cells/mL. 24-well microplates were seeded with 500 μL of cell suspension and incubated at 37 °C and 5% CO_2 for 24 h.

After 24 h incubation, the cells were treated with the newly synthesized compounds and then incubated for further 24 h. 100 μL of PBS solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 5 mg/mL] was added to each well, and incubation was continued for another 4 h. The medium was discarded and the cells were washed with sterile PBS and then the resultant formazan blue crystals were dissolved in 500 μL DMSO and the optical density (OD) was measured at 570 nm (reference filter 690 nm) using an automatic microplate reader. Triplicate readings were obtained for each sample and optical density values of the three wells were averaged for one sample. The results were recorded and expressed as % inhibition in cell growth of Human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa) compared with the blank control where cells incubated with DMSO (Table 1).

3. Results and discussion

3.1. Synthesis of tri-arm 1,3,5-triazine hydrazones

Tri-arm star shaped hydrazones with different substitutions at *para* positions emanating from 1,3,5-triazine core were synthesized as shown in Scheme 1. The initial reaction consists of grafting of three hydroxybenzaldehyde groups onto the 1,3,5-triazine core. The reaction of 2,4,6-trichloro-1,3,5-triazine with the sodium salt of 4-hydroxybenzaldehyde yielded 2,4,6-tris(4-formylphenoxy)-1,3,5-triazine. The second step consists of the condensation of 3 equivalents of benzoic hydrazides **2a**–**i** with the aldehydic functional groups of Tripod in THF for 15–18 h at refluxing temperature and affords the corresponding trifunctionalized hydrazones **3a**–**i**. Due to the limited solubility of 4-hydroxybenzoic hydrazide and 4-nitrobenzoic hydrazide in THF the duration of the reaction for the synthesis of **3e** and **3h** is comparatively more.

All products were generally obtained in high yields and were characterized by FT-IR, ^{1}H, ^{13}C and 2D-HSQC NMR spectroscopy and MALDI-TOF mass spectrometry.

3.2. IR spectral studies

The diagnostic IR bands of hydrazones **3a–i** are presented in the experimental section. The aldehyde carbonyl groups of the core (**1**) which were observed at 1705 cm^{-1} disappeared following hydrazone formation, accompanied by the appearance of imine C=N stretching frequencies in the 1601–1614 cm^{-1} region. A broad band at 3214–3251 cm^{-1} is ascribed to N—H stretching frequency

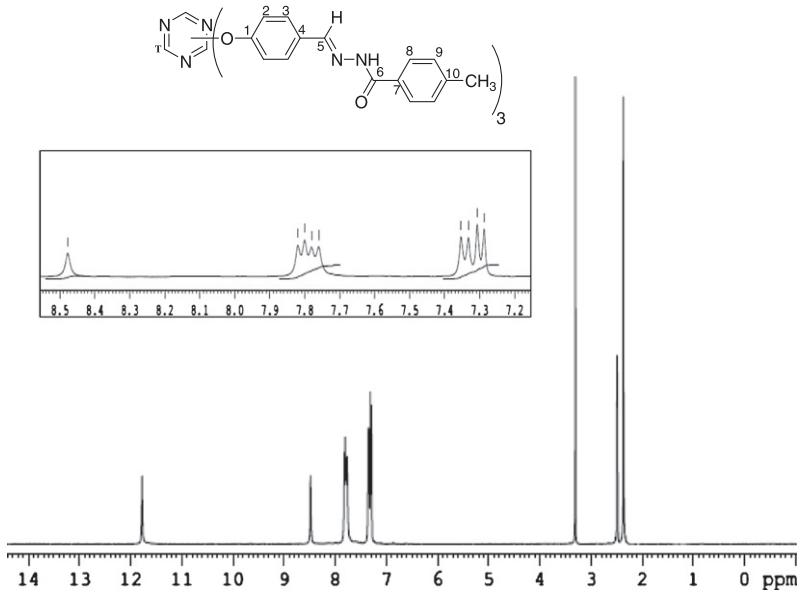


Fig. 1. ^{1}H NMR spectrum of **3g** in DMSO-d_6 (expansion of the aromatic region is shown in the box inset).

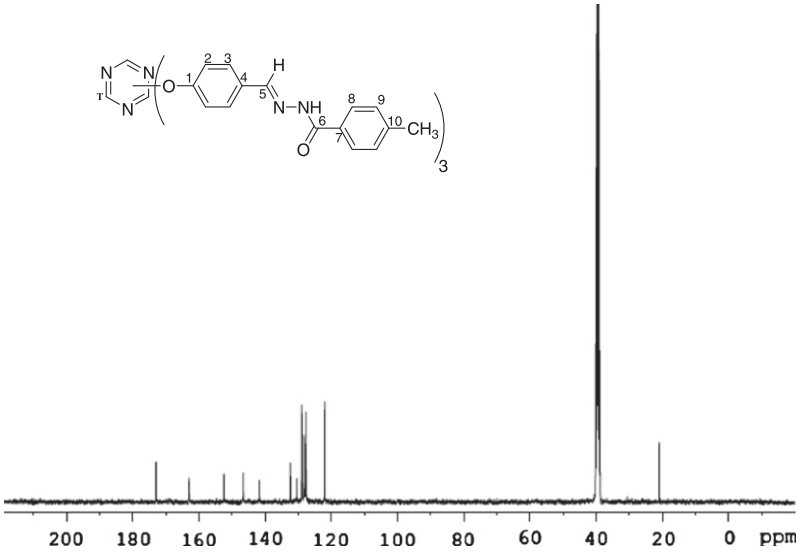


Fig. 2. ^{13}C NMR spectrum of **3g** in DMSO-d_6.

of the amide (—NH—C=O) moiety. A strong band at 1641–1662 cm^{-1} is due to the amide carbonyl (C=O) stretching frequencies and the C–O–Ar absorption is observed as a distinct band at 1363–1374 cm^{-1} this is attributed to the involvement of carbon atom of triazine ring in ether linkage [16,17]. Furthermore, triazine derivatives show another important band in the region 1560 and 1573 cm^{-1} ascribed to C=N stretching vibrations of *s*-triazine ring [16,17]. Thus, the IR spectral data provide strong evidences for the formation of the 1,3,5-triazine hydrazones.

3.3. NMR investigations

According to the NMR spectral data, all the *s*-triazine hydrazone molecules **3a–i** appears to have symmetric structures in solution. Detailed assignment of ^{1}H and ^{13}C NMR resonances presented in experimental section was made based on the 2D-Heteronuclear Single Quantum Coherence (HSQC) NMR analysis. For the description of NMR spectra, we used the arbitrary numbering of atoms as in Figs. 1 and 2.

3.3.1. ^{1}H NMR studies

^{1}H NMR spectrum of **1** showed a singlet at 10 ppm for aldehyde protons in addition to the two doublets at 7.31 and 7.93 ppm for the aromatic protons. The absence of aldehydic proton resonance and appearance of singlet peak for azomethine (—***H***C=N—N—) protons in the range 8.45-8.49 ppm confirms the formation of hydrazones **3a–i**. This is further supported by the resonance for hydrazide (—CO—N***H***—N=C) protons as a broad singlet in the downfield region 11.63–12.10 ppm. The resonances due to aromatic protons appear in the range 6.83 to 8.30 ppm for the triazine derivatives. In case of **3e**, the *p*-hydroxy (—OH) protons resonate as a singlet in the downfield region 10.07 ppm and **3i**, the *p*-amine ($—NH_2$) protons resonate as a singlet in the region 5.46 ppm. A singlet at 3.82 ppm and 2.36 ppm for 3**f** and **3g** accounts for protons of *p*-methoxy ($—OCH_3$) and *p*-methyl ($—CH_3$) groups respectively. A representative ^{1}H NMR spectrum of **3g** is given at Fig. 1.

3.3.2. ^{13}C NMR spectral studies

In the ^{13}C-NMR spectrum of compound **1**, the aldehyde carbon atom is observed at 190.99 ppm. None of the ^{13}C NMR spectra of compounds **3a–i** exhibited any signal that could be attributed to unreacted aldehyde functional group. ^{13}C NMR spectra of hydrazones showed resonance in the range 145.80–148.47 ppm due to the carbons (C^5) of the azomethine (H***C***=N—N) functions. The signal in the region 160.66–163.09 ppm is assigned to amide carbonyl (C^6). A distinct resonance in the range 172.86 to 173.45 ppm is due to the symmetrically substituted carbon atoms of the *s*-triazine ring. ^{13}C NMR analysis agreed with the ^{1}H NMR analysis in demonstrating the architecture of the star shaped hydrazones. The ^{13}C NMR spectrum of **3g** is given at Fig. 2.

From 2D HSQC NMR spectrum, it is clear that the ^{13}C NMR resonances of **3a–i** in the range 152.27–153.19 and 129.64-132.48 ppm are not having directly attached hydrogen atoms and are assigned to C^1 and C^4 respectively. The resonances of C^1 carbons are shifted up field by 2.88–3.80 ppm compared to the corresponding signal at 156.07 ppm in (**1**). The signals of C^2 were observed in the range 121.92–122.54 and C^3 chemical shift were in the range 128.40–132.46 ppm. The *p*-substituents at the periphery of the hydrazone arm do not have much impact on the resonances of these inner aromatic ring carbons hence the variation in their chemical shift values is much less. 2D HSQC NMR spectrum confirms that the C^7 and C^{10} are not having directly bonded hydrogens and the resonances of these carbons are influenced by the substituent at C^{10} carbon. Depending upon the inductive and mesomeric effect of the

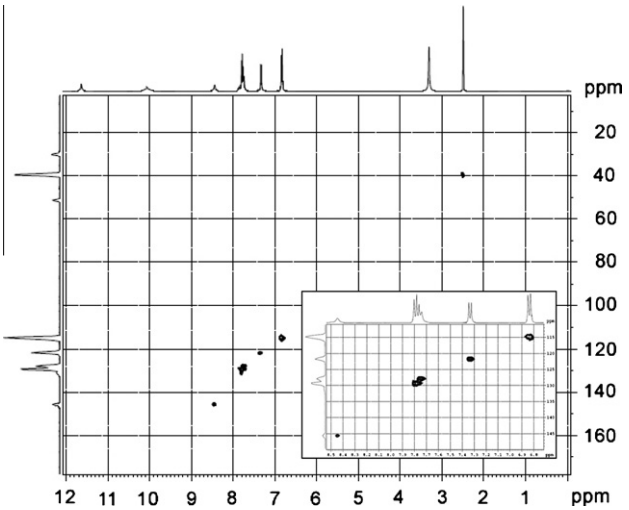


Fig. 3. 2D-HSQC-NMR spectrum of **3e** in DMSO-d_6 (expanded aromatic region is shown in the box inset).

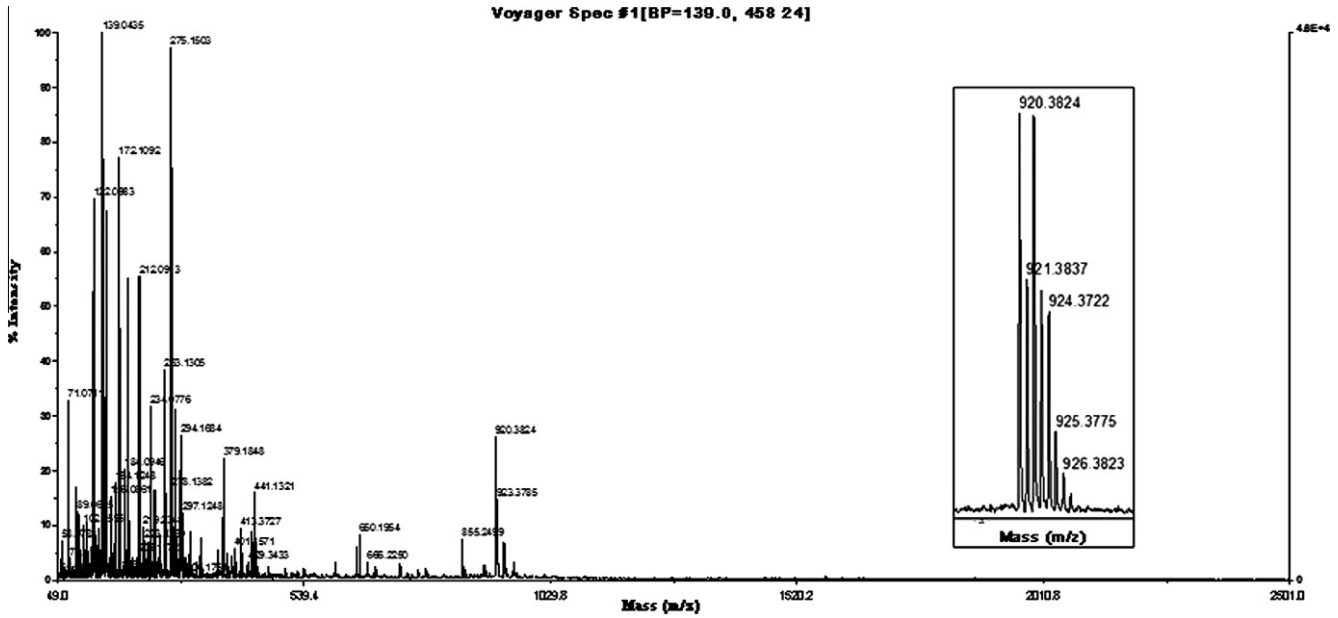


Fig. 4. MALDI-TOF mass spectrum of **3c**. Expansion is shown in the box inset.

substituent attached the C^{10} carbons show large variation in their chemical shift values. The signals due to the C^{10} attached to bromine (**3b**), chlorine (**3c**) and fluorine (**3d**) show downfield shift with increasing electronegativity of halogen substitutions and were observed at 125.50, 136.49 and 165.03 ppm respectively. In case of **3a**, carbons (C^{10}) bonded to hydrogen show resonances at 132.25 while in **3g** they are attached to methyl group and resonates at 141.74 ppm. The resonances arising from C^{10} attached to the hydroxy (**3e**), methoxy (**3f**) nitro (**3h**) and amino (**3i**) groups are observed at 159.18, 162.56, 149.69 and 152.45 ppm respectively. A representative 2D-HSQC NMR spectrum of compound **3e** is shown in Fig. 3.

3.4. Mass spectrometry

The single molecular nature of the hydrazone triazines **3a–i** formed by the complete reaction of all peripheral aldehyde groups of **1** with the benzoic hydrazides was also checked by MALDI-TOF mass spectrometry, which confirmed the expected chemical structures with m/z values corresponding to $(M + Na)^+$ ion. MALDI-TOF mass spectrograph of **3c** is given as a representative in Fig. 4.

3.5. In vitro antiproliferative activity

The synthesized compounds were tested for their antiproliferative activity *in vitro* against human liver carcinoma (HepG2) and human cervix carcinoma (HeLa) cell lines. The resulting data in Table 1 indicates that the compounds behaved differently in relation to the different cell lines. The compounds **3g** and **3h** bearing *p*-methyl and *p*-nitro substituents respectively exhibit highest activity against HepG2 cells. The compounds **3c** and **3i** bearing *p*-chloro and *p*-amino substituents respectively exhibited good activity against HeLa cells. It is interesting to note that the compound **3h** with *p*-nitro substituent exhibited least activity against HeLa cells but highest activity against the HepG2 cells. **3a–i** were not sufficiently effective against the HeLa cell line but exhibited moderate antiproliferative activity against HepG2 cell line.

4. Conclusion

A simple and convenient method for the synthesis of tri-arm star shaped molecules bearing hydrazone functions is reported. 2,4,6-Tris(4-formylphenoxy)-1,3,5-triazine (**1**) possessing three reactive terminal aldehydic functions on the substituents could be readily elaborated to the trifunctionalized hydrazones by condensation with the benzoic hydrazides. IR as well as ^1H, ^{13}C and 2D-HSQC NMR spectral characteristics of triazine hydrazones are consistent with their proposed structures. The ^{13}C NMR spectra of **3a–i** bearing three hydrazone arms exhibited a single peak in the range 172.86–173.45 ppm corresponding to the symmetrically substituted carbon atoms of the *s*-triazine ring. Microanalysis and MALDI-TOF mass spectrometry also proved the proposed chemical structures. The compounds synthesized were tested for their *in vitro* antiproliferative activity against human liver carcinoma (HepG2) and human cervix carcinoma (HeLa) cell lines. The present compounds exhibited lower activity against HeLa cell lines but reasonably moderate *in vitro* cytotoxicity against HepG2 cell lines. The compounds **3a–i** have been identified as trisubstituted symmetric triazines and are important as synthetic and structural models for the reactions and molecular structure of the analogous triazine dendrimers. Efforts on the synthesis and characterization of higher generation triazine hydrazones are in progress and will be reported in future communications.

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Synthesis, antimicrobial and antimycobacterial evaluation of star shaped hydrazones derived from 1,3,5-triazine

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ABSTRACT

Trisubstituted s-triazine hydrazones [N_3C_3(-OC_6H_4-p-CH=N-NH-C(O)-C_6H_4-p-X)$_3$] (X = H, Br, Cl, F) were prepared in excellent yields by the reaction of p-substituted N'-(4-hydroxy-benzylidene)-hydrazides with cyanuric chloride. The structures were elucidated by elemental analysis, FT-IR, ^{1}H, ^{13}C NMR and MALDI-TOF mass spectrometry. These s-triazine hydrazone derivatives were evaluated for their in vitro antimicrobial and antimycobacterial activity using serial dilution method.

Keywords: Triazine; Cyanuric chloride; Hydrazide; Hydrazone; Antimicrobial, Antituberculosis.

INTRODUCTION

Triazine compounds constitute an important class of heterocyclic chemistry and have been studied intensively [1, 2]. Cyanuric Chloride is an inexpensive, commercially available reagent used for the preparation of variety of *s*-triazine derivatives. The ease of displacement of chlorine atoms in cyanuric chloride by various nucleophiles enhances the utility of this reagent for the preparation of mono-, di- and tri substituted 1,3,5-triazine derivatives under controlled temperature conditions [3, 4].

The triazine scaffold has provided the basis for the design of compounds with a wide variety of properties useful in medicinal and agricultural applications [5-7]. Substituted s-triazine derivatives constitute an important class of compounds having antimalarial [8], antiviral [9], anticancer [10, 11] and estrogen receptor modulators [12]. Also, it was reported that some of these compounds possess potent antibacterial and antifungal activities [13-17].

Hydrazones represent one of the most biologically active classes of compounds [18]. Besides being utilizable for a wide range of pharmaceutical important derivatives, hydrazones are also important intermediates in organic synthesis [19]. Hydrazone linkage is extensively utilized for pH-dependent release of drugs from polymer-drug conjugates [20]. Many hydrazone derivatives have been claimed to possess, among others, antibacterial [21, 22] and antifungal [23, 24] activities. Looking at the antimicrobial importance of hydrazone moiety and triazine derivatives it would be worthwhile to design, synthesize some new triazine derivatives bearing hydrazone pharmacophore group and to investigate their possible antibacterial and antifungal activities.

We have previously described the synthesis of star shaped triazine hydrazones by condensation of tri-aldehyde core with aromatic hydrazides [25]. In this article, we illustrate the synthesis of similar triazine hydrazones by the reaction of cyanuric chloride with N'-(4-hydroxybenzylidene)-4-substituted-benzohydrazides. Thus prepared *s*-triazine hydrazones were evaluated for their antimicrobial and antituberculosis activity.

MATERIALS AND METHODS

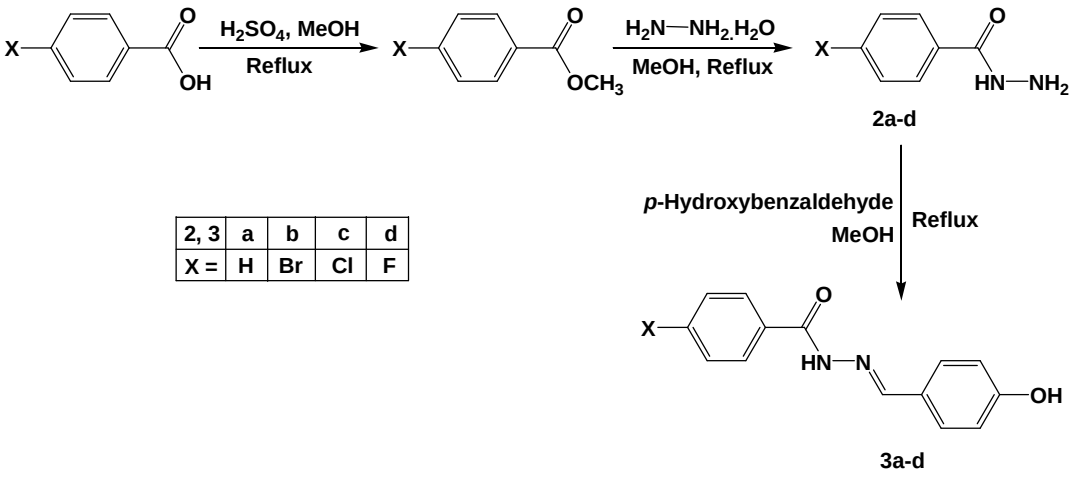
Materials and measurements

The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent with TMS as an internal standard at δ=0 ppm using a BRUKER AV-500 MHz High Resolution Multinuclear FT-NMR Spectrometer at room temperature. IR spectra were recorded on Impact-410 Nicolet (USA) FT-IR spectrometer in 4000–400 cm^{-1} range in a KBr matrix. The mass spectrum was obtained on Shimadzu GCMS-QP2010S and MALDI-TOF mass spectrum was measured with α-cyano-4-hydroxycinnamic acid as the matrix on a Voyager-DE STR spectrometer. Leco Model Truespec CHNS Analyser was used for Elemental (C, H, and N) analyses. Melting points were determined in an open capillary on a Gallenkamp melting point apparatus and are uncorrected.

Solvents were purified by standard methods [26]. Cyanuric chloride (Aldrich) and all other chemicals (sd fine chemicals, India) were used as received. All compounds were routinely checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates.

Synthesis of p-substituted benzoic acid hydrazides (2a-d)

p-substituted benzoic acid hydrazides were synthesized according to the literature method [25].



2, 3	a	b	c	d
X =	H	Br	Cl	F

Scheme 1. Synthesis of 4-substituted N'-(4-hydroxy-benzylidene)-hydrazides

Synthesis of 4-substituted N'-(4-hydroxy-benzylidene)-hydrazides (3a-d)

Reports dealing with the synthesis of N'-(4-hydroxybenzylidene)benzohydrazide (**3a**) [27], N'-(4-hydroxybenzylidene)-4-bromobenzohydrazide (**3b**), N'-(4-hydroxybenzylidene)-4-chlorobenzohydrazide (**3c**) [28], N'-(4-hydroxybenzylidene)-4-fluorobenzohydrazide(**3d**) [29], are well documented. Due to the non-availability of the IR and NMR spectral data in some of these reports, 4-substituted-benzoic acid (4-hydroxy-benzylidene)-hydrazides (**3a-d)** were synthesized as shown in scheme 1 by the following general procedure and their spectral data is presented.

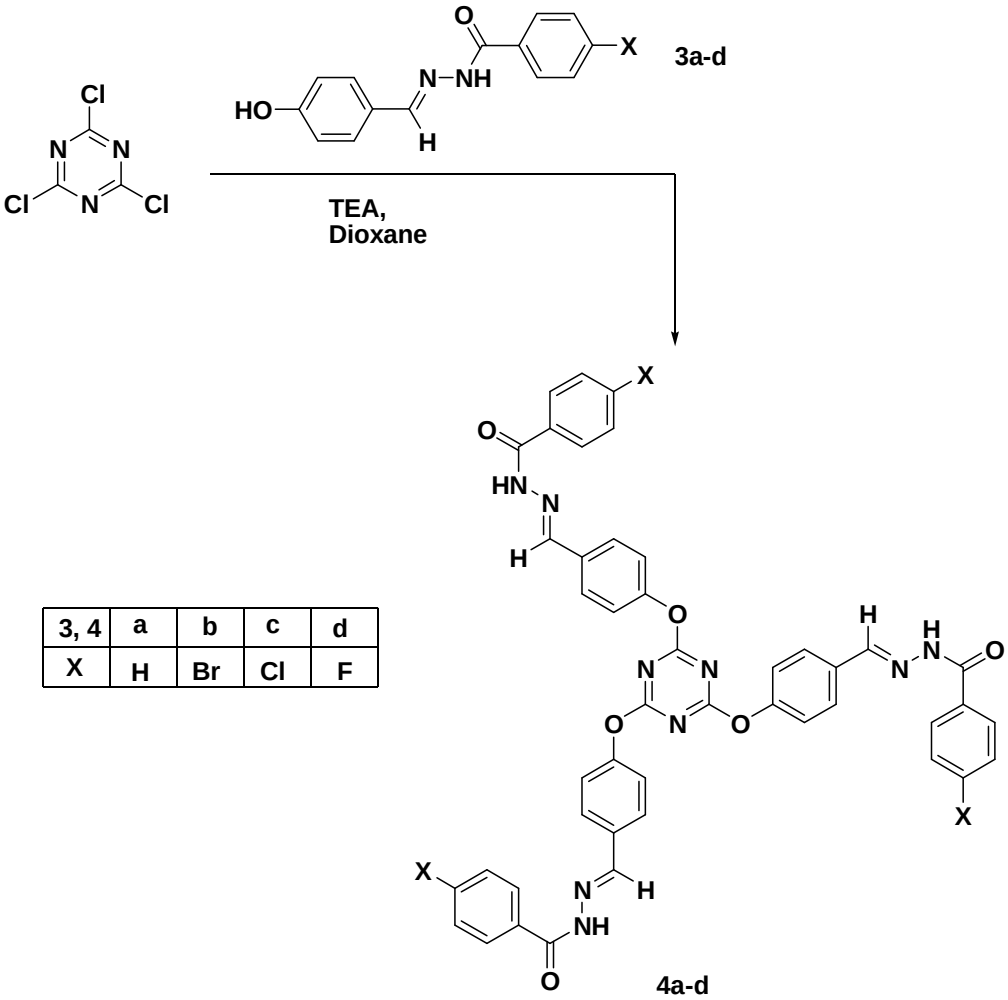
p-Hydroxylbenzaldehyde (1 mmol) was added to a solution of benzoic acid hydrazide (1 mmol) in 50 mL of methanol. The mixture was stirred at refluxing temperature for 3 h and then concentrated under vacuum. The solid product was collected by filtration under suction, and dried.

N'-(4-Hydroxybenzylidene)benzohydrazide (3a)

Yield: 86.04 %. IR (KBr) cm^{-1}: 3435 υ (O-H), 3303 υ (N-H), 1653 υ (C=O), 1608 υ (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.80 (d, 6H, $C^{2}H$), 7.32 (d, 6H, $C^{3}H$), 7.71 (d, 6H, $C^{9}H$), 7.74 (d, 6H, $C^{8}H$), 8.38 (s, 3H, $C^{5}H$) 10.08 (s, 3H, OH), 11.73 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 115.91 (C^{2}), 124.02 (C^{8}), 127.05 (C^{9}), 128.36 (C^{3}), 131.15 (C^{4}), 132.06 (C^{10}), 132.85 (C^{7}), 145.86 (C^{5}), 159.54 (C^{1}), 162.88 (C^{6}). MS m/z: 240 (M^{+}). Anal. Calcd. For $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66%. Found: C, 69.90; H, 4.98; N, 11.56%.

N'-(4-Hydroxybenzylidene)-4-bromobenzohydrazide (3b)

Yield: 80.23%. IR (KBr) cm^{-1}: 3438 υ (O-H), 3302 υ (N-H), 1658 υ (C=O), 1604 υ (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.84 (d, 6H, $C^{2}H$), 7.74 (d, 6H, $C^{3}H$), 7.78 (d, 6H, $C^{9}H$), 7.85 (d, 6H, $C^{8}H$), 8.34 (s, 3H, $C^{5}H$) 9.95 (s, 3H, OH), 11.34 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 115.69 (C^{2}), 125.49 (C^{10}), 128.43 (C^{8}), 129.63 (C^{3}), 129.89 (C^{4}), 131.14 (C^{9}), 133.35 (C^{7}), 146.68 (C^{5}), 159.94 (C^{1}), 162.37 (C^{6}). MS m/z: 319 (M^{+}). Anal. Calcd. For $C_{14}H_{11}BrN_2O_2$: C, 52.69; H, 3.47; Br, 25.04; N, 8.78%. Found: C, 52.61; H, 3.53; Br, 25.10; N, 8.73%.



3, 4	a	b	c	d
X	H	Br	Cl	F

Scheme 2. Synthetic route for1,3,5- triazine hydrazones

N'-(4-Hydroxybenzylidene)-4-chlorobenzohydrazide (3c)
Yield: 80.66 %. IR (KBr) cm^{-1}: 3444 υ (O-H), 3304 υ (N-H), 1652 υ (C=O), 1602 υ (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.86 (d, 6H, C^2H), 7.54 (d, 6H, C^3H), 7.74 (d, 6H, C^9H), 7.81 (d, 6H, C^8H), 8.42 (s, 3H, C^5H) 10.17 (s, 3H, OH), 11.74 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 114.93 (C^2), 123.62 (C^8), 128.82 (C^3), 129.61 (C^4), 133.37 (C^9), 134.16 (C^7), 136.29 (C^{10}), 145.33 (C^5), 160.66 (C^1), 162.66 (C^6). MS m/z: 274 (M^+). Anal. Calcd. For $C_{14}H_{11}ClN_2O_2$: C, 61.21; H, 4.04; Cl, 12.91; N, 10.20%. Found: C, 61.29; H, 4.10; Cl, 12.85; N, 10.15%.

N'-(4-Hydroxybenzylidene)-4-fluorobenzohydrazide (3d)
Yield: 84.09 %. IR (KBr) cm^{-1}: 3304 υ (O-H), 3305 υ (N-H), 1653 υ (C=O), 1605 υ (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.86 (d, 6H, C^2H), 7.34 (d, 6H, C^9H), 7.68 (d, 6H, C^3H), 7.94 (d, 6H, C^8H), 8.37 (s, 3H, C^5H) 10.13 (s, 3H, OH), 11.70 (s, 3H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 114.98 (C^9), 115.36 (C^2), 128.18 (C^8), 129.57 (C^3), 130.09 (C^4), 131.87 (C^7), 146.43 (C^5), 160.15 (C^1), 163.18 (C^6), 166.13 (C^{10}). MS m/z: 258 (M^+). Anal. Calcd. For $C_{14}H_{11}FN_2O_2$: C, 65.11; H, 4.29; F, 7.36; N, 10.85%. Found: C, 65.07; H, 4.30; F, 7.31; N, 10.88%.

Synthesis of triazine hydrazone **[N_3C_3(-OC_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-X)$_3$]**
[X=H (4a); X=Br (4b); X=Cl (4c); X=F (4d)].
Cyanuric chloride (0.06 g, 1 mmol) was added to a mixture of N'-(4-hydroxy-benzylidene)-hydrazide (**3a)** (0.242 g, 0.32 mmol) and triethylamine in 50 mL of dioxane. The mixture was refluxed for 36 h and then concentrated under vaccum. The solid was filtered under suction and washed with water and then with minimum amount of warm THF. The resulting solid (**4a**) was dried in vacuo at 40°C for 4 h. The same general procedure was followed for the compounds **4b-d** with 36-40 h at refluxing temperature**.** The spectral and analytical data of (**4a-d**) are in good agreement with literature values for similar compounds reported in our earlier communication [25].

Biological study

Protocols for Antimicrobial activity

The synthesized compounds were assayed for their *in vitro* antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144), Gram-negative bacteria *Escherichia coli* (ATCC 87261) and fungi *Candida albicans* (ATCC 2091). Minimum inhibitory concentrations (MIC) for test compounds as well as for reference standards were determined using serial dilution method as per the standard protocols [30-31]. Ciprofloxacin and Flucinozole were used as reference clinical standards for the antibacterial and antifungal activities, respectively. Test compounds and reference drugs were dissolved in 0.5 mL DMSO. Final concentrations of 100 to 0.2 µg/mL were prepared by serial two fold dilutions with Muller-hinton broth for the bacteria and Potato Dextrose Media for the fungi. The final DMSO concentration was adjusted to 5%. Preliminary experiments demonstrated that DMSO had no effect on the microorganism in the concentrations studied. Each of the 10 test tubes was inoculated with a suspension of microorganism to be tested and incubated for 24-48 h at 35 ºC in an ambient air incubator. At the end of the incubation period, the tubes were visually examined for the turbidity. The lowest concentration of the test compound that inhibited visible growth of microorganisms after incubation at 35 ºC for 24 h for bacteria or 48 h for fungi was taken as the MIC value. The antimicrobial activity tests were run in triplicate.

Protocols for Anti-tubercular activity

Test compounds were evaluated for *in vitro* anti-mycobacterial activity. The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.

The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs [32]. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. Test compounds were compared with reference drugs isoniazid.

RESULTS AND DISCUSSION

Synthesis of tri-arm 1,3,5-triazine hydrazones

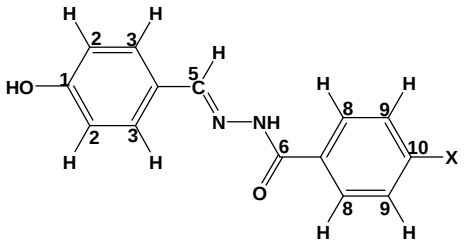
Tri-arm star shaped hydrazones with different substitutions at para positions emanating from 1,3,5-triazine core were synthesized as shown in Scheme 2. The condensation of benzoic hydrazide **2a**–**d** with 4-hydroxybenzaldehyde in tetrahydrofuran affords the corresponding hydrazones **3a-d**. The hydrazones **3a–d** bearing phenolic hydroxy functionality were then treated with cyanuric chloride in dioxane in presence of triethylamine for 36-40 h at refluxing temperature to obtain tri-arm 1,3,5-triazine hydrazones **4a–d.** The target compounds were obtained in high yields and were characterized by FT-IR, ^{1}H, ^{13}C spectroscopy, elemental analysis and MALDI-TOF mass spectrometry.

IR spectral studies

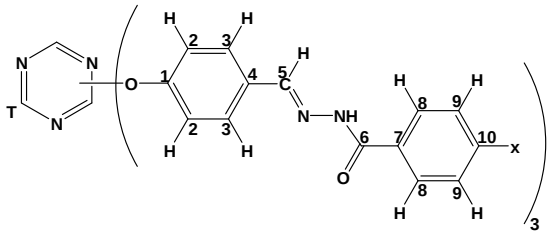
The formation hydrazones **3a-d** is confirmed by the appearance of imine C=N stretching frequencies in 1602-1608 cm^{-1} region. A broad band at 3214-3251 cm^{-1} is ascribed to N-H stretching frequency of the amide (-NH-C=O) moiety. A strong band at 1652-1658 cm^{-1} is due to the amide carbonyl (C=O) stretching frequency. The OH vibration observed at 3404–3444 cm^{-1} in **3a**–**d** are absent in **4a–d**. C_T-O-Ar absorption is observed as a distinct band at 1363-1374 cm^{-1} in **4a–d**, this is attributed to the involvement of carbon atom of triazine ring in ether linkage. Furthermore, triazine derivatives show another important band in the region 1567 and 1573 cm^{-1} ascribed to C=N stretching vibrations of *s*-triazine ring. Thus, the IR spectral data provide strong evidences for the formation of the 1,3,5-triazine hydrazones.

NMR investigations

The numbering used for NMR assignments is presented in figure 1. According to the NMR spectral data, all the triazine hydrazone (**4a-d**) molecules appear to have symmetric structures in solution.



I



II

Figure 1. Numbering pattern for NMR assignments of (I) 3a-d and (II) 4a-d

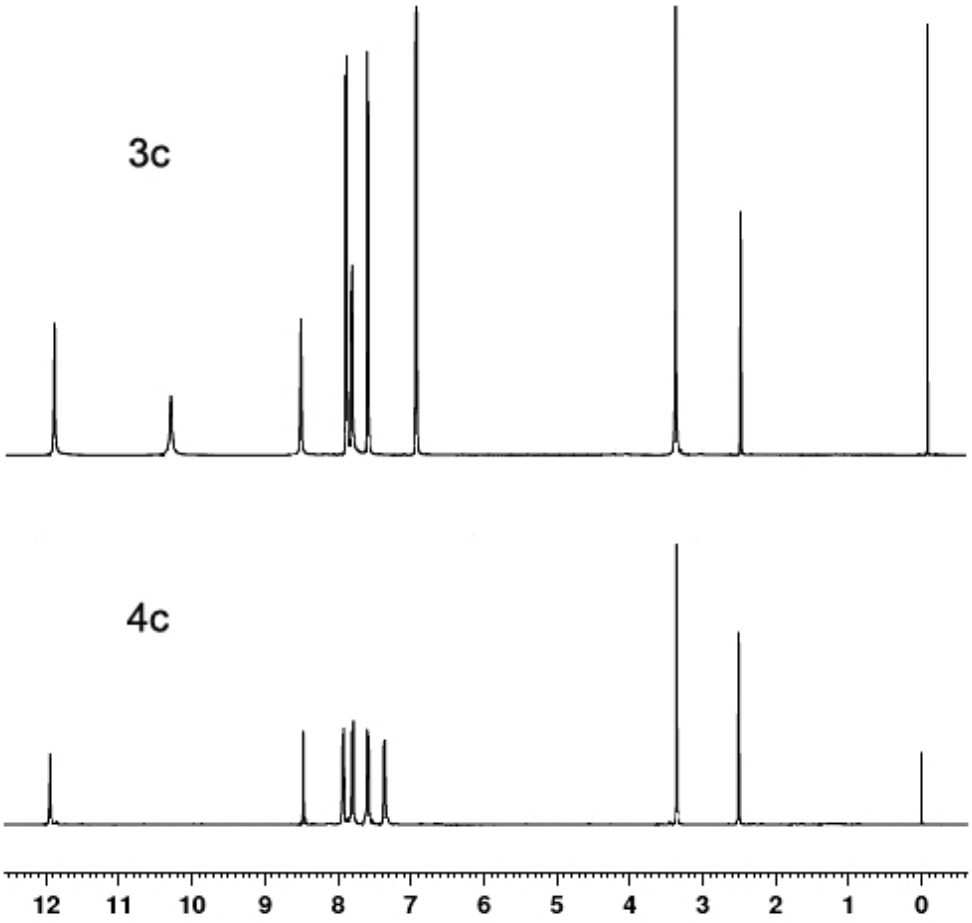


Figure 2. ^{1}H Nmr spectra of 3c and 4c

The appearance of a singlet for azomethine proton (-N-N=C***H***-) in the range 8.34-8.38 ppm confirms the formation of hydrazones **3a-d**. This is further supported by the resonance for hydrazide (-CO-N***H***-N=C-) protons as a broad singlet in the downfield region 11.34-11.76 ppm. The resonances due to aromatic protons appear in the range 6.80 to 7.99 ppm. A singlet at 9.95–10.13 ppm assigned to the phenolic hydroxy group of **3a-d** is absent in **4a-d** indicating the formation of ether linkage in the latter. This is further confirmed by a considerable change in the resonances of $C^{2}H$ protons. Other protons of **4a–d** have appeared in their usual pattern but with a slight variation in their chemical shift compared to the corresponding hydrazones **3a**–**d**. ^{1}H NMR spectra of **3c** and **4c** are given at Figure 2.

^{13}C NMR spectra of hydrazones **3a-d** exhibit resonances in the range 146.80-147.17 ppm due to the carbons (C^5) of the azomethine (-N-N=***C***H-) functionality. The amide carbonyl carbon (C^6) signal is observed in the region 161.92-163.09 ppm.

The resonance of C^1 carbons are observed at 159.42–160.15 ppm while in case of **4a-d** these resonances are observed in the range 152.22–152.44 ppm. The resonances of C^2 carbons of **4a-d** are observed downfield compared to the corresponding signal at 115.36-115.91 ppm in **3a**–**d**. Resonances of other carbons of **4a–d** exhibited slight variation in their chemical shift compared to the hydrazones **3a**–**d**. ^{13}C NMR spectrum of **3b** and **4b** are displayed in Figure 3.

Mass spectrometry

The single molecular nature of the hydrazone triazines **4a-d** was also checked by MALDI-TOF mass spectrometry, which confirmed the expected chemical structures with m/z values corresponding to $(M+Na)^+$ ion.

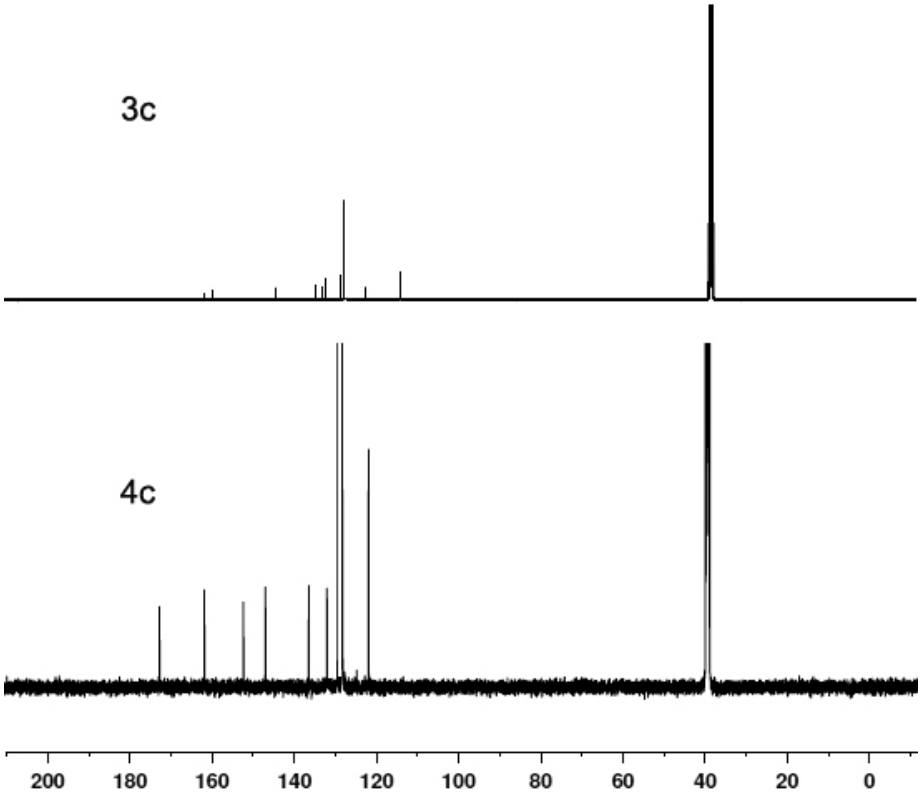


Figure 3. ^{13}C Nmr spectra of 3c and 4c

Biological activity

In order to determine the *in vitro* antimicrobial activity, the synthesized compounds 4a-d were screened against Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144), Gram-negative bacteria *Escherichia coli* (ATCC 87261) and a fungal strain *Candida albicans* (ATCC 2091) using a two fold serial dilution technique. The investigation of antibacterial and antifungal screening data (Table 1) reveal that all the tested compounds exhibit moderate inhibition against the strains used. Antibacterial activity of compounds **4b**, **4c** is higher than other two compounds against *S.aureus*, while **4c**, **4d** have shown good activity against *E.coli*. Compounds **4a**, **4b**, **4c** were found to be more active against *C.albicans* fungal strain. Compound **4c** is found to be active against all the tested strains. However, activity exhibited by all synthesized compounds is less compared to the standard drugs used in the study.

Table 1. *In-vitro* antimicrobial and antimycobacterial activities of 4a-d (MIC in µg/mL)

Compounds	Bacterial		Fungal	Antituberculosis
	S. aureus	*E. coli*	*C. albicans*	*$H_{37}Rv$*
4a	12.5	25	0.8	25
4b	1.6	12.5	0.8	12.5
4c	1.6	1.6	0.8	6.25
4d	6.25	1.6	6.25	6.25
Ciprofloxacin	0.2	0.2	--	--
Flucanazole	--	--	0.4	--
Isoniazide	--	--	--	0.8

The anti-mycobacterial activity of synthesized compounds of **4a-d** were assessed against M. tuberculosis ***$H_{37}Rv$*** at several µg concentrations. The Minimum Inhibitory Concentrations (MIC) of compounds was compared with Isoniazid, the standard anti-TB drug. All the tested compounds have shown poor inhibition compared to the standard used. Among the compounds studied **4b** and **4c** have shown comparatively better inhibition.

CONCLUSION

An alternative method for the synthesis of tri-arm star shaped molecules bearing hydrazone functions is reported. 2,4,6-Tris(4-formylphenoxy)-1,3,5-triazine (**1**) possessing three reactive terminal aldehydic functions on the substituents could be readily elaborated to the trifunctionalized hydrazones by condensation with the benzoic hydrazides. IR as well as ^{1}H, ^{13}C NMR spectral characteristics of triazine hydrazones are consistent with their proposed structures. Microanalysis and MALDI-TOF mass spectrometry also proved the proposed chemical structures. The tri-arm star shaped hydrazones were evaluated *in vitro* for their antimicrobial and antimycobacterial activity using a two-fold serial dilution method and exhibited moderate inhibition against the strains. The compounds **4a-d** have been identified as fully substituted symmetric triazines and are important as synthetic and structural models for the reactions and molecular structure of the analogous triazine dendrimers. Efforts on the synthesis and characterization of higher generation triazine hydrazones are in progress and will be reported in future communications.

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