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ORIGINAL ARTICLE

Synthesis and biological evaluation of novel *s*-triazine based aryl/heteroaryl entities: Design, rationale and comparative study

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Abstract The urgent need in search of new biological entities to fight back with recent drug-resistant microbial flora, has led us report a library of *s*-triazine derivatives. The intermediate 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile 3 was substituted with various thiophenol, phenol, aniline and piperazine/piperidine/morpholine moieties to furnish the final 35 target compounds i.e. (**4a**–**j**), (**5a**–**j**), (**6a**–**g**) and (**7a**–**h**), respectively. These compounds were screened for in vitro antibacterial evaluation against bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 619, *Escherichia coli* MTCC 739, and *Pseudomonas aeruginosa* MTCC 741) and antifungal activity against fungi (*Candida albicans* MTCC 183, *Aspergillus niger* MTCC 282, and *Aspergillus clavatus* MTCC 1323). The title compounds were further subjected for antituberculosis activity against *Mycobacterium tuberculosis* **H37Rv** strain using the BACTEC MGIT method. In this biological evaluation, thiophenol derivatives were found to be more active than the rest (i.e. -Thiophenol > -piperazine > -Aniline > -phenol). The final compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis.

1. Introduction

Multidrug resistant (MDR) strains, a rapid development of pathogens causing a severe resistance toward currently available standard drugs, pose a frightening threat by increasing severe opportunistic microbial infections in past decades (Gootz, 2010; Niccolai and Tarsi, 1997; Overbye and Barrett, 2005). Such resistant organisms were due for a dramatic and alarming increase in microbial infections which results in pressing problem worldwide. On the other hand, MDR-Tuberculosis (TB) and extensively drug-resistant XDR-TB, caused by some mycobacteria of the *Mycobacterium tuberculosis* complex which most commonly affect the lungs, emerged as one of the most infectious diseases in the recent era (Ducati et al., 2006; Gandhi et al., 2010; Udwadia et al., 2012). The latest statistics of World health Organization (WHO) reported that about one third of the human population were infected with TB which showed the urgent need to combat such dilemma (2012).

Surprisingly, 8.7 million new cases of TB were reported in 2011 from which 13% co-infected with HIV (Human Immuno

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Deficiency); 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 0.4 million people allied to HIV-positive which scores around 25% death due to TB (2013). In view of the above, consequences of these problems highlight the urgent need to develop new agents with specific activity with increased potency to sustain a pool of new bioactive entities. Therefore, design and synthesis of new compounds likely to be unaffected by existing resistance mechanisms are an area of immense significance for medicinal chemists.

Owing to a wide range of biological applications, *s*-triazine nucleus has received an immense attention among chemists through fertile source of pharmacological activities such as antibacterial (Bhushan Singh et al., 2012; Gahtori et al., 2012b; Kumar Ghosh et al., 2012), antimalarial (Gahtori et al., 2012a), antiprotozoal (Baliani et al., 2005), antifungal (Singh et al., 2012), anticancer (Menicagli et al., 2004), antimycobacterial (Patel et al., 2012), and antiviral (Chen et al., 2012). In addition to this several *s*-triazine derivatives bearing p-amino benzonitrile moiety have been found to possess an enhanced antimicrobial profile and improved antitubercular (Patel et al., 2011b) and profound anticancer activity (Patel et al., 2011a) as well. Consequences of such potential effects of triazine and an imperative need in search of new chemical entities lead us to synthesize some biologically efficient molecules.

Recently our research group has reported 2,4,6-trisubstituted triazine derivatives endowing promising biological activity (Modh et al., 2012a,b,c, 2013a,b; Patel et al., 2012, 2011a,b); hence it is worthy to synthesize novel compounds which elicit a series of antimicrobial and anti tuberculosis agents. Recent studies have confirmed that several *s*-triazine derivatives bearing morpholine, piperidine and some piperazine moieties are effective against *M. tuberculosis* H37Rv strain (Sunduru et al., 2010). Prompted by such facts it is worthy to envisage that combination of such bioactive moieties in a compact system may arise with new biologically active agents. We introduced synthetic strategy to acquire triazine nucleus with biolabile derivatives viz. phenol, thiophenol, aniline and piperazine/piperidine/morpholine. Target compounds were rationalized and designed using the hits obtained from the (piperazinyl/piperidinyl)-*s*-triazines derivatives (Patel et al., 2011a), which were previously reported for their antimicrobial, antimycobacterial and anticancer activities besides this, compound R129385 (Das et al., 2004) with *s*-triazine nucleus was reported as an effective antiviral agent (Fig. 1). Adopting all such criteria, herewith, a library of 35 *s*-triazine based compounds were synthesized and evaluated for their biological potential which may lead to future prospects in drug design and discovery.

2. Experimental section

2.1. Materials and methods

All chemicals as well as solvents were procured from sigma Aldrich, Merck and Fluka. Solvents taken were of analytical grade and used without further purification. All reactions were routinely checked by TLC. TLC was performed on aluminum-backed silica gel plates (Silica gel 60 F254 grade, Merck DC) with spots visualized by UV light. Column chromatography was performed on silica gel LC 60A (70–200 μ). Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. FT-IR spectra were recorded on a perkin–Elmer 257 spectrometer using KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz model spectrometer using DMSO-*d6* as a solvent and TMS as an internal standard. The chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. Purity of all tested compounds was ensured on the basis of their elemental analyses (C, H, N) and were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany). The electron spray mass spectra were recorded on a triple quadrupole mass spectrometer with the ionization potential of 70 eV Fig. 2.

2.2. Chemistry: general methods

2.2.1. Synthesis of 2,4-dichloro-6-methoxy-1,3,5-triazine (2)

A mixture of 2,4,6-trichloro-1,3,5-triazine **1** (5.0 g, mol) and sodium bicarbonate (2.5 g, 0.02982 mol) in methanol (10 mL) was stirred at 0–5 °C for 4 h. The progress of the reaction was monitored by TLC using hexane:ethyl acetate (4:1) solvent system as an eluent. After the completion of the reaction, the reaction mass was poured into crushed ice. The solid was separated, washed with cold water, dried and recrystallized from ethanol to give compound **2** (Dudley et al., 1951). Yield: 79%; m.p. 88–90 °C; IR (KBr cm^{-1}): 2815 (–OCH_3), 826 (C_3N_3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 3.77 (s, 3H–Ar–OCH_3); ^{13}C NMR 179.8, 167.5, 58.3; ESI-MS (M + 1): 180.99.

2.2.2. Synthesis of 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (3)

To a stirred solution of compound **2** (5.0 g, 0.02778 mol) and sodium bicarbonate (2.56 g, 0.03056 mol) in THF (20 mL), a solution of 4-amino benzonitrile (3.28 g, 0.02778 mol) was added and stirred for 6 h at room temperature. The progress of the reaction was monitored by TLC using toluene:acetone (4:1) solvent system as an eluent. After the completion of the reaction, resultant mixture was poured into crushed ice. The solid product obtained was filtered, washed with distilled water, dried and purified by column chromatography using toluene:acetone solvent system as an eluent. Yield: 85%; m.p.167 °C; IR (KBr cm^{-1}): 3372 (N–H), 2210 (C═N), 845 (C3N3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*): DMSO-*d6*: δ 2.97 (s, 3H–Ar–OCH_3), 9.8 (s,1H, –NH); ^{13}C NMR 177.1, 169.2, 168.9, 144.6, 135.9, 119.7, 118.5, 105.1, 54.8; ESI-MS (*m*/*z*): 262.67.

2.2.3. General procedure for the preparation of ((4-methoxy-6-(substituted phenylthio)-1,3,5-triazin-2-yl)amino)benzonitrile (4a–j)

A stirred mixture of appropriate thiophenol (0.0191 mol), 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile **3** (5.0 g, 0.0191 mol) and anhydrous K_2CO_3 (2.92 g, 0.0211 mol) in DMF (20 mL) was refluxed for 20 h. The progress of the reaction was monitored by TLC using toluene:acetone (7:3) solvent system as an eluent. After the completion of the reaction, the reaction mass was poured into ice. The