Synthesis and in vitro antimicrobial activity of N’-(4-(arylamino)-6-(pyridin-2-ylamino)-1,3,5-triazin-2-yl)benzohydrazide

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Abstract: A variety of N’-(4-(arylamino)-6-(pyrazin-2-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazide, 7a-j were synthesized by using 2-aminopyridine, isonicotic acid hydrazide and cyanuric chloride. And the structures of these compounds were confirmed by IR, NMR (1H & 13C) spectral analysis. The newly synthesized compounds were also evaluated for antimicrobial activity against variety of bacterial strains and some of these compounds have shown significant antibacterial and antifungal activities.

Keywords: 2-aminopyridine, isonicotic acid hydrazide, s-triazine, antibacterial activity.

Introduction

The extraordinary progress represented by the arrival of antibiotics has changed the medical prognosis of minor and major infections.1 Any bacterial species acquired resistance to the most common classes of antibiotics. Bacterial resistance continues to develop and pose a significant threat both in hospitals and more recently in the community.2 A relevant report on resistant antibacterial agents for human medicine is provided by World Health Organization. The panel agreed that the list of Critically Important antibacterial agents should be updated regularly as new information becomes available, including data on resistance patterns, new and emerging diseases and the development of new drugs.3

During the last few years the potential of s-triazine derivatives in agrochemical and medicinal properties have been subjected to investigation. Literature survey reveals that amino substituted s-triazine derivatives are associated with number of pronounced antibacterial activities4-7 against gram positive (B. subtilis, B. sphaericus, S. aureus etc) and gram negative organism (E. coli, K. aerogenes, P. aeruginosa etc). The biological activity is a function of physicochemical properties of the targeted molecule and this assessment is made of the sorts of chemicals that might fit into an active site,8.9 To randomly explore the novel compounds10-14, our idea was to combine, 2-aminopyridine, isonicotic acid hydrazide, s-triazine nucleus using cyanuric chloride and various amines. Substituted-s-triazines, derivatives remain attractive, with their significant biological activities15-18 and further incorporation of these derivatives with commercial drug viz. isoniazid, could give access to a wide array of structures, which can be expected to show interesting antibacterial activities, thus, herein, we report the synthesis and antimicrobial activity of a variety of novel s-triazine derivatives.

Experimental

All the melting points were taken in open capillaries tube and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel – G coated Al – plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (υmax in cm⁻¹) were recorded on Shimadzu FTIR spectrophotometer using KBr or Nujol technique.1H & 13C NMR spectra on a Bruker’s WM 400 FT MHz NMR instrument using CDCl3 or DMSO-d6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The elemental analysis (C, H, N) of compounds was performed on Carlo Erba – 1108 elemental analyzer.

N’-(4-(arylamino)-6-(pyridin-2-ylamino)-1,3,5-triazin-2-yl)benzohydrazide 7a-j

The designed compounds were prepared in one pot by three steps reaction, first step consists of nucleophilic substitution19 of one chlorine atom of cyanuric chloride, 1 in presence of acetone with 2-aminopyridine, 2 to synthesize compound 3 (0-5°C, 0.5-1.0 hour), second step involves further substitution19 of
second chlorine atom in presence of acetone with isonicotinic acid hydrazide, 4 to give compound 5 (30-35°C, 3.0-3.5 hour), while third chlorine atom was substituted with various aromatic amines, 6a-j (reflux, 5-6 hours) in presence of aqueous dioxane to obtain a series of compounds, 7a-j (Scheme 1). The progress of reaction was monitored by TLC using acetone:toluene (8:2) as eluent. After completion of reaction, the stirring was stopped and the solution was treated with crushed ice. The product obtained was filtered and dried. The crude products were purified by crystallization from acetone to give the title compounds. The physical and analytical data of novel compounds are given in Table 1.

![Chemical structures of compounds](image)

**Scheme-1: Synthetic route to s-triazine derivatives 7a-j.**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>Melting point(°C)</th>
<th>Yield (%)</th>
<th>λmax# nm (DMF)</th>
<th>Rf Value</th>
<th>Anal. Calcd. (found)/ %</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>C</td>
<td>H</td>
<td></td>
<td></td>
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<tr>
<td>7a</td>
<td>H</td>
<td>222 ~ 24</td>
<td>61 ~ 63</td>
<td>270</td>
<td>0.77</td>
<td>63.31 (56.78) 4.55 (4.01) 28.12 (35.18)</td>
</tr>
<tr>
<td>7b</td>
<td>2-NO₂</td>
<td>266 ~ 67</td>
<td>62 ~ 64</td>
<td>261</td>
<td>0.81</td>
<td>56.88 (56.47) 3.86 (3.61) 28.43 (28.40)</td>
</tr>
<tr>
<td>7c</td>
<td>3-NO₂</td>
<td>266 ~ 67</td>
<td>61 ~ 63</td>
<td>262</td>
<td>0.82</td>
<td>56.88 (56.77) 3.86 (3.61) 28.43 (28.40)</td>
</tr>
<tr>
<td>7d</td>
<td>4-NO₂</td>
<td>268 ~ 69</td>
<td>62 ~ 63</td>
<td>264</td>
<td>0.83</td>
<td>56.88 (56.92) 3.86 (3.66) 28.43 (28.77)</td>
</tr>
<tr>
<td>7e</td>
<td>2-Cl</td>
<td>248 ~ 69</td>
<td>65 ~ 67</td>
<td>272</td>
<td>0.71</td>
<td>58.27 (58.61) 3.96 (3.66) 25.89 (25.99)</td>
</tr>
<tr>
<td>7f</td>
<td>3-Cl</td>
<td>242 ~ 43</td>
<td>64 ~ 65</td>
<td>274</td>
<td>0.74</td>
<td>58.27 (58.50) 3.96 (3.77) 25.89 (25.66)</td>
</tr>
<tr>
<td>7g</td>
<td>4-Cl</td>
<td>241 ~ 72</td>
<td>65 ~ 67</td>
<td>279</td>
<td>0.77</td>
<td>58.27 (58.50) 3.96 (3.79) 25.89 (25.77)</td>
</tr>
<tr>
<td>7h</td>
<td>2-CH₃</td>
<td>238 ~ 39</td>
<td>67 ~ 69</td>
<td>281</td>
<td>0.67</td>
<td>64.07 (64.09) 4.89 (4.49) 27.17 (27.29)</td>
</tr>
<tr>
<td>7i</td>
<td>3-CH₃</td>
<td>235 ~ 36</td>
<td>68 ~ 69</td>
<td>282</td>
<td>0.69</td>
<td>64.07 (64.11) 4.89 (4.50) 27.17 (27.19)</td>
</tr>
<tr>
<td>7j</td>
<td>4-CH₃</td>
<td>237 ~ 38</td>
<td>67 ~ 69</td>
<td>287</td>
<td>0.77</td>
<td>64.07 (64.32) 4.89 (4.91) 27.17 (27.27)</td>
</tr>
</tbody>
</table>
Spectral data of synthesized compounds, 7a-j.

7a: IR (νmax in cm⁻¹): 1670 (C=O of amide, C-O str), 3340(NH) & 1325(CN), 3085(Aromatic CH str). ¹H NMR δ ppm: 10.19(s, 1H, CONH-, D₂O exchangeable), 4.1(s, 1H, NH), 9.24(s, 1H, NH-Ar), 7.1-8.1 (m, 13H, Ar-H). ¹³C NMR δ ppm: 119.1-147.2(Ar-C), 164.7, 168.1, 176.9(C=N of s-triazine), 163.27(CO). ¹H NMR δ ppm: 10.19(s, 1H, CONH, D₂O exchangeable), 4.0(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.2-8.3 (m, 13H, Ar-H). ¹³C NMR δ ppm: 119.2-149.3(Ar-C), 164.8, 168.2, 176.0(C=N of s-triazine), 163.27(CO).

8.9 (m, 12H, Ar-H).

7b: IR (νmax in cm⁻¹): 1672(=C=O of amide, C=O str), 3345(NH) & 1322(CN), 3081(Aromatic CH str). ¹H NMR δ ppm: 10.10(s, 1H, CONH-, D₂O exchangeable), 4.0(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.2-8.3 (m, 13H, Ar-H). ¹³C NMR δ ppm: 119.5-147.7(Ar-C), 165.1, 168.7, 177.2(C=N of s-triazine), 163.11(CO).

8.9 (m, 12H, Ar-H).

7c: IR (νmax in cm⁻¹): 1670(=C=O of amide, C=O str), 3340(NH) & 1320(CN), 3084(Aromatic CH str). ¹H NMR δ ppm: 10.11(s, 1H, CONH-, D₂O exchangeable), 4.2(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.0-8.2 (m, 13H, Ar-H). ¹³C NMR δ ppm: 119.5-147.7(Ar-C), 165.1, 168.7, 177.2(C=N of s-triazine), 163.11(CO).

7d: IR (νmax in cm⁻¹): 1670(=C=O of amide, C=O str), 3340(NH) & 1320(CN), 1519, 1377(NO₂), 3077(Aromatic CH str), 1517, 1537(=C=O str). ¹H NMR δ ppm: 10.15(s, 1H, CONH, D₂O exchangeable), 4.1(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.0-8.2 (m, 12H, Ar-H). ¹³C NMR δ ppm: 111.7-147.7(Ar-C), 164.7, 168.7, 176.9(C=N of s-triazine), 164.5(CO).

7e: IR (νmax in cm⁻¹): 1675(=C=O of amide, C=O str), 3310(NH) & 1325(CN), 3090(Aromatic CH str), 835(Cl-C str). ¹H NMR δ ppm: 10.14(s, 1H, CONH, D₂O exchangeable), 4.0(s, 1H, NH), 9.27(s, 1H, NH-Ar), 7.7-8.9 (m, 12H, Ar-H). ¹³C NMR δ ppm: 111.9-147.9(Ar-C), 163.2, 169.9, 176.7(C=N of s-triazine), 164.5(CO).

7f: IR (νmax in cm⁻¹): 1676(=C=O of amide, C=O str), 3315(NH) & 1320(CN), 3095(Aromatic CH str), 830(C-Cl str). ¹H NMR δ ppm: 10.15(s, 1H, CONH, D₂O exchangeable), 4.0(s, 1H, NH), 9.25(s, 1H, NH-, 7.7-8.9 (m, 12H, Ar-H). ¹³C NMR δ ppm: 111.9-147.7(Ar-C), 163.0, 169.7, 176.9(C=N of s-triazine), 164.0(CO).

7g: IR (νmax in cm⁻¹): 1677(=C=O of amide, C=O str), 3319(NH) & 1327(CN), 3099(Aromatic CH str), 837(C-Cl str). ¹H NMR δ ppm: 10.19(s, 1H, CONH, D₂O exchangeable), 4.7(s, 1H, NH), 9.29(s, 1H, NH-Ar), 7.7-8.9 (m, 12H, Ar-H). ¹³C NMR δ ppm: 111.7-147.9(Ar-C), 163.7, 169.9, 176.7(C=N of s-triazine), 164.9(CO).

7h: IR (νmax in cm⁻¹): 1672(=C=O of amide, C=O str), 3315(NH) & 1327(CN), 3099(Aromatic CH str), 832(C-Cl str). ¹H NMR δ ppm: 10.15(s, 1H, CONH, D₂O exchangeable), 4.7(s, 1H, NH), 9.29(s, 1H, NH-Ar), 7.2-8.5 (m, 12H, Ar-H). ¹³C NMR δ ppm: 111.7-149.7(Ar-C), 163.5, 169.7, 176.9(C=N of s-triazine), 164.9(CO).

7i: IR (νmax in cm⁻¹): 1674(=C=O of amide, C=O str), 3322(NH) & 1322(CN), 3091(Aromatic CH str), 1311(CH₃, C-H bend). ¹H NMR δ ppm: 10.11(s, 1H, CONH, D₂O exchangeable), 4.1(s, 1H, NH), 9.11(s, 1H, NH-Ar), 7.1-8.1 (m, 12H, Ar-H). ¹³C NMR δ ppm: 119.1-147.1(Ar-C), 160.1, 171.9, 179.1(C=N of s-triazine), 164.1(CO).

8.9 (m, 12H, Ar-H).

7j: IR (νmax in cm⁻¹): 1675(=C=O of amide, C=O str), 3320(NH) & 1320(CN), 3089(Aromatic CH str), 1309(CH₃, C-H bend). ¹H NMR δ ppm: 10.18(s, 1H, CONH, D₂O exchangeable), 4.0(s, 1H, NH), 9.21(s, 1H, NH-Ar), 7.9-8.7 (m, 12H, Ar-H). ¹³C NMR δ ppm: 119.1-147.9(Ar-C), 160.2, 171.7, 179.9(C=N of s-triazine), 164.2(CO).

Antibacterial activity

The in vitro antibacterial screening of all the compounds was evaluated against selected (Table 1) Gram-positive organisms viz. Bacillus subtilis(MTCC 441), Bacillus subtilis(MTCC 441), Staphylococcus aureus(MTCC 96) and Gram-negative organisms viz. Chromobacterium violaceum (MTCC 2656), Klebsiella aerogenes (MTCC 39), Pseudomonas aeruginos(MTCC 741), Salomonia paratyphi A(MTCC 735) and Escherichia coli(MTCC 443) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards.

Standard antibacterial agent like Benzyl Penicillin and Streptomycin were also screened under identical conditions for comparison.

Antifungal activity

A. awamori, A. niger and C. albicans was employed for testing antifungal activity using the cup-plate method. The culture was maintained on Sabouraud’s agar slants. Fifteen milliliters of sterilized Sabouraud’s agar medium was spread in a Petri dish (13 cm in diameter) and allowed to set for 30 min. Five milliliters of sterilized Sabouraud’s agar medium was inoculated with 72 h old 0.2 ml suspension of fungal spores in a test-tube and spread over the previously settled layer of Sabouraud’s agar medium in the Petri dish. The cups (8 mm in diameter) were punched in the Petri dish and filled with 0.05 ml (40 µg) of a solution of the sample in DMF. The plates were incubated at 30 °C for 48 h. After the completion of the incubation period, the zones of inhibition of growth in millimeter were measured. Along with the test solutions in each Petri dish one cup was filled up with solvent, which acts as the control. Standard antifungal agent like Griseofulvin was also screened under identical conditions for comparison. The zones of inhibition are recorded in Table 3.

Results and discussion

In vitro antibacterial activity data of s-triazine derivatives (Table 1) against tested organisms displayed significant activity with a wide degree of variation. It is found that compound 7e displayed substantial activity against B. subtilis and remaining compounds are significantly active. Also 7b, 7f, 7i and 7j are equipotent against B. sphaericus compared to reference compound. Rest of the compounds has exhibited significant to substantial activity against the same strain. Substantial activity is achieved in case of compounds 7d against S. aureus and...
the remaining compounds are significantly active against the same species. All the s-triazine derivatives have exhibited significant to moderate activity against Gram-negative bacteria. Derivatives 7g and 7j have exhibited substantial activity against C. violaceum. Remaining triazine derivatives in this series, compounds 7a and 7h displayed least activity against all the tested organisms. Against Salomonella paratyphi A, compounds 7i and 7j has been found to possess significant activity, comparatively weak activity has been reported by remaining compounds. E. coli was found to be more susceptible than rest of the other strains of bacteria, among them compounds 7c, 7d and 7j were showing significant activity for the same strain. All s-triazine derivatives in this communication are inactive towards P. aeruginosa, also decreased activity is observed in case of K. aerogenes with all the s-triazines. From in vitro antifungal activity (Table 2), data reveals that all the newly synthesized compounds displayed moderate to significant activity in comparison to standards. Thus, it is obvious from the structure-activity profile of substituted s-triazines; a small structural variation may induce an effect on antibacterial activity.
Conclusion
Trisubstituted s-triazine derivatives, 7a-j was synthesized and characterized for their structure elucidation. Antibacterial and antifungal studies of these compounds indicated that compounds were found to be showing comparable activity against some bacteria compared to standard antibiotic drugs. The produced compounds have good microbial toxicity due to presence of three pharmaco logically active nucleus viz. s-triazine, pyrazine and isoniazid, so such compounds may give good comparable anti-Tuberculosis effect, which will be studied in details hereafter.

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References

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