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ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.1, No.3 , pp 616-620, July-Sept 2009

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 (^{1}H & ^{13}C) 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 activities[4-7] 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 compounds[10-14], 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 activities[15-18] 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.

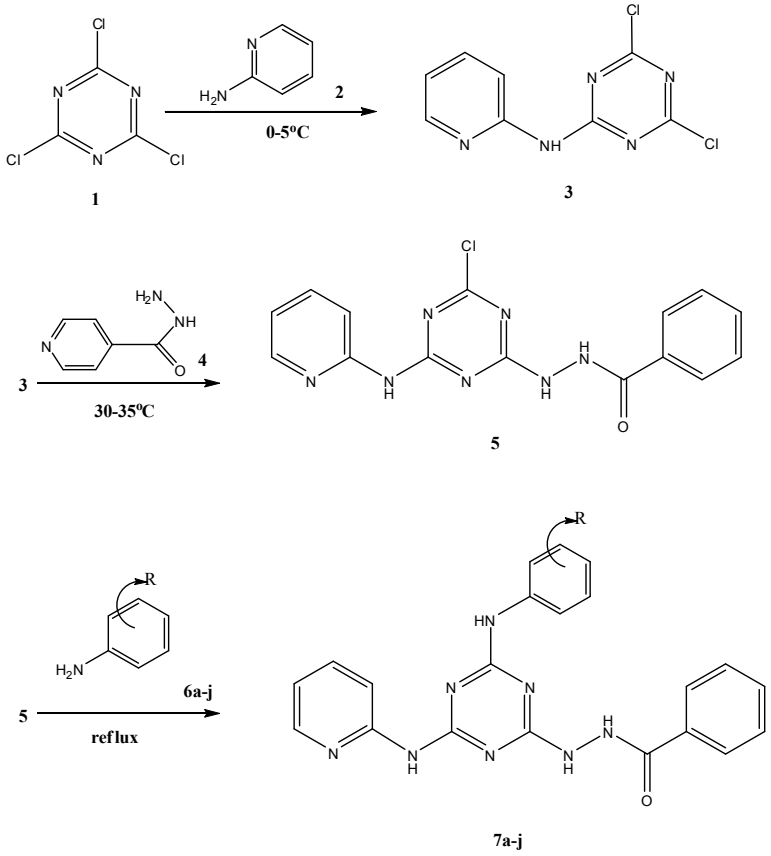
Experimental

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 (ν_{max} in cm^{-1}) were recorded on Shimadzu FTIR spectrophotometer using KBr or Nujol technique. ^{1}H & ^{13}C NMR spectra on a Bruker's WM 400 FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 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 substitution[19] 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 substitution[19] 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[20] with various aromatic amines, **6a-j** (reflux, 5-6 hours) in presence of aqueous dioxane[20] 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**.



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

Table – 1: Characterization data of 7a-j.

Compd.	R	Melting point(°C)	Yield (%)	λmax# nm (DMF)	Rf Value	Anal. Calcd. (found)/ %		
						C	H	N
7a	H	222 ~ 24	61 ~ 63	270	0.77	63.31 (56.78)	4.55 (4.01)	28.12 (35.18)
7b	2-NO_2	266 ~ 67	62 ~ 64	261	0.81	56.88 (56.47)	3.86 (3.61)	28.43 (28.40)
7c	3-NO_2	266 ~ 67	61 ~ 63	262	0.82	56.88 (56.77)	3.86 (3.61)	28.43 (28.40)
7d	4-NO_2	268 ~ 69	62 ~ 63	264	0.83	56.88 (56.92)	3.86 (3.66)	28.43 (28.77)
7e	2-Cl	248 ~ 69	65 ~ 67	272	0.71	58.27 (58.61)	3.96 (3.66)	25.89 (25.99)
7f	3-Cl	242 ~ 43	64 ~ 65	274	0.74	58.27 (58.50)	3.96 (3.77)	25.89 (25.66)
7g	4-Cl	241 ~ 72	65 ~ 67	279	0.77	58.27 (58.50)	3.96 (3.79)	25.89 (25.77)
7h	2-CH_3	238 ~ 39	67 ~ 69	281	0.67	64.07 (64.09)	4.89 (4.49)	27.17 (27.29)
7i	3-CH_3	235 ~ 36	68 ~ 69	282	0.69	64.07 (64.11)	4.89 (4.50)	27.17 (27.19)
7j	4-CH_3	237 ~ 38	67 ~ 69	287	0.77	64.07 (64.32)	4.89 (4.91)	27.17 (27.27)

Spectral data of synthesized compounds, 7a-j.

7a: IR (ν_{max} in cm^{-1}):1670((>C=O of amide, C=O str), 3340(NH) &1325(CN), 3085(Aromatic CH str). **^{1}H NMR δ ppm** :10.19(s, 1H, CONH-, D_2O exchangeable), 4.1(s, 1H, NH), 9.24(s, 1H, NH-Ar), 7.1-8.1 (m, 13H, Ar-H). **^{13}C NMR δ ppm**: 119.1-147.2(Ar-C), 164.7, 168.1, 176.9(C=N of s-triazine), 163.27(CO).

7b: IR (ν_{max} in cm^{-1}):1672((>C=O of amide, C=O str), 3345(NH) &1327(CN), 1517, 1371(NO_2), 3081(Aromatic CH str). **^{1}H NMR δ ppm** :10.10(s, 1H, CONH-, D_2O exchangeable), 4.0(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.2-8.3 (m, 13H, Ar-H). **^{13}C NMR δ ppm**: 119.2-149.3(Ar-C), 164.8, 168.2, 176.0(C=N of s-triazine), 163.27(CO).

7c: IR (ν_{max} in cm^{-1}):1671((>C=O of amide, C=O str), 3342(NH) &1326(CN), 1519, 1377(NO_2), 3084(Aromatic CH str). **^{1}H NMR δ ppm** :10.11(s, 1H, CONH-, D_2O exchangeable), 4.2(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.0-8.2 (m, 13H, Ar-H). **^{13}C 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 (ν_{max} in cm^{-1}):1670((>C=O of amide, C=O str), 3340(NH) &1320(CN), 1519, 1377(NO_2), 3077 (Aromatic CH str), 1517, 1371 (-NO_2, N=O str.). **^{1}H NMR δ ppm** :10.15(s, 1H, CONH, D_2O exchangeable), 4.1(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.0-8.2 (m, 12H, Ar-H). **^{13}C NMR δ ppm**: 111.7-147.7(Ar-C), 164.7, 168.7, 176.9(C=N of s-triazine), 164.77(CO).

7e: IR (ν_{max} in cm^{-1}):1675((>C=O of amide, C=O str), 3310(NH) &1325(CN), 3090 (Aromatic CH str), 835(C-Cl str). **^{1}H NMR δ ppm** :10.14(s, 1H, CONH, D_2O exchangeable), 4.0(s, 1H, NH), 9.27(s, 1H, NH-Ar), 7.7-8.9 (m, 12H, Ar-H). **^{13}C 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 (ν_{max} in cm^{-1}):1676((>C=O of amide, C=O str), 3315(NH) &1320(CN), 3095 (Aromatic CH str), 830(C-Cl str). **^{1}H NMR δ ppm** :10.15(s, 1H, CONH, D_2O exchangeable), 4.0(s, 1H, NH), 9.25(s, 1H, NH-Ar), 7.7-8.9 (m, 12H, Ar-H). **^{13}C 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 (ν_{max} in cm^{-1}):1677((>C=O of amide, C=O str), 3319(NH) &1327(CN), 3099 (Aromatic CH str), 837(C-Cl str). **^{1}H NMR δ ppm** :10.19(s, 1H, CONH, D_2O exchangeable), 4.7(s, 1H, NH), 9.29(s, 1H, NH-Ar), 7.7-8.9 (m, 12H, Ar-H). **^{13}C 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 (ν_{max} in cm^{-1}):1672((>C=O of amide, C=O str), 3315(NH) &1327(CN), 3099 (Aromatic CH str), 832(C-Cl str). **^{1}H NMR δ ppm** :10.15(s, 1H, CONH, D_2O exchangeable), 4.7(s, 1H, NH), 9.29(s, 1H, NH-Ar), 7.2-8.5 (m, 12H, Ar-H). **^{13}C NMR δ ppm**: 111.7-149.9(Ar-C), 163.5, 169.7, 176.9(C=N of s-triazine), 164.9 (CO).

7i: IR (ν_{max} in cm^{-1}):1674((>C=O of amide, C=O str), 3322(NH) &1322(CN), 3091 (Aromatic CH str), 1311 (CH_3, C-H bend.). **^{1}H NMR δ ppm** :10.11(s, 1H, CONH, D_2O exchangeable), 4.1(s, 1H, NH), 9.11(s, 1H, NH-Ar), 7.1-8.1 (m, 12H, Ar-H). **^{13}C NMR δ ppm**: 119.1-147.1(Ar-C), 160.1, 171.1, 179.1(C=N of s-triazine), 164.1 (CO).

7j: IR (ν_{max} in cm^{-1}):1675((>C=O of amide, C=O str), 3320(NH) &1320(CN), 3089 (Aromatic CH str), 1309 (CH_3, C-H bend.). **^{1}H NMR δ ppm** :10.18(s, 1H, CONH, D_2O exchangeable), 4.0(s, 1H, NH), 9.21(s, 1H, NH-Ar), 7.9-8.7 (m, 12H, Ar-H). **^{13}C 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[21]

The in vitro antibacterial screening of all the compounds were evaluated against selected (Table 1) Gram-positive organisms viz. *Bacillus subtilis*(MTCC 441), *Bacillus sphaericus*(MTCC 11), *Staphylococcus aureus*(MTCC 96) and Gram-negative organisms viz. *Chromobacterium violaceum* (MTCC 2656), *Klebseilla aerogenes* (MTCC 39), *Pseudomonas aeruginosa*(MTCC 741), *Salomonella paratyphi A*(MTCC 735) and *Escherichia coli*(MTCC 443) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards[21]. Standard antibacterial agent like Benzyl Penicillin and Streptomycin were also screened under identical conditions for comparison.

Antifungal activity[21]

A. awamori, A. niger and C. albicans was employed for testing antifungal activity using the cup–plate method[21]. 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

Table – 2. *In vitro* Antibacterial activity of compounds 3 & 7a-j (Zone of inhibition in mm)

Compd.	*B.s*	*B.sph*	*S.a*	*K.a*	*C.v*	*P.a*	*E.c*	*S.p*
7a	14	17	16	16	17	14	17	17
7b	15	22	17	15	16	17	16	20
7c	17	20	17	17	17	18	20	18
7d	17	20	22	17	18	16	20	18
7e	24	17	17	16	17	16	19	20
7f	20	23	18	14	18	16	19	20
7g	16	16	16	17	23	15	17	18
7h	15	17	16	15	16	17	16	15
7i	17	23	15	14	17	17	18	22
7j	18	24	15	16	24	17	20	23
Benzyl Penicillin	26	28	30	30	26	32	30	28
Strepto-mycin	32	30	30	32	32	29	34	29

* *Negative control: Acetone*

Gram +ve Organisms
B.s: : Bacillus subtilis (MTCC 121),
B.sph.: Bacillus sphaericus (MTCC 11)
S.a. : Staphylococcus aureus (MTCC 96)

Gram –ve Organisms
K.a. : Klebseilla aerogenes (MTCC 39),
C.v. : Chromobacterium violaceum (MTCC 2656),
P.a. :Pseudomonas aeruginosa (MTCC 791),
E.c. : Escherichia coli (MTCC 443)
S.p. : Salomonella paratyphi A (MTCC 735)

Table – 3. *In vitro* Antifungal activity of compounds 3 & 7a-j (Zone of inhibition in mm)

Compd.	*A. awamori*	*A. niger*	*C. albicans*
7[a]	11	10	09
7b	14	21	20
7c	16	18	17
7d	16	14	14
7e	12	09	11
7f	11	12	15
7g	10	20	20
7h	16	18	17
7i	15	19	18
7j	17	18	16
Griseofulvin	22	24	24

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 s-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

Trisubsituted 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 pharmacologically 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.

Acknowledgments

One of the author(JPRaval) is thankful to Prof. P.S.Patel, Director(Research), ARIBAS, New Vallabh Vidyanagar for providing research facilities, Thanks to SICART & Department of Chemistry(Navinbhai), Sardar Patel University, Vallabh Vidyanagar for providing analytical and NMR spectral facilities.

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