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Synthesis and cytotoxic activity of trisubstituted-1,3,5-triazines

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Abstract—1,3,5-Triazine derivatives were screened for phototoxicity as well as the cytotoxic activities against leukemia and adenocarcinoma derived cell lines in comparison to the normal human keratinocytes. A simple and environmentally friendly procedure has been developed for the synthesis of 1,3,5-triazine derivatives under microwave irradiation in the presence of a HY zeolite. The catalyst can be recovered and reused. Thus, the procedure provides a simple and green synthetic methodology under environmentally friendly conditions. Structure–activity relationships between the chemical structures and antimycobacterial and photosynthesis-inhibiting activity of the evaluated compounds are also discussed.

Chemotherapeutic agents that target cellular DNA have been established as one of the most effective classes of drugs used in the clinic for the treatment of cancer. Several subsets of structurally related compounds have been also prepared and evaluated extensively for their cell-killing effects,[1,2] which can be attributed to a number of different molecular mechanisms. Nevertheless, most of these molecules have been designed to exert cytotoxicity by interacting with DNA and subsequently causing extensive DNA damage, leading to induction of cell death.

1,3,5-Triazines (or *s*-triazines) are a class of compounds well known for a long time, and still continue to be the object of considerable interest, mainly due to their applications in different fields, including the production of herbicides and polymer photostabilizers.[3] Some 1,3,5-triazines display important biological properties (Fig. 1); for example hexamethylmelamine (HMM, **1**) and 2-amino-4-morpholino-*s*-triazine (**2**) are used clinically due to their antitumor properties to treat lung, breast, and ovarian cancer, respectively.[4] Hydroxymethylpentamethylmelamine (HMPMM, **3**) is also the hydroxylated metabolite, which corresponds to the major active form of HMM.[5] More recently, similar general structure **4** shows antitumor activity in human cancer and murine leukemia cell line.[5] Among several other 1,3,5-triazine substituted polyamines tested, the substrate **5** presents a good in vitro activity against the protozoan parasite *Trypanosoma brucei*, the causative organism of Human African Trypanosomasis.[6]

Zeolites, heterogeneous catalysts, have been widely utilized as catalysts in the petroleum refining and chemical industries[7] as well as in the preparation of fine chemicals[8] by use of their characteristic properties such as highly acidic and basic nature, high thermal stability, and specific shape selectivity. Main advantages of acidic zeolites over homogeneous acid catalysts can be offered by replacing corrosive and polluting acid catalysts with more environmentally friendly zeolites. Y Zeolites are also reported as effective catalysts in organic chemistry[9–14] and their specificity in gas phase transformations is greatly utilized in industry[15] which encouraged us to investigate the new catalytic possibilities of HY zeolite.

Parallely, the potential application of microwave technology in organic synthesis,[16] and particularly under free-solvent conditions, is increasing rapidly because of its reaction simplicity, lesser environmental impact, and reduced reaction time, providing rapid access to large libraries of diverse molecules.

The development of one-pot reaction has been of great interest in organic synthesis because this methodology provides easy access to highly complex molecules from relatively simple reagents under economically favorable reaction conditions. Thus, the combination of the

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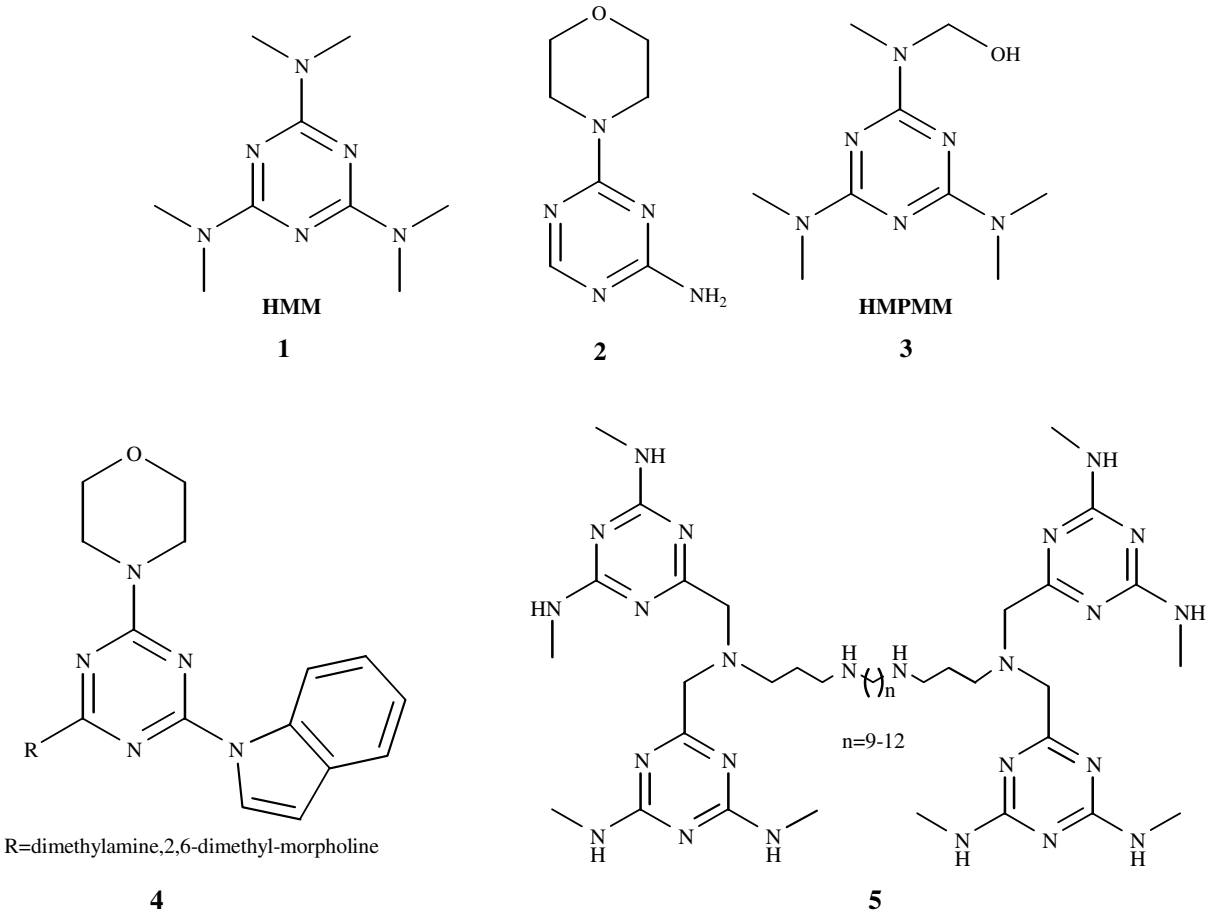
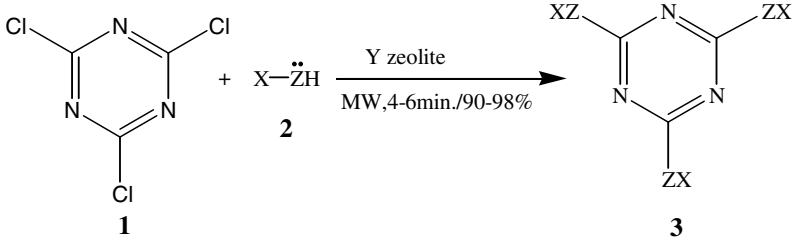


Figure 1. Select examples of biologically active compounds containing the 1,3,5-triazine unit.

one-pot strategy with the use of environmentally friendly zeolite catalysts becomes a powerful means of preparation for specific target compounds to minimize pollutants and to reduce production cost.[17]

The diverse biological activities observed for different molecules containing the 1,3,5-triazine unit prompted us to discover other new potential molecules containing the 1,3,5-triazine unit. As part of a program aimed at achieving simple and environmentally compatible synthetic methodologies for biodynamic heterocycles[18] and substituted triazines,[19] we herein report the one-pot synthesis of 2,4,6-trisubstituted-1,3,5-triazines by reaction of cyanuric chloride with aromatic/aliphatic amines, amide, and water under microwave irradiation using HY zeolite as a catalyst (Scheme 1). We have also investigated cytotoxic activities of the title compounds against leukemia and adenocarcinoma-derived cell lines in comparison to the normal human keratinocytes and found that the introduction of a different substitution results in an improvement of the antiproliferative activity of these compounds.

Although one-pot syntheses of trisubstituted-1,3,5-triazine derivatives are reported in the literature using Pd as catalyst[20], excess of selected amines and potential UV absorbents[21] under volatile and hazardous solvent, the use of highly volatile solvent like dichlorobenzene/



X = Ar/CH_3Ar/$(C_2H_5)_2$/$(CH_3)_2$/CH_3CO/CH_3/OCH_3Ar/NO_2Ar/ClAr/H; Z= O, NH

Scheme 1.

toluene/ether, etc., and Lewis acids such as $ZnCl_2$,[22] $AlCl_3$,[23] $MgCl_2$,[24] and $Mo(CO)_6$[25] or Pd[20] is not favorable for environment.

As an initial attempt to find an optimal reaction condition, a variety of experimental conditions were examined for cyanuric chloride (**1**) by changing catalyst, temperature, amine, and reaction medium (Tables 1 and 2).

Finally, in order to check the possible intervention of specific (non-thermal) MW effects, the results obtained under MW were compared to conventional heating using toluene as solvent. The reaction, in the case of compound **3a**, has been carried out using preheated oil bath, under the same conditions as under MW (time, temperature, vessel) (Table 2). Reactions proceeding with considerable good yields under similar thermal conditions showed there is nonexistence of specific MW effect. The reaction is also tried under neat and solvent conditions with or without microwaves giving lower conversion to the product which showed catalytic effect of zeolite.

It was found that HY zeolite is the best choice of catalyst for the preparation of 2,4,6-trisubstituted-1,3,5-triazines compared with NaY, CeY, and LaY zeolites (**3e**–**g**). Since the number and strength of acid site in zeolite decreases with metal cation exchanged in the order of $Na^+ < Ca^{2+} < La^{3+}$, the decrease in the yield of **3a** according to this order suggests that acid sites on zeolite work as active sites for this reaction. As usual in the catalytic reaction, the increase of the reaction temperature accelerated the conversion of the substrate (**3a**–**d**, Table 1).

The substituents of the aromatic ring also affected the yields as well as the distribution of products. The reaction of various aliphatic/aromatic amines substituted with electron-withdrawing groups (**3a**, **k**, **m**–**o**) provided high conversion in a short reaction time (Table 1), while the reaction with an electron-donating group (**3h**–**j**, **l**) resulted in low conversion (90–92%). The yield of desired 2,4,6-trisubstituted-1,3,5-triazines was found to be increased as the number of electron-withdrawing substituents on the aromatic ring increased.

The phototoxicity of title compounds was investigated first on a cell line of human tumor HL-60 (human promyelocytic leukemia). Table 3 shows the extent of cell survival expressed as GI50, which is the concentration, expressed in mM, which induces 50% of inhibition of cell growth, after irradiation at different UVA doses.

Control experiments with UVA light or compounds alone were carried out without significant cytotoxic effects (data not shown). The results, shown in Table 3, indicate that compound **3a** is not active, instead **3k**, **m**, **n**, **o** exhibit the highest activity. Interestingly, compound **3i** is only slightly active and substitution for a methyl group leads to an inactive derivative **3h**. From this preliminary screening the most active compounds were also evaluated on a human intestinal adenocarcinoma cell line (LoVo) and

Table 1. Substitution reaction of (**1**) to the corresponding 2,4,6-trisubstituted-1,3,5-triazines (**3**) under various reaction conditions

Compound	X	Z	Zeolite	Reaction conditions		Yield (%)[a]
			Type	Temp. (°C)	Time (min)	
3a	C_6H_5	NH	HY	180	4	94
3b	C_6H_5	NH	HY	150	6	65
3c	C_6H_5	NH	HY	120	8	45
3d	C_6H_5	NH	HY	100	9	20
3e	C_6H_5	NH	NaY	180	5	85
3f	C_6H_5	NH	CeY	180	6	82
3g	C_6H_5	NH	LaY	180	6	80
3h	$CH_3C_6H_4$	NH	HY	180	5	90
3i	$(C_2H_5)_2$	N	HY	180	5	92
3j	$(CH_3)_2$	N	HY	180	6	94
3k	CH_3CO	NH	HY	180	4	98
3l	CH_3	NH	HY	180	6	92
3m	$OCH_3C_6H_4$	NH	HY	180	5	96
3n	$NO_2C_6H_4$	NH	HY	180	6	97
3o	ClC_6H_4	NH	HY	180	5	95
3p	H	O	HY	180	4	97

[a] Isolated yields.

Table 2. Comparative study of the synthesis of **3a**

Entry	Medium	Reaction conditions	Reaction temperature (°C)	Time (min)	Yield[a] (%)
1	HY zeolite	MW	180	4	94
2	Neat	MW	180	10	40
3	HY zeolite	Reflux in aniline	180	6	70
4	HY zeolite	Reflux in toluene	110	360	58

[a] Isolated yield.

Table 3. Photocytotoxicity of test compounds against HL-60 cell line, compounds GI_{50}[a] (mM)

Compound	GI_{50}[a] (μm)	
	1.25[b] J cm^{-2}	2.5 J cm^{-2}
3a	>10	10
3h	>10	10
3i	>10	7.5 ± 2.0
3j	>10	7.2 ± 1.9
3k	6.8 ± 0.7	0.8 ± 0.1
3l	>10	10
3m	3.4 ± 0.7	0.6 ± 0.1
3n	2.8 ± 0.7	0.8 ± 0.1
3o	4.3 ± 0.7	0.3 ± 0.1

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[b] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole-Parmer radiometer.

one line of immortalized, not tumorigenic, human keratinocytes (NCTC 2544). From Table 4 it appears that the phototoxicity of the most active compounds, in particular **3k**, is higher in the tumor cell lines in comparison to the normal ones (NCTC 2544). In preliminary experiments devoted to the search for a possible molecular target, compound **3k** was evaluated for its potential capability to induce single strand breaks in a plasmid DNA, as a model.

The obtained results (data not shown) indicate that **3k**, after irradiation in the presence of DNA, is not able to induce any significant damage to DNA thus suggesting that another target at cellular level may be involved in its phototoxic effect. In parallel to the cytotoxic evaluation, flow cytometry was employed to study cell cycle variations upon irradiation. The effects of the most active compound **3k** were evaluated after 24, 48, and 72 h from irradiation in the leukemic cell line. The percentage of the cells in the different phases of the cell cycle is shown in Table 5.

It can be observed that treatment with **3k** in combination with UVA induces a reduction of the S phase at 48 and 72 h after irradiation especially for the highest dose utilized. This is accompanied by a concomitant block in G1 phase. This event is followed at 48 and 72 h after the irradiation by massive induction of apoptosis, as observed by the appearance of a sub G1 peak

Table 4. Photocytotoxicity of test compounds against NCTC 2544 and LoVo cell lines[a]

Compound	Cell line GI_{50}[b] (μm)			
	NCTC 2544		LoVo	
	1.25[c] J cm^{-2}	2.5 J cm^{-2}	1.25[b] J cm^{-2}	2.5 J cm^{-2}
3k	7.2 ± 1.3	2.5 ± 0.7	3.5 ± 0.8	1.89 ± 0.2
3i	>20	>20	>20	12.5 ± 1.9
3j	>20	7.5 ± 2.2	17.2 ± 2.1	10.3 ± 1.3

[a] Human cell lines: NCTC 2544 Human keratinocytes; LoVo intestinaladenocarcinoma.
[b] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[c] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole-Parmer radiometer.

Table 5. Percentage of HL-60 in the different phases of the cell cycle[a]

Treatment	G1	G2	S	Apoptotic cells[b]
Non irradiated cells	39.0	9.0	51.7	0.8
UVA irradiated cells without drug (2.5 J cm^{-2})				
24 h	35.8	13.0	50.3	8.6
48 h	48.9	10.9	40.9	11.7
72 h	34.5	10.5	54.4	9.4
3k 2.5 mM CUVA (2.5 J cm^{-2})				
24 h	29.3	10.8	58.4	13.2
48 h	44.5	10.3	45.0	27.0
72 h	45.2	12.0	43.8	40.5
3k 5.0 mM CUVA (2.5 J cm^{-2})				
24 h	34.2	11.1	54.3	21.0
48 h	50.3	11.7	38.6	26.0
72 h	57.3	6.9	37.1	46.0

[a] The percentage of each phase of the cell cycle (G1, S, and G2/M) was calculated on living cells.
[b] The percentage of apoptotic cells is referred to cell population characterized by the appearance of a sub G1 peak.

(apoptotic cells) that refers to cells with DNA content lower than G1.[26,27] In fact, apoptosis induces the activation of endogenous nucleases, which are responsible for nucleic acid degradation.

In vitro antimycobacterial activity and inhibition of oxygen evolution rate in spinach chloroplasts were also investigated. The highest antituberculotic activity (72% inhibition) against *Mycobacterium tuberculosis* and also the highest lipophilicity (log $P = 6.85$) were shown by the *N*,*N*′,*N*″-(4-methoxyphenyl)-1,3,5-triazine-2,4,6- triamine (**3m**). Some other amides (**3j**, **3l**, **3n**, **3o**) with higher than 20% inhibition were investigated. The negative results of antimycobacterial activity screening allow us to make no conclusions regarding potential structure–activity relationships. Results of their antimycobacterial activity (MIC, % Inhibition) and calculated log *P* values of **3a**, **3h–o** are shown in Table 6.

Additionally some inhibition of chlorophyll production in green algae *Chlorella vulgaris* was studied by the

Table 6. Antimycobacterial activity (MIC, % inhibition), IC_{50} values for inhibition of oxygen evolution rate in spinach chloroplasts by compounds **3a**, **3h–o**, and calculated log *P* values of the compounds in comparison with standards rifampicin (RMP) and DCMU (see Experimental)

Compound	MIC (μg ml^{-1})	% Inhibition	IC_{50} (mmol dm^{-3})	log *P*
3a	>6.25	0	1.072	2.72 ± 0.41
3h	>6.25	0	0.440	3.28 ± 0.40
3i	>6.25	0	0.244	4.41 ± 0.42
3j	>6.25	28	0.486	2.72 ± 0.41
3k	>6.25	11	0.148	3.28 ± 0.40
3l	>6.25	24	0.118	4.41 ± 0.42
3m	>6.25	72	0.241	6.85 ± 0.55
3n	>6.25	20	0.107	5.15 ± 0.50
3o	>6.25	39	0.081	4.03 ± 0.48
RMP	0.125	100		0.37 ± 0.35
DCMUc	—	—	0.0019	2.78 ± 0.38

compounds **3h–m**. Results of their antialgal activity (Fig. 2) are given in Table 7.

In conclusion, we have presented a new economical, safe, environmentally benign solvent-free HY zeolite promoted synthesis of corresponding 2,4,6-trisubstituted 1,3,5-triazines under microwave irradiation.[29,30] The operational simplicity, avoiding the use of solvent and solid support, high yield in significantly very short reaction times, can impose this procedure as a useful and attractive alternative to the currently available methods.

On the basis of the biological evaluation, compound **3k** seems to be very attractive as a potential drug for photochemotherapy.[31] Hence, experiments aimed at defining the target(s) at cellular level and the phototoxicity mechanism are in progress. While the compound **3m** shows highest antituberculotic and antialgal activities.

Human promyelocytic leukemia cells (HL-60) were grown in RPMI-1640 medium (Sigma Co., MO, USA), human keratinocytes (NCTC 2544) were grown in DMEM (Sigma Co., MO, USA), intestinal adenocarcinoma cells (LoVo) were grown in Ham's F12 medium (Sigma Co., MO, USA) all supplemented with 115 U/ml of penicillin G (Invitrogen, Milano, Italy), 115 μg/ml streptomycin (Invitrogen, Milano, Italy) and 10% fetal bovine serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate (Falcon BD) were inoculated with 100 ml of complete medium containing 8×10^3 HL-60 cells or 5×10^3 NCTC 2544 and LoVo cells. The plates were incubated at 37 °C in a humidified 5% incubator for 18 h prior to the experiments. After medium removal, 100 ml of the drug solution, dissolved in DMSO and diluted with Hanks' Balanced Salt Solution (HBSS pH 7.2), was added to each well and incubated at 37 °C for 30 min and then irradiated.

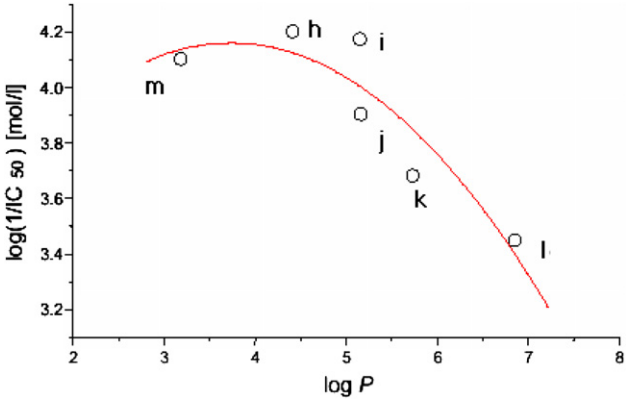


Figure 2. Quasi-parabolic dependence between antialgal activity and log *P* of **3h-m**.

Table 7. IC_{50} values concerning inhibition of chlorophyll production in green algae *Chlorella vulgaris* by the compounds **3h**, **3i**, **3j**, **3k**, **3l**, and **3m**, and calculated log *P* values of the compounds in comparison with standard DCMU (see experimental)

Compound	IC_{50} (mmol dm^{-3})	log *P*
3h	0.063	4.41 ± 0.42
3i	0.067	5.15 ± 0.50
3j	0.125	5.16 ± 0.54
3k	0.208	5.73 ± 0.53
3l	0.356	6.85 ± 0.55
3m	0.079	3.18 ± 0.41
DCMU	0.0073	2.78 ± 0.38

After irradiation, the solution was replaced with the medium, and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] test, as described previously.[32,33]

For flow cytometric analysis of DNA content, 5×105 HL-60 cells in exponential growth were treated at different concentrations of the test compounds for 24, 48, and 72 h. After the incubation period, the cells were centrifuged and fixed with ice-cold ethanol (70%), treated with lysis buffer containing RNAseA, and then stained with propidium iodide. Samples were analyzed on a Becton Coulter Epics XL-MCL flow cytometer. For cell cycle analysis, DNA histograms were analyzed using Multi Cycle for Windows (Phoenix Flow Systems, San Diego, CA).

Antimycobacterial assay. Primary screening of all compounds was conducted at 6.5 or 12.5 μg ml^{-1} against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system.[34] The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls. For the results, see Table 6.

Study of inhibition of chlorophyll production in green algae Chlorella vulgaris. The algae *Chlorella vulgaris* were cultivated statically at room temperature according to Sidóová et al.[34] (photoperiod 16 h light/8 h dark; illumination 4000 lux; pH 7.2). The effect of compounds **3h–m** on algal chlorophyll (Chl) content was determined after 4-day cultivation in the presence of the tested compounds, expressing the response as percentage of the corresponding values obtained for control. The Chl content in the algal suspension was determined spectrophotometrically (Specord UV VIS, Zeiss Jena, Germany) after extraction into *N*,*N*-dimethylformamide according to Inskeep and Bloom. The Chl content in the suspensions at the beginning of cultivation was 0.5 mg dm^{-3}. Because of their low water solubility, the tested compounds were dissolved in DMSO. DMSO concentration in the algal suspensions did not exceed 0.25 v/v% and the control samples contained the same DMSO amount as the suspensions treated with the tested compounds. IC_{50} value for the standard, a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (DIURON), was measured about 7.3 μmol dm^{-3}. For the results, see Table 7.

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29. *Experimental*: All melting points were taken on a Büchi-Tottoli capillary apparatus and are uncorrected; IR spectra (KBr) were recorded on a Magna FT IR–550 spectrophotometer, ^{1}H, ^{19}F, and ^{13}C NMR spectra [$CDCl_3$ + $(CD_3)_2SO$] were taken on a Bruker –300DX spectrometer at 300, 84.25, and 200 MHz, respectively, using TMS as an internal standard for PMR, and mass spectra were recorded on Jeol D–300 spectrometer at an ionization potential of 70 e.v. Microwave-assisted reactions were carried out on a BPL BMO model, operating at 900 W, generating 2450 MHz frequency. Temperature was measured with an IR-sensor and reaction is given as hold times. All reactants were purchased from Aldrich Chemical Co. and were used as received. Log *P* values were computed using a program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto). All the products were characterized by comparison of their ^{1}H NMR spectral data and mixed mp with the reported ones.[28]
30. *Synthesis of 2,4,6-trisubstituted-1,3,5-triazines* (**3a**, **h–p**): A mixture of cyanuric chloride (1.84 g, 0.01 mol) and HY zeolite (2 mg) was finely ground with a mortar and pestle. Aromatic/aliphatic amines, amide (0.04 mol), and water (3 g) (**2**) were added to this mixture in a Pyrex glass vial,

which was placed in a screw capped Teflon vessel. Microwave irradiation (MW domestic type oven 900 W with a frequency 2450 MHz) was applied for 4–9 min at 180 °C. After the completion of reaction (TLC analysis), recyclable zeolite was separated by filtration after eluting the product with ethanol under reduced pressure and found to be pure with no need of further purification.

Compound **3a**: white solid (94%); mp = 228–230 °C; IR (KBr) 3465–3235 (br, NH), 1610 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS) δ 6.42–7.72 (m, 15Ar-H), 8.57 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 115.3–143.4 (aromatic carbons), 172.2 (*C*=N); MS (EI, 70 eV): *m/z* 354 $[M]^+$ (100), 277 (10), 200 (23.8), 124 (1.1), 123 (72.4); Anal. Calcd for $C_{21}H_{18}N_6$ (MW 354): C, 71.17; H, 5.12; N, 23.71. Found: C, 71.04; H, 5.18; N, 23.65.

Compound **3h**: white solid (90%); mp = 250–254 °C; IR (KBr) 3465–3235 (br, NH); 2920–2870 (CH stretching),1625 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS) δ 2.36 (s, 3H, CH_3), 6.36–7.50 (m, 12 Ar-H), 8.89 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 21.3 (C–*C*H_3), 112.5–148.3 (aromatic carbons), 175.3 (*C*=N); MS (EI, 70 eV): *m/z* 396 $[M]^+$ (45), 354 (100), 200 (23.8), 124 (31.9), 123 (72.4); Anal. Calcd for $C_{24}H_{24}N_6$ (MW 396): C, 72.70; H, 6.10; N, 21.20. Found: C, 72.74; H, 6.13; N, 21.15.

Compound **3i**: white solid (92%); mp = 332–335 °C; IR (KBr) 2920 (CH stretching),1620 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ1.70–1.88 (t, 3H, CH_3, *J* = 6.1 Hz), 4.10–4.18 (q, 2H, CH_2, *J* = 6.1 Hz); ^{13}C NMR (300 MHz, DMSO, TMS) δ 18.3 (C–*C*H_3),50.2 (C–*C*H_2),171.9 (*C*=N); MS (EI, 70 eV): *m/z* 294 $[M]^+$ (45), 354 (40), 245 (23.8), 197 (21.1), 123 (100); Anal. Calcd for $C_{15}H_{30}N_6$ (MW 294): C, 61.19; H, 10.27; N, 28.54. Found: C, 61.24; H, 10.32; N, 28.59.

Compound **3j**: white solid (94%); mp = 325–328 °C; IR(KBr) 2910–2850 (CH stretching), 1615 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ 2.45 (s, 3H, CH_3); ^{13}C NMR (300 MHz, DMSO, TMS) δ 25.6 (C–*C*H_3),176.8 (*C*=N), MS (EI, 70 eV): *m/z* 210 $[M]^+$ (49), 195 (25), 145 (41), 123 (100); Anal. Calcd for $C_9H_{18}N_6$ (MW 210):C, 51.41; H, 8.63; N, 39.97. Found: C, 51.35; H, 8.69; N, 40.02.

Compound **3k**: white solid (98%); mp = 295–298 °C. IR (KBr) 2910–2850 (CH stretching), 1610 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ 2.08 (s, 3H, CH_3), 8.74 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 22.5 (C–*C*H_3); 168.3 (*C*=O); 173.6 (*C*=N); MS (EI, 70 eV): *m/z* 252 $[M]^+$ (100), 224 (41), 168 (25); Anal. Calcd for $C_9H_{12}N_6O_3$ (MW 252): C, 42.86; H, 4.80; N, 33.32; O, 19.03. Found: C, 42.77; H, 4.85; N, 33.28; O, 19.06.

Compound **3l**: white solid (92%); mp = 263–265 °C; IR (KBr) 2940–2880 (CH stretching),1615 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ 2.12 (s, 3H, CH_3); 8.74 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 23.2 (C–*C*H_3), 174.6 (*C*=N). MS (EI, 70 eV): *m/z* 168 $[M]^+$ (100), 153 (41);Anal. Calcd for $C_6H_{12}N_6$ (MW 168): C, 42.84; H, 7.19; N, 49.96 Found: C, 42.77; H, 7.14; N, 50.08.

Compound **3m**: white solid (96%); mp = 196–198 °C; IR (KBr) 2920–2870 (CH stretching); 1620 (C=N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ 3.56 (s, 3H, OCH_3), 6.65–7.85 (m, 12 Ar-H) 9.05 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 56.8 (C–OCH_3),113.8–145.3 (aromatic carbons), 173.9 (*C*=N); MS (EI, 70 eV): *m/z* 444 $[M]^+$ (100), 412 (35); Anal. Calcd for $C_{24}H_{24}N_6O_3$ (MW 444): C, 64.85; H, 5.44; N, 18.91; O, 10.80. Found: C, 64.74; H, 5.32; N, 18.84; O, 10.72.

Compound **3n**: white solid (97%); mp = 232–234 °C; IR(KBr) 2915–2875(CH stretching), 1620 (C=N) cm^{-1}; ^1H NMR(400 MHz, CD_3OD, TMS): δ 6.56–7.89 (m, 12 Ar-H), 8.64 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS) δ 111.5–132.3 (aromatic carbons), 140.23 (*C*–NO_2), 173.6 (*C*=N) ; MS (EI, 70 eV): *m/z* 489 $[M]^+$ (100), 459 (25); Anal. Calcd for $C_{21}H_{15}N_9O_6$ (MW 489): C, 51.54; H, 3.09; N, 25.76; O, 19.62. Found: C, 51.42; H, 2.97; N, 25.46; O, 19.50.

Compound **3o**: white solid (95%); mp = 254–256 °C; IR (KBr) 2925–2870 (CH stretching), 1620 (C=N), 760 (Cl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TMS): δ 6.32–7.96 (m, 12 Ar-H), 8.56 (s, 3H, NH*); ^{13}C NMR (300 MHz, DMSO, TMS): δ 112.5–134.6 (aromatic carbons), 138.5 (*C*-Cl), 172.8 (*C*=N); MS (EI, 70 eV): *m/z* 458 $[M]^+$ (100), 423 (31) Anal. Calcd for $C_{21}H_{15}N_6Cl_3$ (MW 458): C, 55.10; H, 3.30; N, 18.36; Cl, 23.34. Found: C, 55.20; H, 3.15; N, 18.45; Cl, 23.45.

Compound **3p**[28]: White crystalline solid (97%); mp = 338–340 °C.

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