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Synthesis and antimicrobial activity of 2-fluorophenyl-4,6-disubstituted [1,3,5]triazines

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ABSTRACT

A series of 2-fluorophenyl-4,6-disubstituted [1,3,5]triazines (**1**) and (**2**) were synthesized and evaluated for their antimicrobial activity against three representative gram-positive bacteria and two fungi. The structure–activity relationship (SAR) demonstrates that the 3- or 4-fluorophenyl component attached directly to the triazine ring was essential for activity. Of these compounds, **14**, **15**, and **25** demonstrated significant activity against all selected organisms compared to control. These compounds were generally nontoxic and may prove useful as antimicrobial agents.

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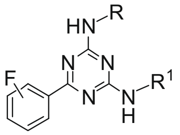
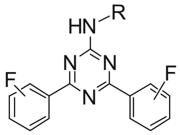
The increasing incidence of infection caused by the rapid development of bacterial resistance to most of the known antibiotics is a serious health problem.[1] While many factors may be responsible for mutations in microbial genomes, it has been widely demonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes.[2] As multidrug-resistant bacterial strains proliferate, the necessity for effective therapy has stimulated research into the design and synthesis of novel antimicrobial molecules.

The chemistry of [1,3,5]triazine compounds has been studied intensively and is the subject of many reviews.[3] The triazine scaffold has provided the basis for the design of biologically relevant molecules with broad biomedical value as therapeutics. For example, triazine compounds possess potent antiprotozoal,[4] anticancer,[5] antimalarial,[6] and antiviral activity.[7] Also, it was reported that some of these compounds possess potent antimicrobial activity.[8] The starting material for these compounds is cyanuric chloride. This is an inexpensive commercially available reagent which makes its use more attractive. Increased interest in this scaffold lies in the different reactivities of the substituent chlorine atoms, which are controlled by temperature.[3] This allows sequential introduction of various substituents into the [1,3,5]triazine ring. As part of an effort towards the discovery of novel therapeutics for the treatment of infectious diseases, a search was initiated to discover small molecules that possess significant antimicrobial activity. In this Letter, the synthesis and antimicrobial activities of 2-fluorophenyl-4,6-disubstituted [1,3,5]triazines are reported as exemplified by general structures **1** and **2**.



1



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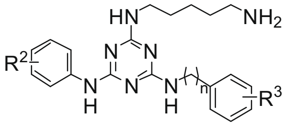
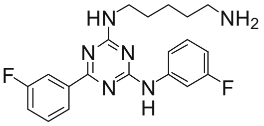
A number of in-house triazine derivatives were screened against gram-positive and gram-negative bacteria, and against fungi. Only compounds of general structure **3**, exemplified by fluorotriazine derivatives **4–6**, demonstrated weak activities.



3; R^2 = F; R^3 = OH, F; n = 0-2
4; R^2 = 3-F; R^3 = 4-OH; n = 2
5; R^2 = 4-F; R^3 = 4-OH; n = 2
6; R^2 = 3-F; R^3 = 3-F; n = 0



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For example, as shown in Table 1, the minimum inhibitory concentrations (MICs) for compound **5** varied between 32 and 128 μg/mL against gram-positive bacteria. Weak MICs were also demonstrated against gram-negative bacteria (64–128 μg/mL)

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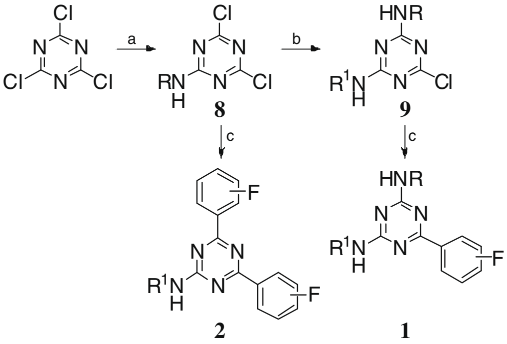
Table 1
Gram-positive antibacterial activity of compounds **5** and **7**(MIC values are in μg/mL)

Entry	Bacterium	Phenotype	MIC compd 5	MIC compd 7	MIC ciprofloxacin
1	*Enterococcus faecalis*		128	8	>16
2	*Enterococcus faecium*	VAN-R	128	4	>16
3	*Staphylococcus aureus*	VAN-R	64	8	>16
4	*Staphylococcus aureus*	M-R	128	8	>16
5	*Staphylococcus cohnii*	CNS	32	2	0.12
6	*Staphylococcus warneri*	CNS	64	4	>16
7	*Staphylococcus epidermidis*	MSSE	64	8	>16
8	*Streptococcus pneumoniae*	MD-R	64	8	1
9	*Streptococcus pneumoniae*		64	8	>16
10	*Propionibac-terium acnes*		32	16	0.5

Compounds **5** and **7** were tested in vitro by Anti-Infective services of Eurofins Medinet, Inc., Herndon, Virginia.
Key: MSSE: methicillin susceptible; M-R: methicillin resistant; VAN-R: vancomycin resistant; CNS: coagulase negative susceptible; MD-R: multidrug-resistant.

and against clinically-relevant fungi (128 μg/mL). The next step was to synthesize analogues in an attempt to enhance the activities of this class of compounds. The fluorophenyl portion appears to be essential and activity declines upon substitution of triazine **3** with other groups (R^2 or $R^3 = H, CH_3, Cl, NH_2$).[9] One possible modification is the introduction of rigidity on one side chain of the triazine ring by linkage directly to the fluorophenyl fragment. As will be seen from the subsequent SAR discussion, this was found to be a preferred variation, since activity is dependent upon distance and relative degrees of freedom at a particular position. An example of this modification is presented by compound **7**. This compound displays weak activity against gram-negative bacteria (MIC values 16–32 μg/mL) which is eightfold higher than the ciprofloxacin control. In addition, compound **7** exhibited moderate activity (MIC value 8 μg/mL) against *Candida albicans* (fungal), as compared to amphotericin B control (MIC 4 μg/mL). However, the best results (Table 1) were obtained against gram-positive bacteria (MIC values (2–16 μg/mL), where triazine **7** exhibited activity at least twofold lower than ciprofloxacin against *Enterococci* and *Staphylococci* sp. (entries 1–4, 6, 7, and 9). Also, this compound did not appear to be affected by the drug resistance phenotype (vancomycin, methicillin, or multidrug-resistant; entries 2–4 and 8). On the basis of these encouraging results, it was decided to further evaluate the activity of analogues of compound **7**.

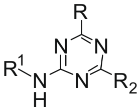
The general synthetic procedure for preparation of triazine compounds[10] **1** and **2** is outlined in Scheme 1. It illustrates the route employed for those derivatives where the key intermediate is the dichlorotriazine **8**. Nucleophilic displacement of chlorine from cyanuric chloride with different aryl or aralkyl amines in the presence of sodium bicarbonate gave dichlorotriazine **8** in high yield (>80%). The latter was treated with aminoalkyl or aralkyl amines and base at room temperature to give the monochlorotriazine **9**. This reaction proceeded in high yield (>85%) and is general for different substituted alkyl amines. The next step was the formation of the aryl–aryl bond between the triazine and the benzene rings using the Suzuki cross-coupling reaction.[11] Thus, treatment of chloro-derivative **9** with *ortho*-, *meta*- or *para*-substituted fluorophenylboronic acid in the presence of $Pd(PPh_3)_4$ and base gave the final product **1** in respectable yields (>65%). This route is practical, amenable to scale-up and offers a general procedure for the preparation of a variety of analogues of 2-(fluorophenyl)-4,6-disubstituted [1,3,5]triazines. A similar approach was applied to synthesize diphenyl triazine derivatives **2**. For example, the chloro intermediate **8** was reacted with 2 equiv of fluorophenylboronic acid under Suzuki condition to give the corresponding fluorotriazine products **2**.

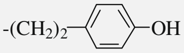
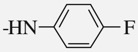
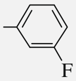
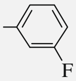
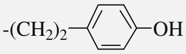
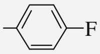
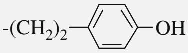
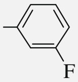
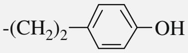
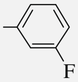
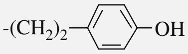
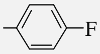
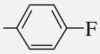
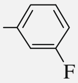
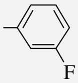
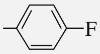
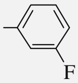
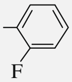
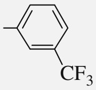
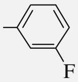
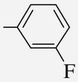
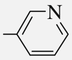
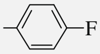
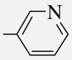
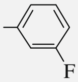
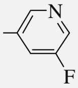


Scheme 1. Reagents and conditions: (a) aryl or aralkyl amine, THF/acetone/H_2O, 5% $NaHCO_3$, 0 °C, 10 min; (b) aminoalkyl or aralkyl amine, 5% $NaHCO_3$, THF/acetone/H_2O, 16 h overnight; (c) fluorophenylboronic acid, $Pd(PPh_3)_4$, 2 M aqueous Na_2CO_3, DME, 90 °C, 16 h.

Compound **7** was selected as a lead structure upon which to define a structure–activity relationship for a 2-(fluorophenyl)-4,6-disubstituted [1,3,5]triazine scaffold. Therefore, analogues were synthesized in which different groups were introduced at positions 4 and 6 of the triazine ring. These compounds were evaluated against three representative gram-positive bacteria: *Staphylococcus epidermidis* (ATCC 155-U), *Enterococcus faecium* (ATCC 49624) and *Bacillus cereus* (ATCC 13061). Since trisubstituted triazines have been reported to possess antifungal activity,[12] these molecules were also tested against two fungi, that is, *C. albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). Table 2 summarizes the results. Ten compounds (**7**, **12**, **14**, **15**, **21**, **24**–**27**, and **30**) displayed significant activity against the bacteria, comparable to streptomycin control, whereas three triazine derivatives (**14**, **15**, and **24**) displayed good activity against *C. albicans*, as compared to amphotericin B control. Only, one compound **28** showed potent activity against *A. niger* compared to the control. As shown in Scheme 1, analogues of the lead compound **7** were synthesized with variations in R (aminoalkyl or aralkyl groups) and R^1 (4-hydroxyphenethyl or aryl substituents), while maintaining the third group as fluorophenyl. Table 2 illustrates the variation of these substituents. Prior work[10] indicated the importance of the carbon chain length (*n*-pentyl) between the amine groups at the R position. In fact, homologation of the chain with one carbon atom (**21**) demonstrated moderate activity. In contrast, an increase in chain length of two carbons (**22**) resulted in reduction of activity. It was observed that good antibacterial activity was obtained with the introduction of rigidity in the diamine fragment (R); for example, isopentyl (**24**), cyclopentylmethyl (**28**), cyclohexyl methyl (**27**) or benzyl amine (**29**) derivatives. However, **24–29** are inactive against fungi except for amines **24** and **28** which respectively demonstrate reasonable and significant activity against *C. albicans* and *A. niger* compared to reference compounds. In general, it can be

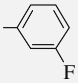
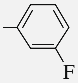
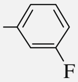
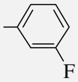
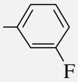
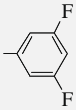
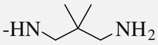
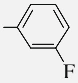
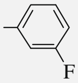
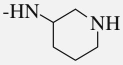
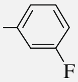
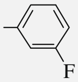
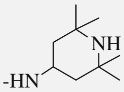
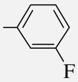
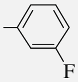
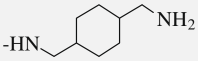
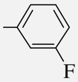
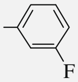
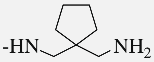
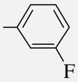
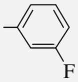
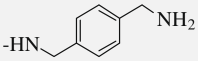
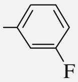
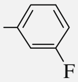
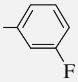
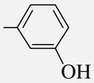
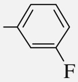
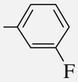
Table 2
In vitro antimicrobial activity of substituted triazines against selected organisms (MIC values are in μg/mL)



Compd	R	R^1	R^2	Antibacterial activity			Antifungal activity	
				E. faecium	*S. epidermidis*	*B. cereus*	*C. albicans*	*A. niger*[a]
5	$-HN(CH_2)_5NH_2$			128	64	16	>100	>100
7	$-HN(CH_2)_5NH_2$			4	8	8	8	8
10	$-HN(CH_2)_5NH_2$			nt	nt	4	>100	>100
11	$-HN(CH_2)_5NH_2$			16	8	8	>100	>100
12	$-N(CH_2CH_2NH_2)_2$			8	4	8	>100	>100
13	$-N(CH_2CH_2NH_2)_2$			16	8	16	32	>100
14	$-HN(CH_2)_5NH_2$			8	2	4	4	16
15	$-HN(CH_2)_5NH_2$			8	2	4	4	8
16	$-HN(CH_2)_5NH_2$			8	4	64	16	>100
17	$-HN(CH_2)_5NH_2$			4	8	16	16	4
18	$-HN(CH_2)_5NH_2$			16	8	>100	>100	>100
19	$-HN(CH_2)_5NH_2$			>100	16	>100	>100	>100
20	$-HN(CH_2)_5NH_2$			32	32	32	>100	>100

(*continued on next page*)

Table 2 (*continued*)

Compd	R	R^1	R^2	Antibacterial activity			Antifungal activity	
				E. faecium	*S. epidermidis*	*B. cereus*	*C. albicans*	*A. niger*[a]
21	$-HN(CH_2)_6NH_2$			4	2	16	16	16
22	$-HN(CH_2)_7NH_2$			16	8	16	64	8
23	$-HN(CH_2)_5NH_2$			8	4	16	16	16
24				8	2	8	4	32
25				4	2	8	8	8
26				4	2	4	32	8
27				8	8	8	64	8
28				32	16	32	8	2
29				8	4	16	16	8
30	$-HN(CH_2)_5NH_2$			8	8	8	32	>100
31		$-(CH_2)_5NH_2$		8	2	nd	4	nd
Streptomycin sulfate				>16	>16	8	—	—
Amphotericin B				—	—	—	1–4[13a]	8[13b]

[a] Similar Results were obtained for compounds tested against *A. niger* using MICs in µg/mL or by disc-diffusion assay.

seen that a rigid amine at R is preferred at this position. Piperidine (**25**) and tetramethylpiperidine (**26**) are the most effective substituents among rigid amines evaluated against bacteria and *A. niger*.

It was next decided to synthesize analogues of triazine **7** in which R^1 = 3-fluorophenyl, R = amino *n*-pentylamine and R^2 was varied with substituted phenyl groups (Table 2). The 2-fluorophenyl function (**16**) resulted in a loss of activity against both bacteria and fungi while 4-fluorophenyl (**15**) displayed good in vitro activity against the tested organisms compared to control. Substitution of the phenyl ring (R^2) with polar functions gave different activities. For example, trifluoromethyl-phenyl (**17**) and 3,5-difluorophenyl (**23**) retained activity against all selected organisms. Also, 3-

hydroxyphenyl (**30**) possessed comparable antibacterial but only weak antifungal activities relative to **7**. In contrast, the corresponding analogue with R^2 = 3-pyridine (**18**) or 5-fluoro-3-pyridine (**20**) displayed a loss of activity against all strains. Interestingly, replacement of 3-fluorophenyl (R^1) of triazine **7** with 4-fluorophenyl (**14**) resulted in an increase in antibacterial activity especially against *S. epidermidis* and *B. cereus*, as well as reasonable activity against *C. albicans* and *A. niger*. Also, an analogue of (**14**) was synthesized in which R^2 was the 3-pyridyl group (**19**). As in the case of compounds **18** and **20**, activity declined against the selected organisms upon replacement of fluorophenyl with 3-pyridyl substituent. Direct substitution of the triazine ring with 3-fluorophenyl at position R and R^2, maintaining pentylamine at R^1 (**31**), resulted in an increase in activity against *S. epidermidis* and *C. albicans* compared to **7**. An analogue of (**14**) was synthesized in which R^1 is tyramine (**11**) instead of 4-fluorophenyl. This compound possessed antibacterial activity comparable with triazine **7** for *S. epidermidis* and *B. cereus*. Similar antifungal results were obtained by replacement of the R^2 substituent of **11** (3-fluorophenyl) by 4-fluorophenyl (compound **10**). The corresponding analogue with R^2 4-fluoroaniline (**5**) yields reduced activity. Interestingly, introduction of rigidity in R of triazine **11** (compound **12**) slightly improves the antibacterial and the antifungal activities. However, replacement of R^2 (3-fluorophenyl) of **12** by 4-fluorophenyl **13** resulted in reduction of activity.

In summary, a number of low molecular weight synthetic molecules of structural type 2-(fluorophenyl)-4,6-disubstituted [1,3,5]triazines (**1**) were prepared and evaluated for their antimicrobial activity against various gram-positive bacteria and fungi. Three lead compounds **14**, **15** and **25** displayed the best activity against the five selected organisms. SAR studies demonstrate the importance of the presence of at least one substituent such as a 3- or 4-fluorophenyl group attached directly to the triazine ring. Also, it can be seen that a rigid diamine at one of the triazine substituents is preferred since activity is increased by introducing rigidity at this position. This class of compounds is safe, nontoxic[9] and displayed promising antimicrobial activity.

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References and notes

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9. Unpublished results.
10. The purity and identity of all the products were monitored by LC/MS at 210 or 250 nm (Agilent 1100) using an analytical C18 column (75 × 4.6 mm, 5 μm) with a gradient of $CH_3CN–H_2O$ (15–99%/10 min) containing 0.01% TFA as the eluant. All compounds demonstrated >98% purity. ^{1}H NMR and ESI-MS characterization data were consistent with the expected structures. For example, data for compound **7**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.12 (d, J = 7.8 Hz, 1H), 8.04 (d, J_{HF} = 9.6 Hz, 1H), 7.72–7.79 (m, 1H), 7.67 (ddd, J = 8.0, 8.0, 5.7 Hz, 1H), 7.51 (ddd, J = 8.2, 8.2, 2.0 Hz, 1H), 7.38–7.45 (m, 2H), 6.96–7.01 (m, 1H), 3.61 (t, J = 7.0 Hz, 2H), 2.95 (t, J = 7.6 Hz, 2H), 1.83–1.95 (m, 2H), 1.73 (tt, J = 7.6, 7.6 Hz, 2H), 1.50–1.58 (m, 2H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −113.45 to −113.35 (m, 1F), −113.99 (s, 1F). LRMS (ESI): *m/z* 385.2 (MH^+). HPLC: t_R = 4.86 min; (254 nm) 100%.
    Data for compound **14**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.09 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.62–7.74 (m, 3H), 7.51 (dd, J = 8.6, 8.0 Hz, 1H), 7.19 (dd, J_{HF} = 8.7 Hz, J_{HH} = 8.7 Hz, 2H), 3.56 (t, J = 7.0 Hz, 2H), 2.93 (t, J = 7.6 Hz, 2H), 1.66–1.79 (m, 4H), 1.33–1.54 (m, 2H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −113.47 (s, 1F), −118.70 (s, 1F). LRMS (ESI): *m/z* 385.2 (MH^+). HPLC: t_R = 4.65 min; (254 nm) 95.0%.
    Data for compound **15**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.33 (dd, J_{HF} = 5.3 Hz, J_{HH} = 8.6 Hz, 2H), 7.73–7.81 (m, 1H), 7.33–7.44 (m, 4H), 6.95–7.00 (m, 1H), 3.60 (t, J = 7.1 Hz, 2H) , 2.94 (t, J = 7.5 Hz, 2H), 1.69–1.83 (m, 4H), 1.50–1.57 (m, 2H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −106.08 (s, 1F), −114.09 (s, 1F). LRMS (ESI): *m/z* 385.2 (MH^+). HPLC: t_R = 4.73 min; (254 nm) 100%.
    Data for compound **24**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.17 (d, J = 7.8 Hz, 1H), 8.07 (d, J_{HF} = 9.6 Hz, 1H), 7.59–7.70 (m, 2H), 7.33–7.52 (m, 3H), 6.96–7.02 (m, 1H), 3.53 (s, 2H), 2.91 (s, 2H), 1.14 (s, 6H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −114.3 to −113.4 (m, 2F). LRMS (ESI): *m/z* 385.2 (MH^+). HPLC: t_R = 4.78 min; (254 nm) 100%.
    Data for compound **25**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.13 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 9.6 Hz, 1H), 7.74–7.84 (m, 1H), 8.04 (ddd, J_{HF} = 5.5 Hz, J_{HH} = 8.1, 8.1 Hz, 1H), 7.31–7.49 (m, 3H), 6.94–6.99 (m, 1H), 3.56–3.62 (m, 1H), 3.43–3.50 (m, 2H), 3.33–3.38 (m, 1H), 2.88–2.96 (m, 1H), 2.80 (t, J = 12.0 Hz, 1H), 2.24–2.33 (m, 1H), 1.96–2.05 (m, 2H), 1.70–1.81 (m, 1H), 1.34–1.46 (m, 1H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −113.90 (s, 1F), −114.26 (s, 1F). LRMS (ESI): *m/z* 397.2 (MH^+). HPLC: t_R = 4.84 min; (254 nm) 97.5%.
    Data for compound **27**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.11 (d, J = 7.8 Hz, 1H), 8.03 (d, J_{HF} = 9.1 Hz, 1H), 7.76–7.83 (m, 1H), 7.67 (ddd, J = 8.0, 8.0, 5.7 Hz, 1H), 7.51 (ddd, J = 8.2, 8.2, 2.0 Hz, 1H), 7.37–7.45 (m, 2H), 6.95–7.00 (m, 1H), 3.45 (d, J = 6.7 Hz, 2H), 2.79 (d, J = 7.0 Hz, 2H), 1.87–2.01 (m, 4H), 1.75–1.85 (m, 1H), 1.59–1.70 (m, 1H), 1.03–1.21 (m, 4H). ^{19}F NMR (CD_3OD, 377 MHz) δ: −113.5 to −113.4 (m, 1F), −114.08 (s, 1F). LRMS (ESI): *m/z* 425.2 (MH^+). HPLC: t_R = 5.09 min; (254 nm) 100%.
    Compound **31**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.39 (br s, 3H), 8.26–8.71 (m, 2H), 8.11–8.16 (m, 2H), 7.67 (ddd, J = 7.8, 7.8, 5.7 Hz, 2H), 7.23–7.31 (m, 2H), 3.63–3.69 (m, 2H), 2.98–3.06 (m, 2H), 1.81–1.86 (m, 2H), 1.70–1.77 (m, 2H), 1.58–1.67 (m, 2H). ^{19}F NMR ($CDCl_3$, 377 MHz) δ: −111.55 to −111.40 (m, 1F), −112.60 to −112.55 (m, 1F). LRMS (ESI): *m/z* 370.2 (MH^+). HPLC: t_R = 5.19 min; (254 nm) 100%.
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