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One pot synthesis and SAR of some novel 3-substituted 5,6-diphenyl-1,2,4-triazines as antifungal agents

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ABSTRACT

An improved protocol for the synthesis of a novel series of 1,2,4-triazines possessing 1,2,3-triazole and piperidine ring using 1-(1-substituted piperidin-4-yl)-1*H*-1,2,3-triazole-4-carbohydrazide, benzil, ammonium acetate and $ZrOCl_2{\cdot}8H_2O$ as a catalyst in ethanol–water has been presented. The yields obtained are in the range of 87–94%. All the synthesized compounds (**4a**–**4l**) are novel and were evaluated for their in vitro antifungal activity. SAR for the series has been developed by comparing their MIC values with miconazole and fluconazole. Based on activity data SAR for the series has been developed. Compound **4c** from the series was equipotent to miconazole against *Candida albicans* (MIC-25), *Aspergillus niger* (MIC-12.5) and *Cryptococcus neoformans* (MIC-25). Compound **4d** was equipotent with miconazole against all tested organisms except *Cryptococcus neoformans*. Also compound **4i** was equipotent with miconazole against *C. albicans*, *A. niger and Fusarium oxysporum*.

1,2,4-Triazines and their analogues have gained considerable attention because of their synthetic, as well as biological utility. 1,2,4-Triazine is an important core found in numerous natural and synthetic biologically active compounds. Well-known antiviral drug azaribine is structurally based on the 1,2,4-triazine scaffold.[1] Also, certain azanucleosides, for example, 6-azacytosine and 6-azauracil, with 1,2,4-triazine heterocycle, have reported in literature as antiviral,[2,3] antitumour[4,5] and antifungal[6] activities. Furthermore, 6-azaisocytosine (3-amino-1,2,4-triazin-5(2*H*)-one), an isosteric isomer of 6-azacytosine and 6-azauracil, is of great biological interest due to its resistance to deaminase. Some condensed derivatives like pyrrolo-1,2,4-triazines are reported as anticancer agents.[7a] In the literature there is a report of 3-heterocycle substituted 1,2,4-triazines which have been prepared from hydrazides.[7b] 1,2,3-Triazole and its derivatives are important heterocycles with different activities like potent antineoplastic,[8] antimicrobial,[9–11] analgesic,[12] anti-inflammatory, local anesthetic,[13] anticonvulsant,[14] antimalarial,[15] anti HIVagents.[16] Some 1,2,3-triazole derivatives were used as DNA cleaving agents[17] and potassium channel activators,[18] cannabinoid CB1 receptor antagonists[19] and antitubercular agents.[20]

Literature reveals that there are no reports of a molecular scaffold containing these two important cores. With this view considering the biological significance of triazine and 1,2,3-triazole and in continuation of our work on synthesis of pharmacologically significant heterocycles,[21] a novel series of 1,2,4-triazines has been synthesized by one pot reaction of benzil, hydrazide and ammonium acetate in ethanol–water using $ZrOCl_2{\cdot}8H_2O$ as a catalyst.

The ester compound **1** was prepared from commercially available *N*-Boc piperidone as described in our previously reported method.[21d] Starting hydrazide compound has been prepared from ester compound **1** using hydrazine hydrate in methanol and further deprotection using trifluoroacetic acid. Deprotected hydrazide compound on alkylation or acylation in presence of triethylamine in tetrahydrofuran gave compounds **2a–2l** as shown in Scheme 1. Corresponding hydrazide compounds, benzil, ammonium acetate were heated at 100 °C in ethanol–water (1:2; v/v) using 10 mol % $ZrOCl_2{\cdot}8H_2O$ (Scheme 2). The reaction has also been tried using other acid catalysts the detail of the reactions with different catalyst is summarized in Table 1. From the table it is found that use of $ZrOCl_2{\cdot}8H_2O$ was more effective.

Catalytic property of $ZrOCl_2{\cdot}8H_2O$ has been studied considering synthesis of (**3a**). Effect of various solvents like *THF*, acetonitrile, ethanol, have also been studied. Among the results obtained, use of 10-mol % $ZrOCl_2{\cdot}8H_2O$ in ethanol–water gave the better yield (94%) for the synthesis of **4a** (Table 2). The use of environmental benign solvent such as water has got very much importance in 'Green Chemistry'. To study this aspect, the reaction was carried out for synthesis of **4a** using 10 mol % $ZrOCl_2{\cdot}8H_2O$, and corresponding substrates in water. The reaction was found to be sluggish and it may be due to the less solubility of substrates. To

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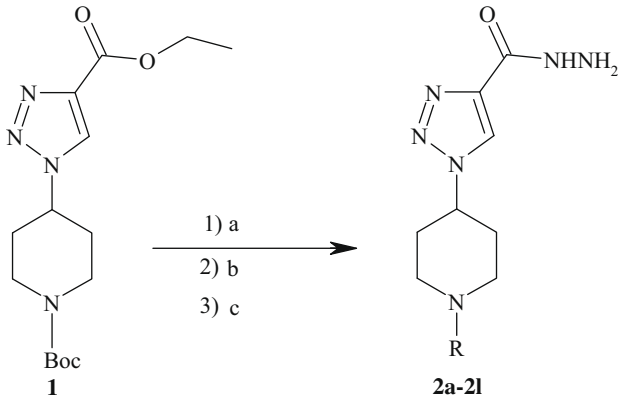


1 2a-2l

Scheme 1. Reagents and conditions: (a) Hydrazine hydrate, methanol, reflux, 8 h; (b) TFA, dichloromethane, rt, 16 h; (c) triethylamine, R–X or RCOX, tetrahydrofuran, 0–5 °C to rt, 2.5 h.

avoid this problem, the ethanol–water (1:2; v/v) solvent was used and found to be effective for synthesis of **4a** (94% in 100 min). The synthetic procedure was extended for synthesis of all the compounds **4a–4l** using different hydrazides, benzil, and ammonium acetate. Results are summarized in Table 3. The yields were obtained in the range of 87–94%. All synthesized derivatives were characterized using mass and ^{1}H NMR.

All the synthesized compounds were screened for in vitro antifungal activity. The antifungal activity was evaluated against different fungal strains such as *Candida albicans* (NCIM3471), *Fusarium oxysporum* (NCIM1332) *Aspergillus flavus* (NCIM539) *Aspergillus niger* (NCIM1196), *Cryptococcus neoformans* (NCIM576).

Table 1
Comparison of the reaction using different catalyst for the synthesis of *tert*-butyl 4-(4-(5,6-diphenyl-1,2,4-triazin-3-yl)-1*H*-1,2,3-triazol-1-yl)piperidine-1-carboxylate (**4a**) using 10 mmol of hydrazide (**2a**), 10 mmol of benzil and 40 mmol of ammonium acetate in ethanol

Catalyst	Mol %	Reaction time	Yield[a] (%)
No catalyst	—	48 h	No product
Bismuth trichloride	20	5 h 40 min	77
Sulfamic acid	20	4 h	80
Oxalic acid	20	3 h 30 min	85
Zinc chloride	20	4 h 30 min	75
Zirconyl chloride	20	1 h 40 min	94

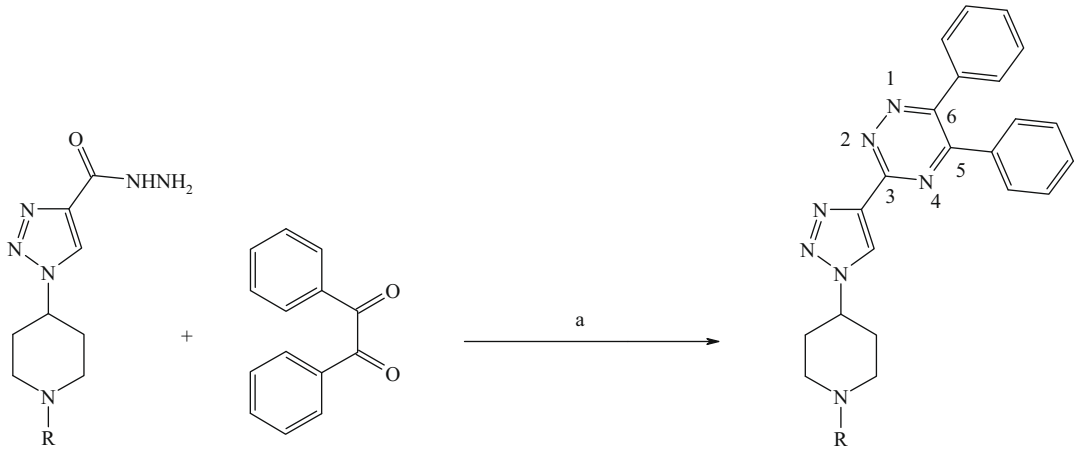
[a] Yields refer to the isolated pure products.

Table 2
Optimization of reaction conditions and the quantity of $ZrOCl_2$ for the synthesis of *tert*-butyl 4-(4-(5,6-diphenyl-1,2,4-triazin-3-yl)-1*H*-1,2,3-triazol-1-yl)piperidine-1-carboxylate (**4a**) at 100 °C

Solvent	Mol % of $ZrOCl_2$	Reaction time	Yield[a] (%)
THF	20	3 h	75
Acetonitrile	20	3 h 30 min	85
Acetonitrile–water (1:1)	20	4 h	70
Ethanol	20	1 h 40 min	94
Ethanol–water (1:2)	20	1 h 40 min	94
Ethanol–water (1:2)	10	1 h 40 min	94
Ethanol–water (1:2)	5	2 h 20 min	82

[a] Yields refer to the isolated pure products.

Minimum inhibitory concentration (MIC) values were determined using standard agar plate method.[22] Miconazole and Fluconazole were used as a standard for the comparison of antifungal activity.



2a-2l 3 4a-4l

4a R = -Boc **4b** R = -H
4c R = $-CH_3$ **4d** R = $-CH_2CH_3$
4e R = $-COCH_3$ **4f** R = $-COC_2H_5$
4g R = $-COC_3H_7$ **4h** R = $-COC_6H_5$
4i R = $-COC_6H_5$, 4 *Cl* **4j** R = $-SO_2CH_3$
4k R = $-SO_2C_6H_5\ CH_3$ **4l** R = $-CH_2C_2H_5$

Scheme 2. Reagents and conditions: (a) $ZrOCl_2{\cdot}8H_2O$ (10 mol %), ammonium acetate, ethanol–water (1:2), 100 °C, 100–120 min.

Table 3
Experimental data of the synthesized compounds **4a–41**

Entry	Time in minutes	Yield* (%)	Molecular formula/molecular weight	Elemental analysis% Found (calcd)		
				C	H	N
4a	100	94	$C_{27}H_{29}N_7O_2$ 483	67.14 (67.06)	6.10 (6.04)	20.28 (20.27)
4b	100	94	$C_{22}H_{21}N_7$ 383	68.87 (68.91)	5.50 (5.52)	25.57 (25.59)
4c	110	90	$C_{23}H_{23}N_7$ 397	69.50 (69.53)	5.83 (5.83)	24.67 (24.67)
4d	110	88	$C_{24}H_{25}N_7$ 412	70.05 (70.00)	6.15 (6.12)	23.82 (23.80)
4e	100	90	$C_{24}H_{23}N_7O$ 425	67.77 (67.75)	5.46 (5.45)	23.04 (23.04)
4f	120	88	$C_{25}H_{25}N_7O$ 439	68.30 (68.32)	5.73 (5.70)	21.30 (21.31)
4g	120	87	$C_{26}H_{27}N_7O$ 453	68.82 (68.85)	6.00 (6.02)	21.60 (21.62)
4h	100	90	$C_{29}H_{25}N_7O$ 487	71.43 (71.44)	5.19 (5.17)	20.10 (20.11)
4i	100	90	$C_{29}H_{24}$ Cl N_7O 522	66.75 (66.73)	4.62 (4.63)	18.90 (18.88)
4j	100	92	$C_{23}H_{23}N_7O_2S$ 461	59.50 (59.51)	5.00 (5.02)	21.25 (21.24)
4k	110	88	$C_{29}H_{27}N_7O_2S$ 537	64.77 (64.79)	5.08 (5.06)	18.24 (18.24)
4l	110	88	$C_{29}H_{27}N_7$ 473	73.57 (73.55)	5.74 (5.75)	20.75 (20.70)

* Yields refer to the isolated pure products.

Dimethyl sulfoxide was used as solvent control. MIC values of the tested compounds are presented in Table 4.

Many of the newly synthesized compounds were found to show good antifungal activity. From the antifungal activity data (Table 4), it is observed that compounds **4c**, **4d** and **4i** are the most active among all tested compounds against most of the tested organisms. N-Protected compound **4a** shows very less antifungal activity comparable to miconazole and fluconazole. Deprotected compound **4b** shows significant rise in activity compared to **4a** (activity increases by double as reflected in reduced MIC). Substitution of methyl group (**4c**) on piperidine nitrogen increases the antifungal activity compared with unsubstituted nitrogen (**4b**). Compound **4c** was equipotent to miconazole (activity comparable to fluconazole) against *C. albicans* (MIC-25), *A. niger* (MIC-12.5) and *C. neoformans* (MIC-25) where as slightly less active against *F. oxysporum* (MIC-30) and *A. flavus* (MIC-17.5). Substitution of ethyl group (**4d**) on nitrogen further enhance activity against *F. oxysporum* (MIC-25) and *A. flavus* (MIC-12.5) whereas there is no effect on activity against *C. albicans*, *A. niger* as evidenced by same MIC. Compound **4d** was equipotent with miconazole against all tested organisms except *C. neoformans* (MIC-35) whereas 2–5 times less active compared to fluconazole. Introduction of acetyl group on nitrogen (**4e**) reduces the antifungal activity compared with unsubstituted piperidine against all tested organisms. There was further decrease in antifungal activity if acetyl group replaced by propionyl (**4f**) and butyryl group (**4g**). Introduction of benzoyl group on nitrogen (**4h**) shows significant enhancement of activity compared with acetyl or propionyl substituent. Compound (**4h**) is marginally less active compared with miconazole. Introduction of *Cl* group on 4 position of Phenyl (**4i**) further increases the antifungal activity compared with unsubstituted phenyl. Compound (**4i**) was equipotent with miconazole against *C. albicans*, *A. niger and F. oxysporum*. Introduction of mesyl group on piperidine nitrogen (**3j**) shows increase in activity against *C. albicans*, *A. flavus* and *F. oxysporum* compared with unsubstituted nitrogen. Replacement of mesyl group by tosyl group (**4k**) reduces the antifungal activity.

Table 4
Antifungal activity of the synthesized compounds

Compound	MIC values in μg/mL[a]				
	C. albicans	*F. oxysporum*	*A. flavus*	*A. niger*	*C. neoformans*
4a	70	*	55	*	100
4b	30	40	37.5	42.5	45
4c	**25**	30	17.5	**12.5**	**25**
4d	**25**	**25**	12.5	**12.5**	35
4e	35	40	20	20	—*
4f	50	50	20	20	—*
4g	62.5	65	35	40	75
4h	30	30	17.5	15	55
4i	**25**	**25**	15	**12.5**	30
4j	50	60	30	*	*
4k	90	100	35	47.5	150
4l	40	60	—*	95	*
Miconazole	25	25	12.5	12.5	25
Fluconazole	5	5	5	10	5

* No activity was observed up to 200 μg/mL.
[a] Values are the average of three readings.

In conclusion, we have developed a new, convenient, simple and efficient method for the synthesis of novel series of 3-(1-(1-substitutedpiperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)-5,6-diphenyl-1,2,4-triazines using $ZrOCl_2 \cdot 8H_2O$ as a catalyst in good yields. The synthesized compounds were tested for in vitro antifungal activity. Based on the activity data SAR for the series has been developed. From the series it is found that compounds **4c**, **4d** and **4i** are the most active compounds from series suggesting that compounds from present series of 1,2,4-triazine with piperidino-1,2,3-triazole on 3rd position bearing substitutions like methyl, ethyl or benzoyl on piperidine nitrogen can serve as a important scaffold for the design and development of new lead as antifungal agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.048.

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