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Graphical Abstract

An eco-friendly water mediated synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids as highly potent anti-bacterial agents

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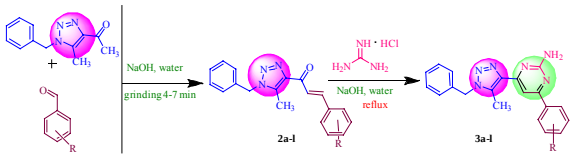

An elegant green synthesis of 1,2,3-triazolyl linked 2-aminopyrimidine hybrids in water has been accomplished for the first time. Their antibacterial activities are comparable with that of tetracycline.

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Original article

An eco-friendly water mediated synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids as highly potent anti-bacterial agents

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ABSTRACT

An elegant and efficient synthesis of novel 1,2,3-triazole fused 2-aminopyrimidine hybrids has been accomplished for the first time in the green solvent *viz*. water. The hybrid molecules exhibit significant anti-bacterial activity when screened against three human pathogens *viz*. *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In comparison to the commercially marketed drug tetracycline, some of them are equally potent and a few are more potent.

1. Introduction

Microbial infections are a growing problem in contemporary medicine. According to statistical evidence provided by WHO, many of the drug treatment breakthroughs of the last century could be lost through the spread of antimicrobial resistance [1]. For instance, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are important pathogens causing invasive diseases such as sepsis, meningitis, necrotising fasciitis, pneumonia [2], nosocomial pneumonia [3], cystic fibrosis, acute leukemia, organ transplants, and intravenous-drug addiction [4]. Some of these pathogens have been reported to develop resistance [5] to the well-known commercially available drugs. As a result, many infectious diseases may one day become uncontrollable and could rapidly spread throughout the world. Consequently, the discovery of potent antibiotic drugs is considered to be one of the greatest scientific and medical goals.

There is a growing interest pertaining to the synthesis of bioactive heterocyclic compounds in pharmacy. Among various heterocyclic compounds, pyrimidine derivatives have apparently gained considerable importance owing to their varied biological activities such as adenosine receptor antagonist [6], anti-inflammatory [7], CDK inhibitor [8], calcium channel antagonist [9], anti-tumor [10] activities, *etc*. On the other hand, a promising diverse pharmacological activity is shown by the 1,2,3-triazole nucleus such as anti-biotic, anti-fungal [11], anti-cancer [12], HIV protease inhibitor [13], and chemotherapeutic activities [14], *etc*.

In view of the bioactivity profiles of the individual heterocycles, the synthesis of hybrid molecules containing both of the above said moieties in a single frame was attempted. Thus, the present study was undertaken to synthesize and investigate the anti-microbial activities of a series of novel 1,2,3-triazolyl-2-aminopyrimidine hybrids against three human pathogenic bacteria *viz*. *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa,* the details of which are presented *vide infra*.

2. Experimental

Typical procedure: A mixture of (*E*)-1-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-phenylprop-2-en-1-one, **2a** (0.2 g, 0.65 mmol), guanidine hydrochloride (0.18 g, 1.88 mmol) and NaOH (0.04 g, 0.75 mmol) in water or ethanol (10 mL) was refluxed for 30-40 min. Then, the reaction mixture was poured onto excess crushed ice and neutralized with dilute hydrochloric acid. The precipitated 1,2,3-triazolyl-2-aminopyrimidine (**3a**) was filtered and recrystallized from ethanol. Yield 0.19 g (85%) in ethanol, 0.18 g (82%) in water.

4-(1-Benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-6-phenylpyrimidin-2-amine (**3a**): Obtained as white solid in 82% yield, mp 152 °C; IR (KBr, cm^{-1}): *v* 3411, 3192, 1677, 1552, 1462, 1355, 1181, 834, 729, 700; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12-8.09 (m, 2H, ArH), 7.98 (s, 1H, =CH), 7.49-7.18 (m, 8H, ArH), 5.56 (s, 2H, C_6H_5-CH_2), 5.07 (brs, 2H, NH_2), 2.62 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ

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165.87, 163.14, 160.56, 142.30, 137.57, 134.51, 134.00, 133.94, 130.48, 130.42, 129.06, 128.71, 128.66, 128.41, 127.17, 104.50, 51.80, 9.82. MS (ESI): *m/z* 343.33 (M + H); Anal. Calcd. for $C_{20}H_{18}N_6$: C, 70.16; H, 5.30; N, 24.54; Found: C, 70.09; H, 5.32; N, 24.50.

By a similar procedure, 4-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-6-arylpyrimidin-2-amines (**3b-l**) were synthesized. Their characterization data and copies of NMR (1D& 2D), FT-IR and LC-MS spectra of representative compounds can be found in Supporting information.

3. Results and discussion

Over the past decades, many protocols have been developed for the synthesis of pyrimidine derivatives [15], and most of these involve the use of hazardous organic solvents [16-17]. Hence, eco-fiendly organic reactions that occur under solvent-free conditions or use water have attained much importance and are of current interest. In particular, as a reaction medium, water offers many practical and economic advantages including low cost, environmental compatibility, and safety among all available solvents, thus leading to environmentally-friendly chemical processes [18]. In view of this, recently we have accomplished the synthesis of amides and various heterocycles/hybrid heterocycles under solvent-free/water medium conditions [19-30].

Perusal of literature suggests that there are no reports on the synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids. In connection with our goal to conduct organic reactions in water medium and in continuation of our earlier report on the water promoted synthesis of 1,2,3-triazolyl-pyrimidine-2-thione hybrids [28] as a highly potent antibacterial agent, we hereby submit the first report on the eco-friendly synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids and their anti-bacterial activity.

At the outset, synthesis of 1,2,3-triazolyl-2-aminopyrimidine (**3a**) was attempted while varying the bases (Scheme1, Table 1) *i.e.* a mixture of 1,2,3-triazolyl chalcone [26] (**2a**, 1 equiv.), guanidine hydrochloride (3 equiv) and base (1.5 equiv) was refluxed in ethanol or water (10 mL). Completion of the reaction was monitored by TLC. It is noted that basicity is one of the key points behind the success of the reaction (Table 1). Moreover, water seems to be the proper solvent to fulfill the reaction by achieving the best solubility of the salts *viz*. guanidine hydrochloride and sodium hydroxide, thus promoting the reaction. The yields in ethanol and water were comparable.

All twelve compounds exhibit different levels of inhibition against the previously mentioned human pathogens (both Gram-positive & Gram-negative) (Scheme 2, Table 2). Among them, compounds coded **3a** (-H), **3b** (-4OMe), **3c** (-2,4 diOMe), **3g** (-2OMe), **3i** (-4F), **3k** (-2,4 diCl) (MIC 2 µg/mL) and **3d** (-4Br) (MIC 5 µg/mL) were more potent against *P. aeruginosa* than tetracycline (MIC 10 µg/mL). Meanwhile, the compounds **3j** (-3OMe) and **3l** (2-thiophenyl) show equal inhibition (MIC 10 µg/mL) to tetracycline. However, the rest of the compounds showed lower levels of inhibitions (MIC 25-35µg/mL). In the case of *Klebsiella pneumonia*, compounds **3b** and **3c** were equally potent (MIC 2 µg/mL) as tetracycline. The compounds **3a** and **3e** (-3Br) exhibited moderate potency (MIC 5 µg/mL) and the others showed less potency (MIC >5µg/mL). On the other hand, all the compounds exhibited less potency (MIC ≥30 µg/mL) towards *Staphylococcus aureus* than tetracycline (MIC 15 µg/mL). This may be attributed to the thick peptidoglycan layer of the cell wall of Gram positive *S. aureus*. Also, the binding of the cell-surface proteins of the Gram positive *S. aureus* might have inactivated the compounds. The above results project **3b** and **3c** as highly potent compounds with remarkable antibacterial activity in comparison to the commercial control.

Structure–activity relationship (SAR) in these compounds demonstrated that compounds **3b** and **3c** with substituents *viz*. -4OMe and -2, 4 diOMe in the phenyl ring attached to the C-6 carbon of 2-amino pyrimidine moiety are more potent against all the tested pathogens. Subsequently, various substituents were introduced in either of the *-ortho*, *-meta* or *-para* position of the phenyl ring to evaluate their effect on biological activity. Their order of activity compared to compound (**3a**) with the unsubstituted phenyl ring linked to C-6 carbon of 2-amino pyrimidine is in the order -4OMe ≥ -2,4-diOMe > -H > -4Br > -2OMe > -4F > -2,4 diCl > -3OMe > -thiophene > -3Br. However, compounds **3f** with -4Me and **3h** with -4Cl substituents did not show notable activity against all the tested pathogens.

4. Conclusion

In conclusion, an elegant, environmentally-friendly water promoted synthesis of a library of 1,2,3-triazolyl-2-aminopyrimidine hybrids has been achieved. All the synthesized compounds were evaluated for antibacterial activity. Many of them exhibited potent activity, and some of them were equally potent or of higher potency than the commercially marketed tetracycline.

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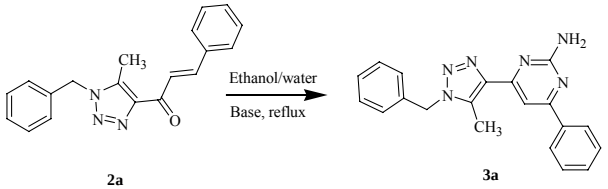
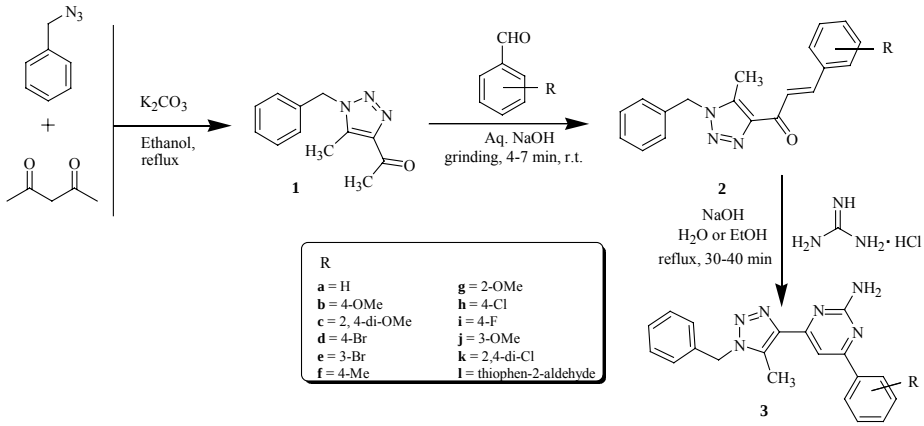


2a **3a**

Scheme 1. Optimization for the synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids (**3a**).



1 **2** **3**

Scheme 2. Synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids (**3**).

Table 1
Optimization for the synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids (**3a**).

Entry	Base	Time	Solvent	Yield[a]
1	Et_3N	6 h	Ethanol	31
2	Et_3N	6 h	water	35
3	Pyridine	8 h	Ethanol	-
4	Pyridine	8 h	Water	-
5	Na_2CO_3	6 h	Ethanol	33
6	Na_2CO_3	6 h	Water	29
7	NaOH	30 min	Ethanol	85
8	NaOH	40 min	Water	82

[a] Isolated yield

Table 2
Synthesis of 1,2,3-triazolyl-2-aminopyrimidine hybrids (**3a-l**)[a] in ethanol and water and their *in vitro* anti-bacterial activity against three human pathogens.

Entry	Hybrids (**3a-l**)	Ethanol		Water		Minimum inhibitory concentration (MIC) (µg/mL)		
		Time (min)	Yield[b] (%)	Time (min)	Yield[b] (%)	*S.aureus* MTCC 3160	*P.aeruginosa* MTCC 2488	*K.pneumoniae* MTCC 3384
1	**3a**	30	85	40	82	NA	2	5
2	**3b**	30	83	40	87	30	2	2
3	**3c**	30	78	40	82	30	2	2
4	**3d**	30	63	40	65	40	5	25
5	**3e**	30	62	40	61	35	25	5
6	**3f**	30	81	40	82	NA	30	15
7	**3g**	30	73	40	75	40	2	25
8	**3h**	30	83	40	81	NA	35	40
9	**3i**	30	79	40	74	NA	2	NA
10	**3j**	30	75	40	78	35	10	25
11	**3k**	30	72	40	79	NA	5	35
12	**3l**	30	83	40	85	35	10	25
	Tetracycline					15	10	2
	DMSO					NA	NA	NA

[a] Synthesized compounds were completely characterized by NMR (1D & 2D), IR and mass spectral techniques.
[b] Isolated yield.