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An efficient one-pot synthesis of thiochromeno[3,4-*d*]pyrimidines derivatives: Inducing ROS dependent antibacterial and anti-biofilm activities

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ABSTRACT

An efficient synthesis of thiochromeno[3,4-*d*]pyrimidine derivatives has been achieved successfully *via* a one-pot three-component reaction of thiochrome-4-one, aromatic aldehyde and thiourea in the presence of 1-butyl-3-methyl imidazolium hydrogen sulphate [Bmim]HSO_4. This new protocol has the advantages of environmental friendliness, high yields, short reaction times, and convenient operation. Furthermore, among all the tested derivatives, compounds **4b** and **4c** exhibited promising antibacterial, minimum bactericidal concentration and anti-biofilm activities against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121. The compound **4c** also showed promising intracellular ROS accumulation in *Staphylococcus aureus* MLS16 MTCC 2940 comparable to that of ciprofloxacin resulting in apoptotic cell death of the bacterium.

1. Introduction

In the recent years, the treatment of bacterial infections has become a major challenge in the realm of conventional antibiotic therapy. In parallel, the disease causing microbes have gained resistance to several antibiotics and are causing serious health care problems [1]. It is reported that a high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is a major nosocomial pathogen [2]. Thus, antimicrobial resistance has gained serious attention as a global health threat [3]. According to the World Health Organization (WHO), the infections caused by resistant microorganisms often fail to respond to conventional antibiotic therapy, resulting in prolonged illness and greater risk of death. The primary reason is the inappropriate and irrational use of currently available antimicrobial agents which has led to the emergence of highly resistant pathogenic microbes [4]. The increased threat from the drug-resistant Gram-positive and -negative bacterial strains has emphasized the urgent need and perusal to identify new antimicrobial agents with high efficiency, low toxicity, broad spectrum and safety [5].

In the present circumstances, the pyrimidine derivatives play a prominent role in organic and medicinal chemistry [6,7]. They exist in a wide variety of natural products and in rationally designed pharmaceutical agents [8,9]. Pyrimidines have become integral units to a large number of drug substances with activities including antimicrobial [10], antioxidants [11], anticonvulsant [12], antidepressant [13] anticancer [14], and anti-biofilm [15]. In view of the diverse pharmacological properties of these compounds some of the well-known biologically potent thiochromen pyrimidine scaffolds (**I**, **II** and **III**) [16–18] are shown in Fig. 1.

In the context of sustainable chemistry [19–21], ionic liquids (ILs) have gained considerable attention in several branches of the chemical industry as potential "green" substitutes for conventional organic solvents [22,23]. The green aspect of ILs is mainly due to non-flammability and reduced air pollution [24–26]. The introduction of structural functionalities on the cationic or anionic part has made it possible to design new ILs with targeted properties [27]. In the recent years, ionic liquids (ILs) especially the acidic types have gained a renewed interest in the area of heterocyclic synthesis [28,29]. Nowadays, ILs is being used in multicomponent reactions (MCRs) and their properties have also been investigated in organic synthesis [30–33]. Hammam et al. [34] have described the reported methods of few compounds which suffer from drawbacks such as harsh reaction conditions, unsatisfactory yields, prolonged reaction times, and cumbersome product isolation procedures. Hence, our goals to use a green, one-pot synthetic

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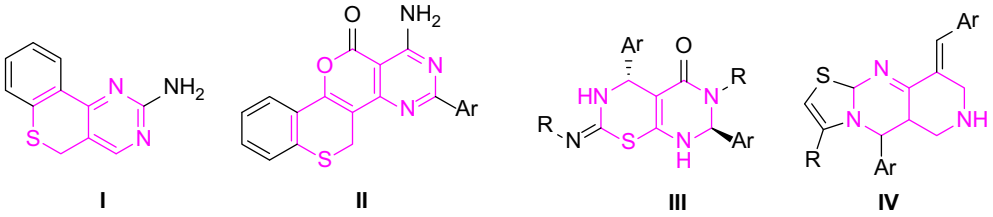


Fig. 1. Biologically potent thiochromen pyrimidine scaffolds.

approach of the above title compounds which are a welcome goal due to the wide spectrum of biological activities.

As a part of our ongoing research in the area of MCRs and ILs, targeted towards the synthesis of novel heterocyclic compounds [35–38], we herein report a three-component domino processes for the synthesis of thiochromeno[3,4-*d*]pyrimidine derivatives and were further screened for antibacterial, minimum bactericidal concentration and anti-biofilm activities.

2. Results and discussion

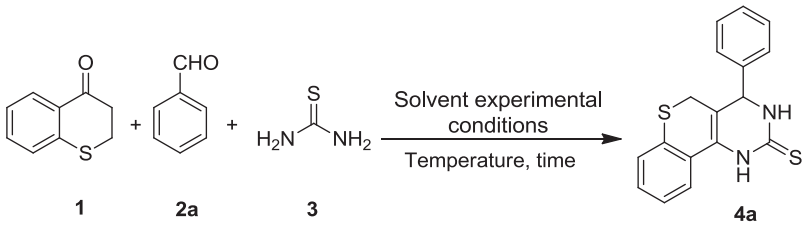
2.1. Chemistry

Highly volatile and toxic organic solvents were replaced by a green medium namely [Bmim]HSO_4 in the present research program. Thiochromeno[3,4-*d*]pyrimidine derivatives (**4a**-**n**) were obtained in good yields. The conditions were optimized for the designed protocol based on the reaction of thiochroman-4-one **1** (1 mmol), aromatic aldehyde **2a**-**n** (1 mmol) and thiourea **3** (1 mmol) in the presence of [Bmim]HSO_4. Initially, a one-pot three component reaction with thiochroman-4-one **1**, benzaldehyde **2a** and thiourea **3** was taken up as a model reaction to optimize the reaction conditions and the results are summarized in Table 1. When the reaction was carried out under neat conditions it did not give the require products even after 24 h (Table 1 entry 1). Later the reaction was carried out with organic solvents such as MeOH, EtOH, MeCN and DMF which resulted in poor yields (Table 1, entries 2–6). It was found that when acid was applied, the acidic mixture of all the reactants under reflux conditions gave **4a** in 35% yields (Table 1, entry 6). While, studying the scope and efficiency of the reaction with different ILs in this MCR, it was observed that almost all of the investigated ILs such as [Bmim]BF_4, [Bmim]Br, [Bmim]PF_6 and [Bmim]HSO_4 were capable of promoting the synthesis of desired compound **4a**. Good results were achieved by carrying out the reaction using [Bmim]HSO_4 ionic liquid and the derived product was obtained in good isolated yields when compared to the results obtained using ionic liquid analogous tetrafluoroborate, bromide and hexafluorophosphate (Table 1, entries 7–9). The yield of product **4a** was improved and the reaction time was shortened as the temperature was increased from room temperature to 70 °C (Table 1, entries 10–14). No further improvement was observed at 80 °C (Table 1, entry 15).

Therefore, a temperature of 70 °C was considered as the most suitable optimal reaction temperature for performing all these reactions. It was inferred from the above results that the ionic liquid medium is an essential and crucial factor for promoting the reaction.

To demonstrate the generality of this method, the reaction was investigated under optimized conditions, and the results are

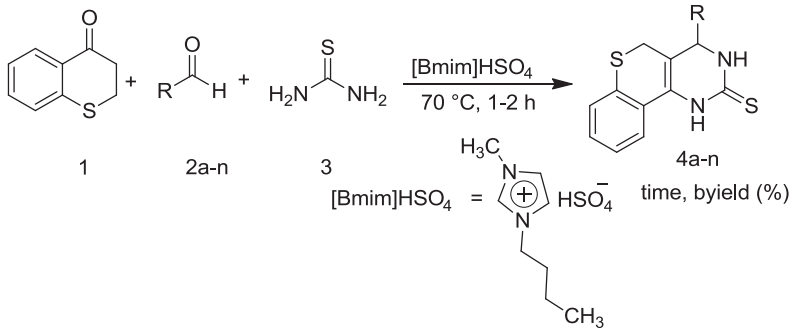
Table 1
Optimization of reaction parameters for the synthesis of compound **4a**.



Entry[a]	Solvent	Temp (°C)	Time (h)	Yield[b] (%)
1	Neat	70	24	NR
2	MeOH	70	12	08
3	EtOH	70	12	12
4	CH_3CN	70	10	06
5	DMF	70	14	0
6	AcOH	70	8	35
7	[Bmim]BF_4	70	2	70
8	[Bmim]Br	70	2	68
9	[Bmim]PF_6	70	3	52
10	**[Bmim]HSO_4**	**70**	**1**	**92**
11	[Bmim]HSO_4	rt	3	38
12	[Bmim]HSO_4	40	3	48
13	[Bmim]HSO_4	50	3	62
14	[Bmim]HSO_4	60	2	84
15	[Bmim]HSO_4	80	1	92

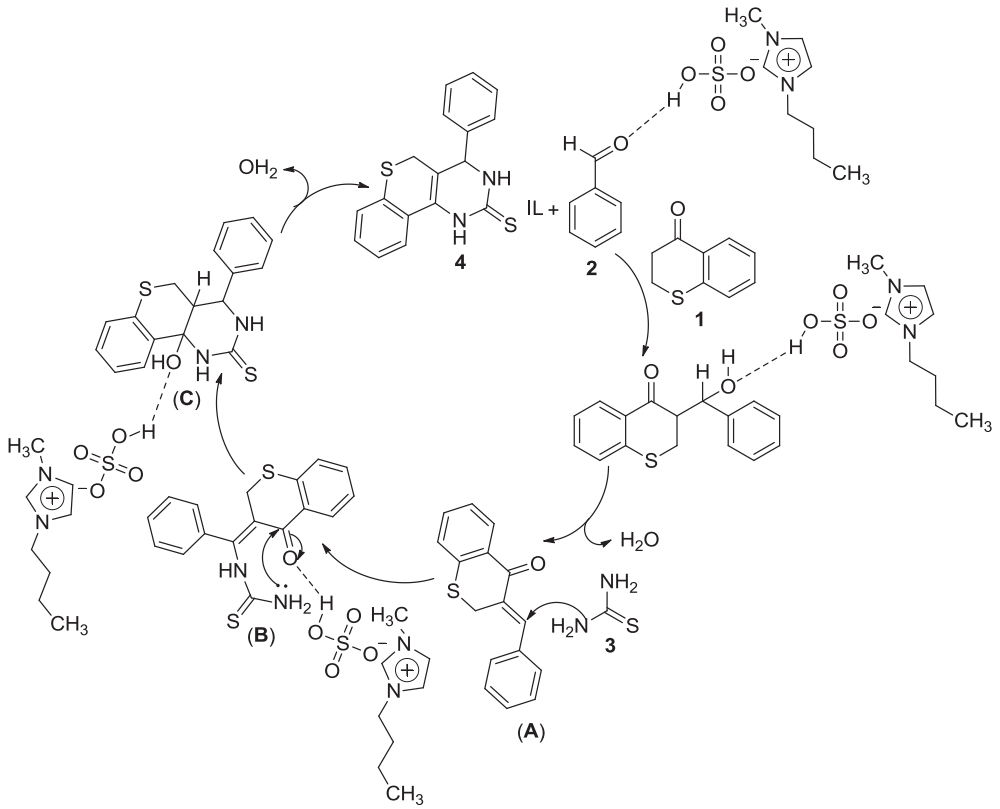
[a] Reaction conditions: thiochroman-4-one **1** (1 mmol), benzaldehyde **2a** (1 mmol) and thiourea **3** (1 mmol) and solvent (2 mL).
[b] Yields of the isolated products.

Table 2
Synthesis of thiochromeno[3,4-*d*]pyrimidine derivatives (**4a**-**n**).[a]



Entry	R	Product	Time (h)	Yield (%)
1	C_6H_5	4a	1	92
2	4-Me-C_6H_4	4b	1	90
3	4-OMe-C_6H_4	4c	1	92
4	4-Cl-C_6H_4	4d	1	92
5	4-F-C_6H_4	4e	1.5	88
6	4-NO_2-C_6H_4	4f	1	90
7	3-NO_2-C_6H_4	4g	1	86
8	2-Cl-C_6H_4	4h	1.5	84
9	$C_{10}H_7$	4i	1.5	86
10	6-OMe-$C_{10}H_6$	4j	1.5	90
11	Pyrazole	4k	2	84
12	4-NO_2-Pyrazole	4l	2	84
13	4-Cl-Pyrazole	4m	2	84
14	4-OCH_3-Pyrazole	4n	2	84

[a] Reaction conditions: thiochroman-4-one **1** (1 mmol), aromatic aldehydes **2a**-**n** (1 mmol) and thiourea **3** (1 mmol) and [Bmim]HSO_4 (2 mL).
[b] Yields of the isolated products.



Scheme 1. Proposed mechanism for the formation of compound **4a**.

Table 3
Antibacterial activity of the synthesized thiochromeno[3,4-*d*]pyrimidines (**4a**-**n**).

Test compound	Minimum inhibitory concentration (μg/mL)						
	Staphylococcus aureus MTCC 96	*Bacillus subtilis* MTCC 121	*Staphylococcus aureus* MLS16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453
4a	125	125	125	125	125	125	125
4b	7.8	15.6	7.8	125	125	125	125
4c	3.9	7.8	3.9	125	125	125	125
4d	125	125	125	125	125	125	125
4e	125	125	125	125	125	125	125
4f	125	125	125	125	125	125	125
4g	125	125	125	125	125	125	25
4h	125	125	125	125	125	125	125
4i	125	125	125	125	125	125	125
4j	125	125	125	125	125	125	125
4k	125	125	125	125	125	125	>125
4l	125	125	125	125	125	125	125
4m	125	125	125	125	125	125	125
4n	125	125	125	125	125	125	125
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Note: Compounds (**4a**, **4d**-**4n**) showed MIC values of 125 μg/mL.

illustrated in Table 2. It was observed that a wide range of aromatic aldehydes (**2a**-**n**) bearing either electron-donating or electron-withdrawing groups could be employed as coupling partners with thiochroman-4-one **1** and thiourea **3** and they were smoothly transformed to the corresponding thiochromeno[3,4-*d*]pyrimidines (**4a**-**n**) in good to moderate yields. All para, meta, and ortho-substituted aldehydes were easily converted into the desired products, indicating that steric bulk did not have any significant impact on the reactivity. Aldehydes bearing pyrazole group furnished the corresponding product in good yields. The tolerance of functional group such as chloro, fluoro, nitro, methyl, methoxy in this method provides an opportunity for further chemical transformation. Several pharmaceutically relevant scaffolds could be easily generated with the help of this green protocol. Although the proposed mechanism of this reaction remains to be fully clarified, the formation of compound **4** is explained by the reaction sequence in Scheme 1. In the first step, a hydrogen bond formation between the hydrogen atom of [Bmim]HSO_4 and carbonyl group of benzaldehyde **2** produced a complex which upon condensation with thiochroman-4-one **1** gave the α,β-unsaturated intermediate (**A**). The formation of intermediate (**B**) took place by a condensation between α,β-unsaturated intermediate **A** and thiourea **3**. Subsequently, intermediate **B** underwent intramolecular cyclization to form another intermediate (**C**). In this last step, the intermediate **C** underwent dehydration affording the final product **4**.

Ionic liquid recyclability is one of the most necessary features and thus makes it useful for commercial applications. Consequently, we studied the recyclability of the [Bmim]HSO_4 ionic liquid in the above model reaction. After completion of the reaction, the mixture was transferred into ice cold water and stirred thoroughly. The solid product thus obtained was isolated by filtration, and the filtrate having ionic liquid was extracted with ethyl acetate (2 × 20 mL) to eliminate the non-ionic organic impurities. Then the water was evaporated under reduced pressure and the recovered ionic liquid was dried under vacuum and recycled for four times in consequent reactions without evident changes in the product yields.

2.2. Biological evaluation

All the synthesized compounds were evaluated for various biological activities such as antibacterial, minimum bactericidal concentration (MBC), anti-biofilm and accumulation of intracellular ROS in *Staphylococcus aureus* MLS-16 MTCC 2940 biofilms.

2.2.1. Antibacterial activity

Compounds **4a**-**n** were screened for antibacterial activity [39] *in vitro* against different Gram-positive and Gram-negative bacterial strains such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530. Among all the derivatives screened, compound **4c** showed promising activity (MIC value of 3.9 μg/mL against *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS16 MTCC 2940 and 7.8 μg/mL against *Bacillus subtilis* MTCC 121; while compound **4b** exhibited promising activity (MIC value 7.8 μg/mL) specifically towards *Staphylococcus aureus MTCC* 96 and *Staphylococcus aureus* MLS16 MTCC 2940 and MIC value of 15.6 μg/mL was observed against *Bacillus subtilis* MTCC 121. However, all the other tested compounds (**4a** and **4d**-**4n**) showed MIC values of >125 μg/mL against all the tested bacterial strains. Based on the structure-activity relationship of the synthesized derivatives, it was observed that the compound **4c** has a methoxy substituent attached to the basic thiochromeno[3,4-*d*]pyrimidine scaffold, which has an electron donating property which probably may be contributing to the antibacterial activity. In case of compound **4b**, a methyl substituent is attached to the basic thiochromeno[3,4-*d*]pyrimidine scaffold, having electron donating property is probably contributing to the antibacterial activity. The antibacterial activity results to this regard are tabulated in Table 3.

Table 4
Minimum Bactericidal Concentration (MBC) of the synthesized thiochromeno[3,4-*d*] pyrimidines (**4a**-**n**).

Test compounds	Minimum bactericidal concentration (μg/ml)		
	Staphylococcus aureus MTCC 96	*Bacillus subtilis* MTCC 121	*Staphylococcus aureus* MLS16 MTCC 2940
4b	15.6	31.2	15.6
4c	3.9	15.6	7.8
Ciprofloxacin (Standard control)	1.9	0.9	0.9

Table 5
Biofilm inhibition assay of the synthesized compounds (**4b**-**c**).

Test compounds	IC_{50} values in (μg/mL)		
	Staphylococcus aureus MTCC 96	*Bacillus subtilis* MTCC 121	*Staphylococcus aureus* MLS16 MTCC 2940
4b	8.8 ± 0.32	10.5 ± 0.24	11.3 ± 0.18
4c	2.1 ± 0.44	8.1 ± 0.29	4.5 ± 0.22
Ciprofloxacin (Standard control)	0.6 ± 0.06	0.5 ± 0.09	0.4 ± 0.07

2.2.1.1. Minimum bactericidal concentration (MBC). Based on the antibacterial activity results, the compounds **4b** and **4c** were screened for the minimum bactericidal concentration [40] against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121 in comparison to ciprofloxacin as standard. Compounds **4b** and **4c** consistently showed promising minimum bactericidal concentration against all the tested bacterial strains. The activity data to this regard is shown in Table 4.

2.2.2. Biofilm inhibition assay

A biofilm is a structured consortium of bacteria embedded in a self-produced polymeric matrix consisting of polysaccharides, protein and DNA. Bacterial biofilms cause chronic infections in humans *via* hospital and community environments since they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the body's defense system [41]. In the medical sector, bacteria colonize through adhesion mechanism and result in biofilm formation on several biomedical implants such as stents, heart valves, vascular grafts and catheters [42]. In this context, the novel compounds that can specifically target and inhibit the biofilm formation would be of significance in comparison to the rational use of antibiotics and/or biocides. Considering these facts, a further step was undertaken to investigate whether these compounds exhibit a specific anti-biofilm activity or whether this observation was simply related to a general toxic effect on the Gram-positive bacterial strains. To this regard, the compounds **4b** and **4c** were screened for anti-biofilm activity [43] against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121, which are common and important nosocomial pathogens having biofilm forming ability. The results summarized in Table 5, clearly reveal that not much information on the structure-activity relationship (SAR) can be highlighted at this stage; however, it is observed that compound **4c** exhibited promising activity (IC_{50} values ranged between 2.1 and 8.1 μg/mL) towards all the tested bacterial species, while compound **4b** showed good activity (IC_{50} values ranged between 8.8 and 11.3 μg/mL) against all the tested bacterial strains. The basic thiochromeno[3,4-*d*]pyrimidine scaffold of these compounds possesses different substituents which exhibit electron donating or electron withdrawing properties which antagonize the biofilm formation and probably may be contributing to the anti-biofilm activity. The activity data to this regard is shown in Table 5.

In the present study, the biofilm formation in different bacterial strains including *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121 was examined in a 96 well microtitre plate. It was noticed that these strains formed biofilms at either the bottom of the well or at the air-liquid interface [44]. For performing the assay, the strains needs to be cultured to attain the required biomass concentration (1.5×10^6 cfu/mL) after 24 h incubation and when the test compounds were added at the minimum inhibitory concentration (MIC) they exhibited inhibitory effect on the biofilm formation and also showed drastic reduction in the bacterial cell growth, which was quantified based on the crystal violet assay [43]. From a mechanistic perspective, the test compounds caused the disruption and detachment of the biofilm from the surface due to the destabilization of the EPS, which results in the dispersal of the bacterial cells from the biofilm. The dispersed cells were more susceptible to these test compounds resulting in microbial cell death which was quantified as a decrease in the microbial population as compared to the untreated biofilms. However, the addition of test compound below the sub-inhibitory concentration (that is less than MIC value), did not exhibit much reduction in the bacterial cell growth as compared to the untreated cells. The results on the cell biomass and cell growth measurements are depicted in Table 6 and Fig. 2, respectively. Some of the published reports illustrate the potential of few small molecule scaffolds such as halogenated furanones [45], sponge-derived natural alkaloid derivatives like oroidin and bromoageliferin [46–49], 2-aminoimidazoles and imidazopyridinium salts [50] and dichlorocarbazol derivative [51] which caused the disruption of bacterial chemical signaling and biofilm formation in some pathogenic bacteria. These molecules also structurally resembled bacterial acyl-homoserine lactone (AHL) quorum-sensing molecules [52,53] and effectively interfered with the quorum signaling, the subsequent gene expression and the swarming phenotype [54–56].

Table 6
Cell biomass measurement of the *Staphylococcus aureus* MTCC 96 biofilms treated with the synthesized compounds (**4b** and **4c**).

Time	Cell dry weight (g/mL)				
	Control	**4b** (MIC)	**4b** (<MIC)	**4c** (MIC)	**4c** (<MIC)
0	0.042	0.042	0.042	0.042	0.042
12	0.85	0.64	0.81	0.58	0.75
24	1.2	0.71	1.08	0.64	1.01

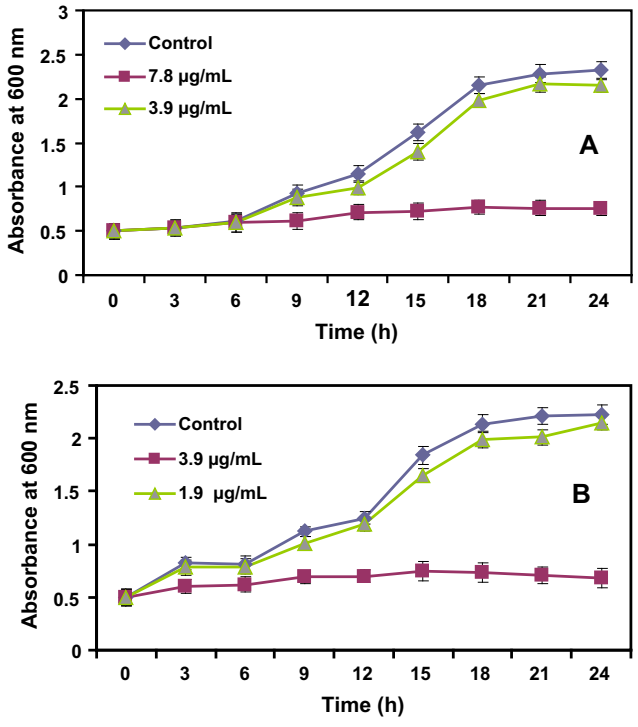


Fig. 2. Cell growth measurement of *Staphylococcus aureus* MTCC 96 treated with the synthesized compounds (A: compound **4b**) and (B: compound **4c**).

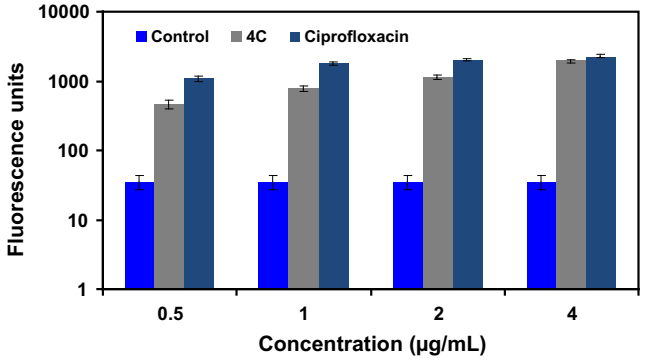


Fig. 3. Intracellular Reactive Oxygen Species (ROS) accumulation in *Staphylococcus aureus* MLS16 (MTCC 2940) biofilms.

2.2.3. Accumulation of intracellular ROS in Staphylococcus aureus MLS16 MTCC 2940 biofilms

In order to elucidate whether oxidative stress in involved in the apoptotic cell death, the intracellular ROS accumulation within the cells of mature biofilms was measured using the fluorescent probe 2′,7′-dichlorofluorescein-diacetate (DCFH-DA), a general ROS fluorescent probe. The DCFH-DA dye conversion mainly depends on the metabolically active cells in the biofilm. DCFH-DA is deacetylated in the cells where it can react quantitatively with intracellular radicals (mainly H_2O_2) to get converted to its fluorescent product (DCF), which is retained within the cells and thus provides an index of oxidation in the cell cytosol. In the present study, the accumulation of intracellular Reactive Oxygen Species (ROS) in mature *Staphylococcus aureus* MLS16 biofilms was measured using DCFH-DA dye [57]. The accumulation of intracellular ROS plays a major role in apoptotic mediated cell death [58]. After treatment with compound **4c**, the ROS levels were significantly accumulated in the tested *Staphylococcus aureus* MLS16 strain (Fig. 3). At a concentration of 4 µg/ml, the compound **4c** treated biofilms showed increased levels of intracellular ROS accumulation which was equal to the standard drug ciprofloxacin. The ROS measurements were also carried out separately in both sessile cells and in the supernatant. The ROS induced increase in fluorescence was observed only for the sessile cells, suggesting that the ROS accumulation is of intracellular origin. This accumulated ROS may be responsible for the bactericidal activity. Oxygen-containing free radical molecules and their precursors formed in biological systems are collectively termed as ROS, which include superoxides ($O_2^{\cdot-}$), peroxides (H_2O_2 and ROOH) and free radicals ($HO^{\cdot}$ and $RO^{\cdot}$). Some of the bactericidal drugs stimulate free radical formation via the Fenton reaction [59]. Recent studies suggest that when bacteria is under oxidative stress, these free radicals contribute to arrest in the cell growth or bacterial mediated cell death by damaging the specific essential metabolic enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately leading to cell lysis and death [60].

3. Conclusion

In conclusion, we have successfully developed a new and efficient route for the synthesis of biologically important thiochromeno[3,4-*d*]pyrimidine derivatives in [Bmim]HSO_4 ionic liquid. The significant advantages of the methodology are higher yields, milder reaction condition, shorter reaction time, convenient procedure, and environmentally friendly green protocols. Among all the tested derivatives, only compounds **4b** and **4c** exhibited promising antibacterial, minimum bactericidal concentration and anti-biofilm activities against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121. Compound **4c** showed promising intracellular ROS accumulation in *Staphylococcus aureus* MLS16 MTCC 2940 comparable to that of ciprofloxacin suggesting that the bacterium underwent apoptotic cell death.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2016.08.006.

References

[1] K. Onuma, T. Tanabe, H. Sato, Antimicrobial resistance of *Staphylococcus pseudintermedius* isolates from healthy dogs and dogs affected with pyoderma in Japan, Vet. Dermatol. 23 (2012) 17–22.
[2] A.S. Haddadin, S.A. Fappiano, P.A. Lipsett, Methicillin resistant *Staphylococcus aureus* (MRSA) in the intensive care unit, Postgr. Med J. 78 (2002) 385–392.
[3] W.H. Organization, Antimicrobial Resistance: Global Report on Surveillance, WHO, 2014.
[4] A.K. Jindal, K. Pandya, I.D. Khan, Antimicrobial resistance: a public health challenge, Med. J. Armed Forces India 71 (2015) 178–181.
[5] C.E. Nord, R. Finch, S.R. Norrby, C.E. Nord, R. Finch, Lack of development of new antimicrobial drugs: a potential serious threat to public health, Lancet. Infect. Dis. 5 (2005) 115–119.
[6] V. Yerragunta, P. Patil, V. Anusha, T. Kumaraswamy, D. Suman, T. Samhitha, Pyrimidine and its biological activity: a review, PharmaTutor 1 (2013) 39–44.
[7] M. Sharma, V. Deekshith, A. Semwal, D. Sriram, P. Yogeeswari, Discovery of tetrahydropyrido[4,3-*d*]pyrimidine derivatives for the treatment of neuropathic pain, Bioorg. Chem. 52 (2014) 69–76.
[8] A. Khalil, C. Mathé, C. Périgaud, Rapid synthesis of 2′,3′-dideoxy-3′β-fluoro-pyrimidine nucleosides from 2′-deoxypyrimidine nucleosides, Bioorg. Chem. 38 (2010) 271–274.
[9] C.V.N.S. Varaprasad, K.S. Ramasamy, J.-L. Girardet, E. Gunic, V. Lai, W. Zhong, et al., Synthesis of pyrrolo[2,3-*d*]pyrimidine nucleoside derivatives as potential anti-HCV agents, Bioorg. Chem. 35 (2007) 25–34.
[10] B. Ramesh, T. Sumana, Synthesis and antimicrobial screening of some new pyrimidine derivatives, Int. J. Pharm. Sci. 1 (2009) 320–322.
[11] J.J. Hanthorn, R. Amorati, L. Valgimigli, D.A. Pratt, The reactivity of air-stable pyridine- and pyrimidine-containing diarylamine antioxidants, J. Org. Chem. 77 (2012) 6895–6907.
[12] S. Ben Wang, X.Q. Deng, Y. Zheng, Y.P. Yuan, Z.S. Quan, L.P. Guan, Synthesis and evaluation of anticonvulsant and antidepressant activities of 5-alkoxytetrazolo [1,5-*c*]thieno[2,3-*e*]pyrimidine derivatives, Eur. J. Med. Chem. 56 (2012) 139–144.
[13] J. Eguchi, Y. Inomata, T. Yuasa, M. Egawa, K. Saito, Pharmacological profile of the novel antidepressant 4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl) thieno-[2,3-*d*]pyrimidine monohydrate hydrochloride, Arzneimittelforschung 47 (1997) 1337–1347.
[14] A. Kamal, D. Dastagiri, M. Janaki Ramaiah, J. Surendranadha Reddy, E. Vijaya Bharathi, M. Kashi Reddy, et al., Synthesis and apoptosis inducing ability of new anilino substituted pyrimidine sulfonamides as potential anticancer agents, Eur. J. Med. Chem. 46 (2011) 5817–5824.
[15] P. Nagender, G. Malla Reddy, R. Naresh Kumar, Y. Poornachandra, C. Ganesh Kumar, B. Narsaiah, Synthesis, cytotoxicity, antimicrobial and anti-biofilm activities of novel pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives, Bioorg. Med. Chem. Lett. 24 (2014) 2905–2908.
[16] R. Srivastava, A. Mishra, R. Pratap, D.S. Bhakuni, R.C. Srimal, Antithrombotic effect of thiopurinol, Thromb. Res. 54 (1989) 741–749.
[17] J. Liu, X. Wei, E. La Kim, X. Lin, X.W. Yang, X. Zhou, et al., Erratum: Fumigatosides A-D, four new glucosidated pyrazinoquinazoline indole alkaloids from a jellyfish-derived fungus *Aspergillus fumigatus*, Org. Lett. 16 (2014) 2574.
[18] S.V. Ryabukhin, A.S. Plaskon, E.N. Ostapchuk, D.M. Volochnyuk, O.V. Shishkin, A.N. Shivanyuk, et al., A one-step fusion of 1,3-thiazine and pyrimidine cycles, Org. Lett. 9 (2007) 4215–4218.
[19] R. Pratap, V.J. Ram, Natural and synthetic chromenes, fused chromenes, and versatility of dihydrobenzo [*h*] chromenes in organic synthesis, Chem. Rev. 114 (2014) 10476–10526.

[20] M.C. Bryan, B. Dillon, L.G. Hamann, G.J. Hughes, M.E. Kopach, E.A. Peterson, et al., Sustainable practices in medicinal chemistry: current state and future directions, J. Med. Chem. 56 (2013) 6007–6021.
[21] M. Poliakoff, P. Licence, Sustainable technology: green chemistry, Nature 450 (2007) 810–812.
[22] K.G. Steinhauser, S. Richter, P. Greiner, J. Penning, M. Angrick, K.G. Steinhäuser, et al., Principles and perspectives, Environ. Sci. Pollut. Res. 11 (2004) 284–290.
[23] H. Olivier-Bourbigou, L. Magna, D. Morvan, Ionic liquids and catalysis: recent progress from knowledge to applications, Appl. Catal. A: Gen. 373 (2010) 1–56.
[24] N. Isambert, M. del M. Sanchez Duque, J. Plaquevent, Y. Génisson, J. Rodriguez, T. Constantieux, Multicomponent reactions and ionic liquids: a perfect synergy for eco-compatible heterocyclic synthesis, Chem. Soc. Rev. 40 (2011) 1347–1357.
[25] Y. Gu, Multicomponent reactions in unconventional solvents: state of the art, Green Chem. 14 (2012) 2091.
[26] A. Kadam, B. Dawane, M. Pawar, H. Shegokar, K. Patil, R. Meshram, et al., Development of novel pyrazolone derivatives as inhibitors of aldose reductase: an eco-friendly one-pot synthesis, experimental screening and in silico analysis, Bioorg. Chem. 53 (2014) 67–74.
[27] V. Ruiz, T. Huynh, S.R. Sivakkumar, A.G. Pandolfo, Ionic liquid–solvent mixtures as supercapacitor electrolytes for extreme temperature operation, RSC Adv. 2 (2012) 5591–5598.
[28] H.-P. Zhu, F. Yang, J. Tang, M.-Y. He, Brønsted acidic ionic liquid 1-methylimidazolium tetrafluoroborate: a green catalyst and recyclable medium for esterification, Green Chem. 5 (2003) 38–39.
[29] S. Safaei, I. Mohammadpoor-Baltork, A.R. Khosropour, M. Moghadam, S. Tangestaninejad, V. Mirkhani, et al., Application of a multi-SO3H Brønsted acidic ionic liquid in water: a highly efficient and reusable catalyst for the regioselective and scaled-up synthesis of pyrazoles under mild conditions, RSC Adv. 2 (2012) 5610–5616.
[30] Z. El Asri, Y. Génisson, F. Guillen, O. Baslé, N. Isambert, M. del Mar Sanchez Duque, et al., Multicomponent reactions in ionic liquids: convenient and ecocompatible access to the 2,6-DABCO core, Green Chem. 13 (2011) 2549–2552.
[31] G. Brahmachari, S. Das, L-Proline catalyzed multicomponent one-pot synthesis of gem-diheteroarylmethane derivatives using facile grinding operation under solvent-free conditions at room temperature, RSC Adv. 4 (2014) 7380–7388.
[32] R.D. Rogers, K.R. Seddon, Ionic liquids–solvents of the future, Science 302 (2003) 792–793.
[33] H.G.O. Alvim, G.A. Bataglion, L.M. Ramos, A.L. De Oliveira, H.C.B. De Oliveira, M. N. Eberlin, et al., Task-specific ionic liquid incorporating anionic heteropolyacid-catalyzed Hantzsch and Mannich multicomponent reactions. Ionic liquid effect probed by ESI-MS(/MS), Tetrahedron 70 (2014) 3306–3313.
[34] A.E.F.G. Hammam, A.F.M. Fahm, A.G.E. Amr, A.M. Mohamed, Synthesis of novel tricyclic, synthesis of novel tricyclic heterocyclic compounds as potential anticancer agents using chromanon and thiochromanone as synthons, Indian J. Chem. 42B (2003) 1985–1993.
[35] L. Suresh, Y. Poornachandra, S. Kanakaraju, C. Ganesh Kumar, G.V.P. Chandramouli, One-pot three-component domino protocol for the synthesis of novel pyrano[2,3-*d*]pyrimidines as antimicrobial and anti-biofilm agents, Org. Biomol. Chem. 13 (2015) 7294–7306.
[36] P. Sagar Vijay Kumar, L. Suresh, G.V.P. Chandramouli, Ionic liquid catalyzed multicomponent synthesis, antifungal activity, docking studies and in-silico ADMET properties of novel fused chromeno-pyrazolo-phthalazine derivatives, J. Saudi Chem. Soc. (2015), http://dx.doi.org/10.1016/j.jscs.2015.08.001.
[37] P.S.V. Kumar, L. Suresh, G. Bhargavi, S. Basavoju, G.V.P. Chandramouli, Ionic liquid-promoted green protocol for the synthesis of novel naphthalimide-based acridine-1,8-dione derivatives via a multicomponent approach, ACS Sustain. Chem. Eng. 3 (2015) 2944–2950.
[38] P. Sagar Vijay Kumar, L. Suresh, T. Vinodkumar, B.M. Reddy, G.V.P. Chandramouli, Zirconium doped ceria nanoparticles: an efficient and reusable catalyst for a green multicomponent synthesis of novel phenyldiazenyl–chromene derivatives using aqueous medium, ACS Sustain. Chem. Eng. 4 (2016) 2376–2386.
[39] D. Amsterdam, Susceptibility testing of antimicrobials in liquid media, Antibiot. Lab. Med (1996) 52–111.
[40] National Committee for Clinical Laboratory Standards, NCCLS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard, fifth ed., NCCLS, Wayne, PA, 2000.
[41] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Bacterial biofilms: from the natural environment to infectious diseases, Nat. Rev. Microbiol. 2 (2004) 95–108.
[42] J.W. Costerton, L. Montanaro, C.R. Arciola, Biofilm in implant infections: its production and regulation, Int. J. Artif. Organs 28 (2005) 1062–1068.
[43] A. Kamal, A. Rahim, S. Riyaz, Y. Poornachandra, M. Balakrishna, C.G. Kumar, et al., Regioselective synthesis, antimicrobial evaluation and theoretical studies of 2-styryl quinolines, Org. Biomol. Chem. 13 (2015) 1347–1357.
[44] G.A. O'Toole, Microtitre dish biofilm formation assay, J. Vis. Exp. 47 (2011) 2437, http://dx.doi.org/10.3791/2437.
[45] M. Hentzer, K. Riedel, T.B. Rasmussen, A. Heydorn, J.B. Andersen, M.R. Parsek, S. A. Rice, L. Eberl, S. Molin, N. Høiby, S. Kjelleberg, M. Givskov, Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound, Microbiology 148 (2002) 87–102.
[46] H.P. Steenackers, J. Levin, J.C. Janssens, A. De Weerdt, J. Balzarini, J. Vanderleyden, D.E. De Vos, S.C. De Keersmaecker, Structure-activity relationship of brominated 3-alkyl-5-methylene-2(5H)-furanones and alkylmaleic anhydrides as inhibitors of *Salmonella* biofilm formation and quorum sensing regulated bioluminescence in *Vibrio harveyi*, Bioorg. Med. Chem. 18 (2010) 5224–5233.
[47] J.J. Richards, T.E. Ballard, C. Melander, Inhibition and dispersion of *Pseudomonas aeruginosa* biofilms with reverse amide 2-aminoimidazole oroidin analogues, Org. Biomol. Chem. 6 (2008) 1356–1363.
[48] S.A. Rogers, C. Melander, Construction and screening of a 2-aminoimidazole library identifies a small molecule capable of inhibiting and dispersing bacterial biofilms across order, class, and phylum, Angew. Chem. Int. Ed. 47 (2008) 5229–5231.
[49] J.J. Richards, T.E. Ballard, R.W. Huigens, C. Melander, Synthesis and screening of an oroidin library against *Pseudomonas aeruginosa* biofilms, ChemBioChem 9 (2008) 1267–1279.
[50] H.P.L. Steenackers, D.S. Ermolat'ev, B. Savaliya, A. De Weerdt, D. De Coster, A. Shah, E.V. Van der Eycken, D.E. De Vos, J. Vanderleyden, S.C.J. De Keersmaecker, Structure–activity relationship of 2-hydroxy-2-aryl-2,3-dihydro-imidazo[1,2-a]pyrimidinium salts and 2N-substituted 4(5)-aryl-2-amino-1H-imidazoles as inhibitors of biofilm formation by *Salmonella typhimurium* and *Pseudomonas aeruginosa*, Bioorg. Med. Chem. 19 (2011) 3462–3473.
[51] V. Liebens, E. Gerits, W.J. Knapen, T. Swings, S. Beullens, H.P. Steenackers, S. Robijns, A. Lippell, A.J. O'Neill, M. Veber, M. Fröhlich, A. Krona, M. Lövenklev, R. Corbau, A. Marchand, P. Chaltin, K. De Brucker, K. Thevissen, B.P. Cammue, M. Fauvart, N. Verstraeten, J. Michiels, Identification and characterization of an anti-pseudomonal dichlorocarbazol derivative displaying anti-biofilm activity, Bioorg. Med. Chem. Lett. 24 (2014) 5404–5408.
[52] M. Givskov, R. de Nys, M. Manefield, L. Gram, R. Maximilien, L. Eberl, S. Molin, P. Steinberg, S. Kjelleberg, Eukaryotic interference with homoserine lactone-mediated prokaryotic signaling, J. Bacteriol. 178 (1996) 6618–6622.
[53] L. Gram, R. de Nys, R. Maximilien, M. Givskov, P. Steinberg, S. Kjelleberg, Inhibitory effects of secondary metabolites from the red alga *Delisea pulchra* on swarming motility of *Proteus mirabilis*, Appl. Environ. Microbiol. 62 (1996) 4284–4287.
[54] M. Manefield, R. de Nys, N. Kumar, R. Read, M. Givskov, P. Steinberg, S. Kjelleberg, Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein, Microbiology 145 (1999) 283–291.
[55] M. Manefield, T.B. Rasmussen, M. Henzter, J.B. Andersen, P. Steinberg, S. Kjelleberg, M. Givskov, Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover, Microbiology 148 (2002) 1119–1127.
[56] T.B. Rasmussen, M. Manefield, J.B. Andersen, L. Eberl, U. Anthoni, C. Christophersen, P. Steinberg, S. Kjelleberg, M. Givskov, How *Delisea pulchra* furanones affect quorum sensing and swarming motility in *Serratia liquefaciens* MG1, Microbiology 146 (2000) 3237–3244.
[57] C.G. Kumar, Y. Poornachandra, Biodirected synthesis of Miconazole-conjugated bacterial silver nanoparticles and their application as antifungal agents and drug delivery vehicles, Coll. Surf. B: Biointerfaces 125 (2015) 110–119.
[58] F. Madeo, E. Fröhlich, M. Ligr, M. Grey, S.J. Sigrist, D.H. Wolf, et al., Oxygen stress: a regulator of apoptosis in yeast, J. Cell Biol. 145 (1999) 757–767.
[59] M.A. Kohanski, D.J. Dwyer, B. Hayete, C.A. Lawrence, J.J. Collins, A common mechanism of cellular death induced by bactericidal antibiotics, Cell 130 (2007) 797–810.
[60] S.J. Dixon, B.R. Stockwell, The role of iron and reactive oxygen species in cell death, Nat. Chem. Biol. 10 (2013) 9–17.