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Regioselective synthesis of 3-benzyl substituted pyrimidino chromen-2-ones and evaluation of anti-microbial and anti-biofilm activities

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ABSTRACT

Regioselective synthesis of a number of highly functionalized 3-benzylpyrimidino chromen-2-ones (**4**) were accomplished in a one pot three component reaction in acetic acid and determined their anti-microbial and anti-biofilm activities. Compounds **4o** and **4p** showed an excellent anti-microbial activity against *Micrococcus luteus* MTCC 2470 at a par with standard control (Ciprofloxacin) and exhibited best activity against *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121. Further, compounds **4h**, **4i**, **4m**, **4n** and **4q** showed promising activity against *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121. Whereas, compounds **4m** showed very promising biofilm inhibition activity against *Staphylococcus aureus* MLS 16 MTCC 2940 and **4o**, **4p** showed very potent activity against *Staphylococcus aureus* MTCC 96 at a par with Ciprofloxacin used as standard control.

Chromen-2-ones are an important class of heterocycles which are omnipresent in natural products and synthetic origin as well. Chromen-2-one also called as coumarin known to exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory, antioxidant, antimicrobial and anticoagulant activities.[1–3] Among the coumarins family, 3-substituted-4-hydroxy coumarins have gained popularity. More specifically, 3-benzyl substituted 4-hydroxy coumarins have received much importance due to its abundance in medicinal scaffolds like warfarin, phenprocumon and novobiocin (Fig. 1). Further, 3-aryl substituted coumarins have been attractive research candidates owing to their application as additives in food, perfumes and cosmetics.[4]

Similarly, pyrimidine moiety is an important class of N-containing heterocycles and widely used as key building blocks for pharmaceutical agents. It is known to exhibit wide spectrum of pharmacophores as it acts as analgesic,[5] antifungal,[6] anti-hypertensive and anti-tumor[7] agents (Fig. 1).

Further, natural products with a pyrimidine skeleton such as vitamin B_1[8] and nucleotide bases[9] play an important role in life science studies. In addition, organic compounds with pyrimidine moiety as a core unit are important targets and are known to exhibit various pharmaceutical activities.[10–12]

In view of the biological significance of coumarin and pyrimidine moieties and also as a part of our ongoing research programme on the bioactive coumarin derivatives,[13] we planned to take-up the synthesis of 3-benzyl substituted pyrimidino coumarins as a molecular scaffold and determine anti-microbial and anti-biofilm activities. In this context, literature review at this stage revealed that a number of methods is available on the synthesis of pyranochromenes,[14] benzylpyrazolylcoumarins[15] and pyridine based coumarins.[16] However, it is surprising to note that there are no reports available on a molecular skeleton having pyrimidine and coumarin fragments joined through a benzyl functional. With this background, herein report for the first time, synthesis of highly functionalized 3-benzyl substituted pyrimidino chromen-2-ones (**4**) and their anti-microbial and anti-biofilm activity studies.

Initially a model reaction was conducted with 6-amino-1,3-dimethyl uracil (**1**) (1.0 mmol), 4-cyano benzaldehyde (**2**) (1.1 mmol) and 4-hydroxy-6-methyl coumarin (**3a**) (1.0 mmol) under solvent free condition at 130 °C for 2 h (Table 1, entry 1). Interestingly, the formation of two products **4** and **5** were observed in 30 and 20% yields. Subsequently, a series of reactions were conducted to optimize the reaction conditions for exclusive formation of **4** by

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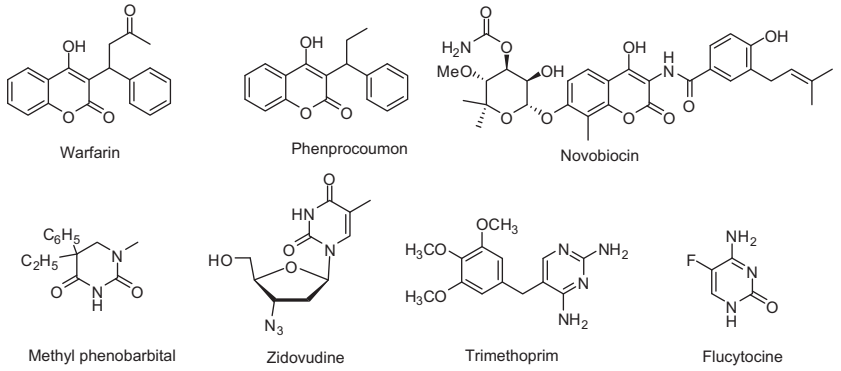
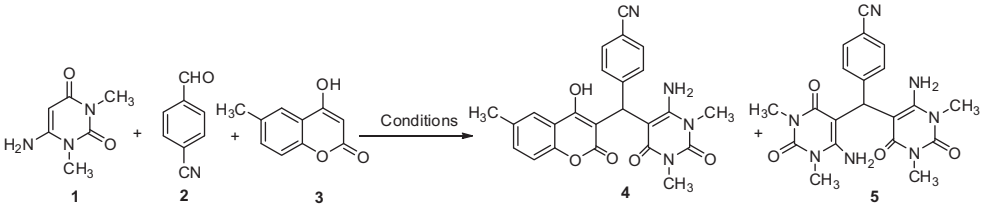


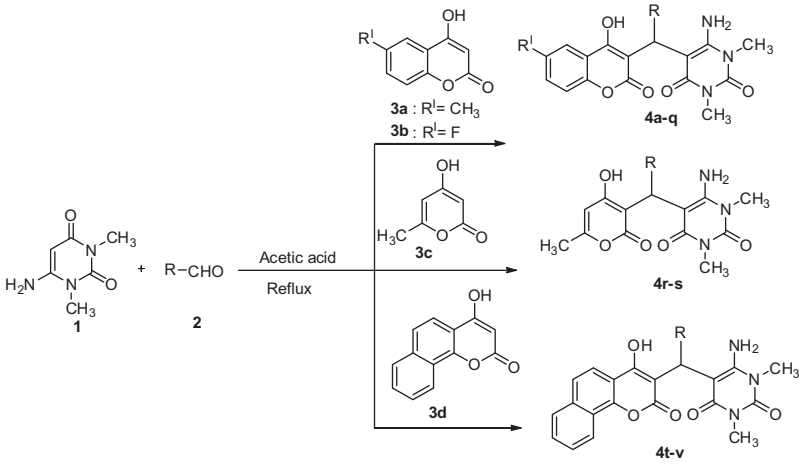
Figure 1. 4-Hydroxy coumarin and pyrimidine moieties containing drugs.

Table 1
Catalysts screening and optimization of reaction conditions for regioselective formation of **4**



Entry	Solvent	Catalyst (mol %)	Time (h)	*T* (°C)	Yield[a] (%) **4**	Yield[a] (%) **5**
1	None	—	2	130	30	20
2	H_2O	PTSA (20)	1	100	70	24
3	Ethanol	PTSA (20)	1	80	55	30
4	Ethanol	$CeCl_3$ (20)	1	80	44	40
5	Ethanol	$FeCl_3$ (20)	1	80	45	38
6	H_2O	$InCl_3$ (20)	1	100	48	40
7	Ethanol	L-Proline (20)	1	80	73	19
8	Ethanol	$SnCl_2{\cdot}2H_2O$ (20)	1	80	60	28
9	AcOH	—	2	RT	30	50
10	AcOH	—	1	80	50	35
11	**AcOH**	**—**	**1**	**120**	**82**	**10**
12	AcOH	—	3	120	79	10

Bold values indicate the higher yields and in fact reflects the preferred reaction parameter.
[a] 4-Hydroxy coumarin (**3a**) (1 mmol), *p*-cyanobenzaldehyde (**2**), (1 mmol), 6-amino uracil (1 mmol) and catalyst were heated to the specified temperature.

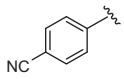
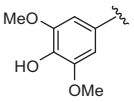
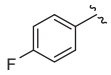
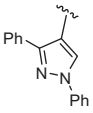
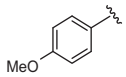
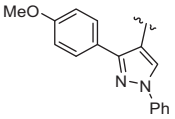
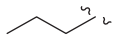
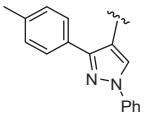
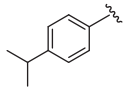
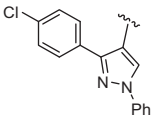
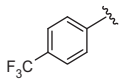
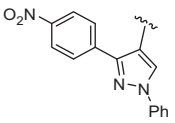
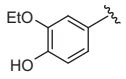
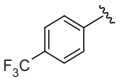
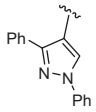
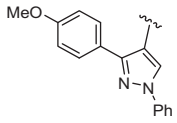
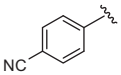
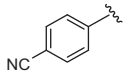
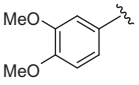
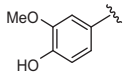
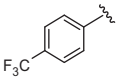


Scheme 1. One pot three component synthesis of pyrimidino chromene derivatives.

employing various Lewis acid catalysts in ethanol/water as a solvent. However, these studies resulted in improved yields of both **4** (44–70%) and **5** (24–40%).

In order to improve the regioselectivity of **4**, a reaction was conducted in acetic acid without added catalyst and found that the yield of compound **4** was dramatically improved. However,

Table 2
Synthesis of 3-benzyl substituted pyrimidino chromen-2-ones (**4a**–**v**) in acetic acid under reflux conditions

Entry	R	Coumarin	Product[a]	Yield[b] (%)	Mp (°C)	Entry	R	Coumarin	Product[a]	Yield[b] (%)	Mp (°C)
1		**3a**	**4a**	76	166–168	12		**3b**	**4l**	72	227–229
2		**3a**	**4b**	82	169–171	13		**3b**	**4m**	84	251–253
3		**3a**	**4c**	74	155–157	14		**3b**	**4n**	75	248–250
4		**3a**	**4d**	75	94–95	15		**3b**	**4o**	79	243–246
5		**3a**	**4e**	77	151–153	16		**3b**	**4p**	77	257–259
6		**3a**	**4f**	80	233–234	17		**3b**	**4q**	82	272–274
7		**3a**	**4g**	69	140–141	18		**3c**	**4r**	78	140–142
8		**3a**	**4h**	78	258–259	19		**3c**	**4s**	70	171–173
9		**3a**	**4i**	76	249–251	20		**3d**	**4t**	74	230–232
10		**3b**	**4j**	80	248–250	21		**3d**	**4u**	78	254–255
11		**3b**	**4k**	70	183–185	22		**3d**	**4v**	76	167–169

[a] All the products are confirmed by NMR , IR and mass spectrometry.
[b] Yields refers to pure products after column chromatography.

experiments in acetic acid were further continued by varying the temperature and best results (82%) were obtained[17] at reflux temperature (Table 1, entry 11). Regioselective formation of **4** in the reflux condition may be attributed to rapid keto-enol tautomerism of compound **3** that may help in predominate reaction with Knoevenagel product of aldehyde and uracil, at the same time, ring deactivation of second molecule of compound **1** as the lone pair electrons on the amine functional are suppose to involve in driving the reaction in favor of **5** are trapped with hydrogen bonding which results in shifting the adjacent π electrons.

Encouraged by the remarkable results obtained in acetic acid, the generality and scope of this coupling protocol was demonstrated by synthesizing a series of 3-benzyl substituted pyrimidino coumarin derivatives **4a**–**v**. A wide range of aldehydes (aromatic, hetero aromatic, aliphatic) and different coumarin derivatives (**3a**, **3b**, **3c**, **3d**) were well tolerated under these reaction condition (Scheme 1) and furnished **4** as a racemic compound in moderate to good yields (Table 2). In order to cyclize the compound **4**, reflux in acetic acid was further continued overnight. However, there was no formation of any cyclized product. Further attempts for cyclization by employing acid catalysts such as PTSA, P_2O_5, H_2SO_4 and $InCl_3$[18,19] in toluene under reflux condition were unsuccessful. Whereas, when PCl_5, $POCl_3$, $SOCl_2$, and NCS employed for cyclization, formation of inseparable mixture of compounds observed and the compound **4** was decomposed.

The structure of **4** was well characterized by proton NMR spectra where $-NH_2$ protons appeared at δ 6.46 (D_2O exchangeable) and two singlet's corresponding to $-NCH_3$ protons appeared at δ 3.57, 3.33 followed by aromatic protons at δ 7.76–7.23. Mass spectra confirmed the molecular ion peak. Further, structure of a representative compound was unambiguously confirmed by single

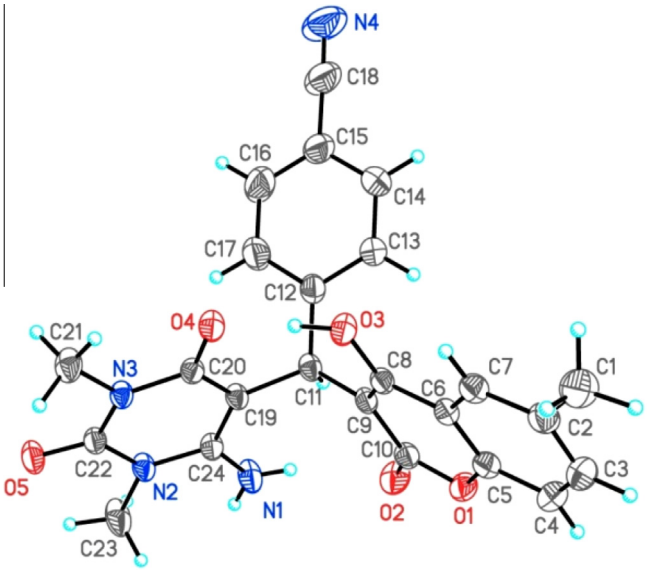


Figure 2. X-ray crystallography structure of **4a**.

crystal X-ray diffraction analysis (Fig. 2) and the data deposited[20] at the CCDC 930245.

Next, compounds in **4** series were subjected to anti-microbial and anti-biofilm activity screening as the symbiosis of coumarin and pyrimidine moieties as a single unit could become a better molecule from a biological activity point of view and also on account of a very few scaffolds such as halogenated furanones,[21] analogues of the sponge derived marine natural alkaloids oroidin and bromoageliferin,[22–25] 2-aminoimidazoles and imidazopyridinium salts[26] have been studied for anti-biofilm activity. The outcome of the anti-microbial activity screening (Table 3) showed that the compounds in **4** series with pyrazolophenyl substitution on the carbon attached to coumarin and pyrimidine ring made a very significant impact against various Gram-positive and Gram-negative bacterial strains. Specifically compounds **4o** and **4p** (entries 15 and 16) showed very promising activity against *Micrococcus luteus* MTCC 2470 at par with standard control (Ciprofloxacin) and exhibited promising activity against *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121. Compounds **4m** and **4v** (entries 13 and 22) showed activity against all the tested Gram-positive strains. Further, compounds **4j**, **4k**, **4r** and **4u** (entries 8, 9, 14 and 17) showed good activity against Gram-positive strains like *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Bacillus*

Table 3
Antimicrobial activity of synthesized compounds **4a–v**

S. No.	Test compounds	Minimum inhibitory concentration (μg/ml)					S.No	Test compounds	Minimum inhibitory concentration (μg/ml)				
		M.l[a]	*S.a*[b]	*S.a*[c]	*B.s*[d]	*P.a*[e]			*M.l*[a]	*S.a*[b]	*S.a*[c]	*B.s*[d]	*P.a*[e]
1	**4a**	>150	>150	150	>150	>150	12	**4l**	18.75	>150	37.50	>150	>150
2	**4b**	>150	>150	150	37.50	>150	13	**4m**	2.34	2.34	1.17	4.68	>150
3	**4c**	75.0	>150	75.0	75.0	>150	14	**4n**	2.34	4.68	>150	2.34	>150
4	**4d**	75.0	75.0	75.0	18.75	>150	15	**4o**	0.58	2.34	>150	1.17	>150
5	**4e**	75.0	>150	150	9.37	>150	16	**4p**	0.58	1.17	>150	1.17	>150
6	**4f**	18.75	>150	18.75	9.37	9.37	17	**4q**	2.34	4.68	>150	2.34	>150
7	**4g**	9.37	75.0	9.37	9.37	9.37	18	**4r**	>150	>150	>150	150	>150
8	**4h**	2.34	4.68	>150	2.34	>150	19	**4s**	>150	>150	>150	150	>150
9	**4i**	2.34	4.68	>150	2.34	>150	20	**4t**	150	>150	37.50	37.50	>150
10	**4j**	>150	75.0	75.0	75.0	>150	21	**4u**	>150	>150	9.37	18.75	>150
11	**4k**	9.37	75.0	37.50	9.37	>150	22	**4v**	18.75	9.37	2.34	9.37	>150
Ciprofloxacin (standard control)		0.58	0.58	0.58	0.58	0.58	Ciprofloxacin (standard control)		0.58	0.58	0.58	0.58	0.58

[a] *Micrococcus luteus* MTCC 2470.
[b] *Staphylococcus aureus* MTCC 96.
[c] *Staphylococcus aureus* MLS-16 MTCC 2940.
[d] *Bacillus subtilis* MTCC 121.
[e] *Pseudomonas aeruginosa* MTCC 2453.

Table 4
Biofilm inhibition assay

S.No.	Test compounds	Minimum biofilm eradication concentration (MBEC) (μg/ml)		
		S.a[a]	*S.a*[b]	*P.a*[c]
1	**4f**	–*	20	20
2	**4g**	–	10	20
3	**4h**	10	–	–
4	**4i**	10	–	–
5	**4k**	–	40	–
6	**4m**	10	4	–
7	**4n**	10	–	–
8	**4o**	4	–	–
9	**4p**	4	–	–
10	**4q**	10	–	–
11	**4v**	10	10	–
Ciprofloxacin (standard control)		4	4	4

* No activity.
[a] *Staphylococcus aureus* MTCC 96.
[b] *Staphylococcus aureus* MLS-16 MTCC 2940.
[c] *Pseudomonas aeruginosa* MTCC 2453.

subtilis MTCC 121. While, compounds **4f** and **4g** (entries 6 and 7) showed good activity against *Pseudomonas aeruginosa* MTCC 2453 along with *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MLS-16 MTCC 2940 and *Bacillus subtilis* MTCC 121.

Subsequently, compounds that have exhibited high anti-microbial activity against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Pseudomonas aeruginosa* MTCC 2453 strains were subjected to anti-biofilm screening studies. The outcome of the study (Table 4) showed that once again the compounds with pyrazolophenyl substitution on the carbon attached to coumarin and pyrimidine ring displayed a very remarkable biofilm inhibition activity. Specifically, compound **4m** has exhibited an excellent activity against *Staphylococcus aureus* MLS 16 MTCC 2940 and **4o**, **4p** showed very potent activity against *Staphylococcus aureus* MTCC 96 at par with

Ciprofloxacin used as standard control. Further, compounds **4h–i**, **4m–n** and **4q**, **4v** showed promising activity against *Staphylococcus aureus* MTCC 96 and **4g**, **4v** displayed very good activity against *Staphylococcus aureus* MLS 16 MTCC 2940.

In conclusion, we have developed an efficient one pot protocol to synthesize a series of novel and highly functionalized 3-benzyl substituted pyrimidino chromen-2-ones (**4**) in a single pot reaction with simple reaction conditions that resulted in high yields with high compatibility and determined their anti-microbial and anti-biofilm activities. Some of the compounds, specifically **4m**, **4o** and **4p** have showed a very potent anti-microbial and biofilm inhibition activities and the data is helpful to design and synthesize more such derivatives to be taken-up for further studies.

Acknowledgments

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Supplementary data

Supplementary data (X-ray crystallographic data, experimental procedure and spectroscopic data of all the compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.038.

References and notes

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17. *General procedure for the preparation of compound* ***4****:* A mixture of hydroxy coumarin (**3a**/**3b**/**3c**/**3d**) (1 mmol), aldehyde (**2**) (1 mmol), 6-amino uracil (**1**) (1 mmol) and acetic acid (5 ml) in 25 ml round bottomed flask was refluxed in a preheated oil bath for 1 h. Then the reaction mixture was evaporated, leftover residual mass taken into ethyl acetate, washed with $NaHCO_3$ solution followed by with water and brine respectively. Organic phase was dried over anhydrous Na_2SO_4, evaporated by rotary evaporator under reduced pressure to give the crude product which was purified by column chromatography over silica gel by using hexane and ethyl acetate as the eluent to furnish **4**.
4-((6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-hydroxy-6-methyl-2-oxo-2H-chromen-3-yl)methyl) benzonitrile (**4a**)*:* mp 169–171 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.27 (s, 1H), 7.76 (s, 1H), 7.58 (d, J = 8.31 Hz, 2H), 7.40 (d, J = 8.31 Hz, 1H), 7.31 (d, J = 8.31 Hz, 2H), 7.28–7.23 (m, 1H), 6.46 (br s, 2H, D_2O exchangeable), 5.74 (s, 1H), 3.57 (s, 3H), 3.33 (s, 3H), 2.42 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 168.0, 165.4, 164.5, 154.9, 150.4, 143.7, 134.4, 133.7, 132.2, 127.2, 124.1, 118.9, 116.6, 116.0, 103.1, 88.4, 36.8, 30.0, 28.7, 20.9 ppm. IR (KBr): 3396, 3215, 2924, 2227, 1699, 1664, 1578, 1507 cm^{-1}; HRMS (ESI) Anal. Calcd for $C_{24}H_{21}O_5N_4$ m/z 445.15065 $[M+H]^+$. Found: 445.15079.
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20. CCDC 930245 contains the supplementary crystallographic data for this Letter. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 (0) 1223 336 033; email: deposit@ccdc.cam.ac.uk].
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