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Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

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To cite this article: M. R. Bhosle, D. B. Wahul, G. M. Bondle, A. Sarkate & S. V. Tiwari (2018): An efficient multicomponent synthesis and *in vitro* anticancer activity of dihydropyranochromene and chromenopyrimidine-2,5-diones, Synthetic Communications, DOI: 10.1080/00397911.2018.1480042

To link to this article: https://doi.org/10.1080/00397911.2018.1480042

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https://doi.org/10.1080/00397911.2018.1480042

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An efficient multicomponent synthesis and *in vitro* anticancer activity of dihydropyranochromene and chromenopyrimidine-2,5-diones

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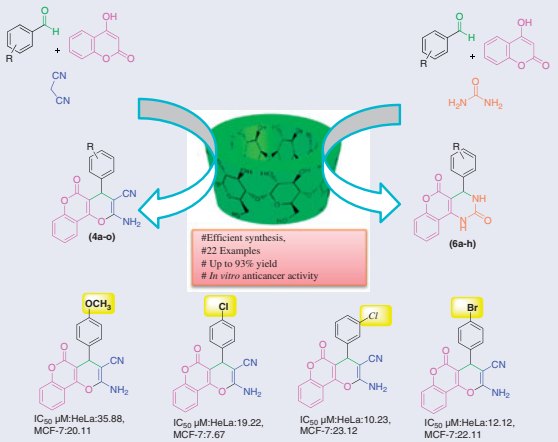
ABSTRACT

A series of dihydropyranochromenes and chromenopyrimidine-2,5-diones having chromene scaffold were synthesized *via* efficient multicomponent protocol in aqueous β-cyclodextrin. The reaction is free of toxic solvents, operating under mild conditions and allows for ease of product isolation, making it more environmentally friendly. All the synthesized compounds biologically evaluated for their potential inhibitory effect on both cervical cancer cell line (HeLa) and human breast adenocarcinoma cell line (MCF-7). Of these compounds, **4d** was found to be the most potent inhibitors of HeLa and MCF-7 demonstrating IC_{50} values of 19 μM and 7 μM. Compounds **4b**, **4e** and **4f** also shown significantly good *in vitro* anticancer activity against HeLa and MCF-7 cancer cell lines.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 20 March 2018

KEYWORDS
Anticancer activity; β-cyclodextrin; chromenopyrimidine-2,5-diones; dihydropyranochromene

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Introduction

Chromenes are synthetically significant structural motifs that can be easily functionalized to access different biologically active analogues. These privileged molecular frameworks were often targeted because of their easily accessible chemical libraries and ability to act as ligands for a diverse array of receptors.[1] The presence of chromene scaffold in the framework of various pharmacologically active compounds represents an interesting template for medicinal chemistry. Many of these compounds are known for their spasmolytic,[1d] diuretic,[2] anti-HIV,[3] antitumor,[4] antimalarial,[5] anti-Alzheimer,[6] and antileukemic[7] properties. In addition, different derivatives of chromenes are known as valuable synthones used for potential biodegradable agrochemicals,[8] cosmetics and pigments.[1] The pyranochromene possess chromene pharmacophore present in a wide variety of natural and synthetic molecules of biological importance.[9] Among the pyranochromenes, dihydropyrano[2,3-c]chromenes form a special sub-class exhibiting diverse biological activities such as anti-microbial,[10] anti-cancer,[11] anti-dyslipidemic[12] anti-hyperglycemic,[13] anti-HIV,[14] antioxidant,[15] antiallergic,[16] xanthine oxidase inhibitory,[17] butyrylcholinesterase, acetylcholinesterase,[18] and Src kinase inhibitory activities (Fig. 1).[19]

Chromenopyrimidine-2,5-dione is fused heterocycle, can be frequently found in an ocean of biologically active molecules as analgesic, antifungal, antibacterial antitumour,[20] cardiotonic,[21] antibronchitic[22] and antifungal activity.[23] Some of them exhibit antihypertensive activity,[24] antimalarial,[25] analgesic[26] antiviral and CNS depressant.

Pyranochromene derivatives are accessible via the one-pot three-component reaction of hydroxycoumarins, carbonyl compounds and active methylenes in the catalytic systems including $Fe_{3-x}Ti_xO_4$ nanoparticles,[27] *N*,*N*-dimethylbenzylamine (DMBA),[28] diammonium hydrogen phosphate (DAHP),[29a] tetrabutylammonium bromide (TBAB),[29b] urea,[30] $H_6P_2W_{18}O_{62}.18H_2O$,[31] MgO,[32] ionic liquids,[33] hexadecyltrimethyl ammonium bromide,[34] SiO_2PrSO_3H,[35] Mg/La mixed metal oxides,[36] nanosilica,[37] α-Fe_2O_3 nanoparticles,[38] and silica-grafted ionic liquid.[39]

A vigilant study dictates that most of the methodologies suffer some drawbacks such as, require forcing conditions, reaction under high temperatures, use of expensive and environmentally harmful catalysts, harsh reaction conditions, formation of a mixture of

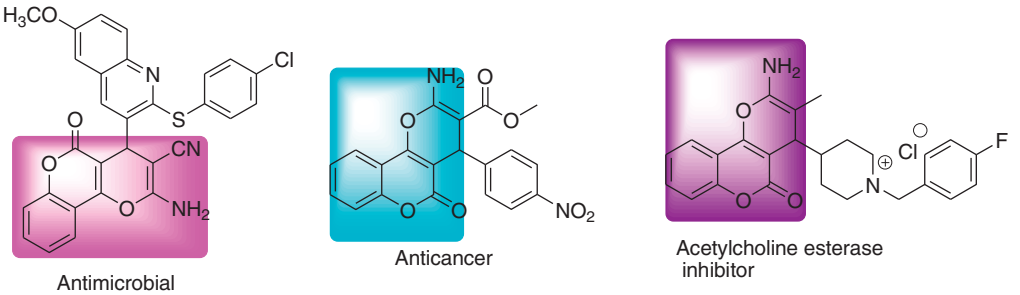


Figure 1. Representative examples of synthetically bioactive dihydropyrano[3,2-c]chromene derivatives.

products, multi-step procedure, longer reaction times and involvement of volatile organic solvents which limits the broad use of these methodologies. Albeit there are methods available for the synthesis of different pyranochromene and chromenopyrimidine-2,5-dione derivatives, their broad utility range has accentuated the need to develop newer synthetic routes for scaffold manipulation of chromene containing pyranochromene and chromenopyrimidine-2,5-dione moiety.

The use of water in multicomponent reactions offers significant environment advantages and has attracted a great deal of interest in recent years,[40] because water is considered as a cheap, environmental safety, high stability, easy handling and green solvent. Owing to the fascinating advantages of water over traditional organic solvents, various types of in water or on water reactions are completely in conformity with the standpoint of green chemistry and the development of green protocols for the syntheses of highly functionalized bio-active motifs in aqueous medium is highly desirable.[41]

Supramolecular catalysis is a discipline in chemistry which involves host–guest type interactions where covalent bonds are not established between the interacting species which can be molecules, ions, or radicals.[42] The most accessible β-cyclodextrin (β-CD) is a cyclic oligosaccharide of D(+)-glucopyranosyl units linked by α-1,4-glycosidic bonds and consist of a hydrophilic outer surface and a hydrophobic central cavity.[43] Cyclodextrins can include water-insoluble organic materials into the cavity in aqueous solution because of these features. Complexation with cyclodextrin is well-known as an effective method for enhancing dissolution properties of poorly soluble organic materials and is, therefore, the molecule of choice for the water-mediated reactions.[44]

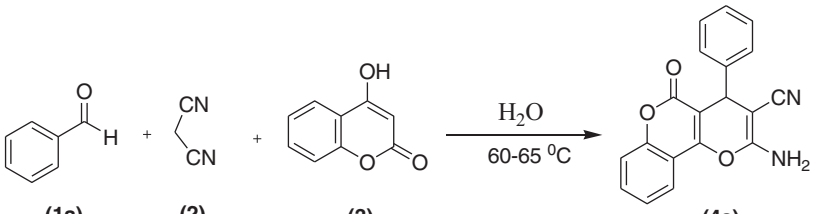
Considering the above-mentioned facts and in continuation of our previous studies on the efficient synthesis of bioactive heterocycles[45] herein, we wish to report a straightforward, efficient, clean, and high yielding MCR protocol for the one-pot facile synthesis of biologically relevant diverse and densely functionalized pyranochromene and chromenopyrimidine-2,5-dione heterocyclic scaffolds and evaluation of their *in vitro* anticancer activity.

Results and discussion

Chemistry

β-Cyclodextrin is attractive as a catalyst since it is useful both from an economic and environmental point of view, apart from being non-toxic, metabolically safe, and readily recoverable and reusable. We chose cyclodextrin as supramolecular catalyst due to its excellent catalytic properties in water. βCD-mediated reactions in water have been found to be a useful tool for economical as well as environmental points of view.[46]

Initially, the three component annulation of 3-nitrobenzaldehyde (**1a**), malononitrile (**2a**) and 4-hydroxy-2H-chromene-2-one (**3**) was examined in the presence of a range of commercially available catalysts in water and conventional heating separately (Scheme 1). While the reaction proceeded in the presence of surfactant like CTAB, SDS and p-TSA (Table 1, entries 2–4), affording the desired product **4a**, the degree of conversion varied from 48 to 64% depending on the nature of catalyst used. Improved conversion was observed when 10 mol% of β-CD was used (Table 1). The poor conversion in the



(1a) (2) (3) (4a)

Scheme 1. Synthesis of 2-amino-4,5-dihydro-5-oxo-4-phenylpyrano[3,2-c]chromene-3-carbonitrile (**4a**) (model reaction).

Table 1. Formation of 2-amino-4,5-dihydro-5-oxo-4-phenylpyrano[3,2-c]chromene-3-carbonitrile (**4a**) using different catalyst in aqueous medium[a].

Entry	Catalyst/solvent	Time (h)	Concentration (mol%)	Yield (%)[b]
1	–/water	15	–	27
2	CTAB/water	7	10	64
3	SDS/water	7	10	59
4	p-TSA/water	7	10	48
5	α-Cyclodextrin/water	9	10	45
6	β-Cyclodextrin/water	2	5, 10, 15, 20	71, 92, 92, 93
7	γ-Cyclodextrin/water	5	20	42

[a]Reaction conditions: benzaldehyde (4 mmol), malanonitrile (4 mmol), 4-hydroxycoumarin (4 mmol), water (20 mL), 60–65 °C.
[b]Isolated yield.

absence of a catalyst (Table 1) indicated its key role in the present MCR. Thus, β-CD was found to be the catalyst of choice and was used for our further studies.

The effect of various reaction parameters, such as the effect of solvents, catalyst concentration, temperature and reaction conditions was evaluated to optimize the reaction. Firstly, the effect of catalyst concentration on the formation of the pyranochromene was investigated (Table 1). Model reaction was performed at different concentrations of β-CD, i.e., 5, 10, 15 and 20 mol% at 60 °C and gave the product in 71, 92, 92 and 93% yield, respectively (Table 1). Thus increasing catalyst concentration up to 20 mol%, product yield is improved to 93% yield. Further increase in the catalyst concentration product yield remains static. It means that the presence of 10 mol% of β-CD was sufficient for catalyzing the reaction effectively in the forward direction.

Therefore, we performed the model reaction using 10 mol% β-CD in water at room temperature the product was obtained in moderate yield (50%). The reaction was found to be sluggish at room temperature; however, by increasing the temperature to 60–65 °C, the corresponding product (**4a**) was obtained in 92% yield within 1 h (Scheme 1).

The role of various solvents (polar protic and aprotic) was examined for the synthesis of pyranochromene. The reactions conducted in polar aprotic solvents, such as acetonitrile and dimethyl formamide, were found to be very slow and resulted in lower product yield under above conditions (Table 2). Next, we tested polar protic solvents such as methanol and ethanol and the yield of the desired products was good; however, an excellent yield was obtained using water as the solvent (Table 2). Water was found to be the best solvent due to high solubility of β-CD in water (Table 2). The more soluble the cyclodextrin in the solvent, the more molecules become available for complexation.

Table 2. Optimization of solvents for synthesis of 2-amino-4,5-dihydro-5-oxo-4-phenylpyrano[3,2-c]chromene-3-carbonitrile (**4a**)[a].

Entry	β-Cyclodextrin loading (mol%)	Solvent	Yield (%)[b]
1	10	Water	92
2	10	EtOH	61
3	10	MeOH	59
4	10	Acetonitrile	42
5	10	DMF	56

[a]Reaction conditions: benzaldehyde (**1a**) (4 mmol), malononitrile (**2**) (4 mmol), 4-hydroxycoumarin (**3**) (4 mmol), at 60–65 °C.
[b]Isolated yield.

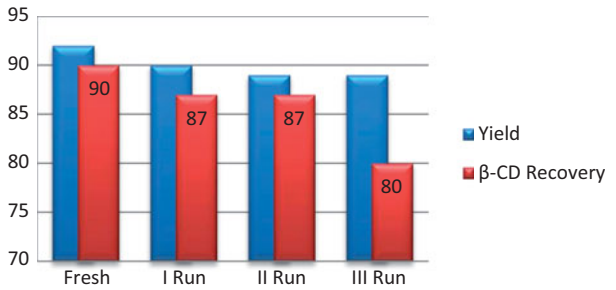
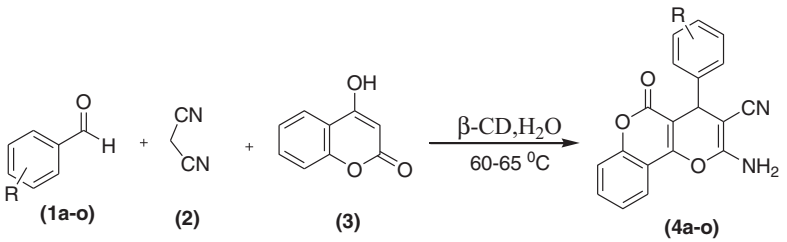


Figure 2. Recycling and recovery of β-CD and its effect on yield.



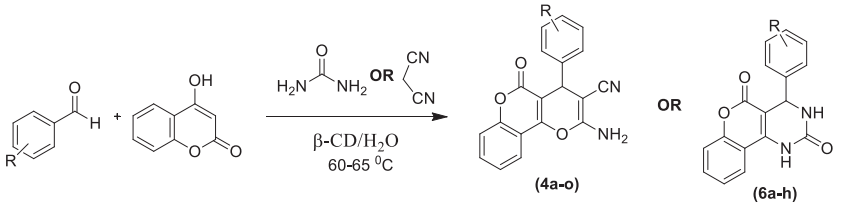
Scheme 2. Synthesis of 2-amino-4,5-dihydro-5-oxo-4-(substitutedphenyl) pyrano[3,2-c]chromene-3-carbonitriles (**4a-o**).

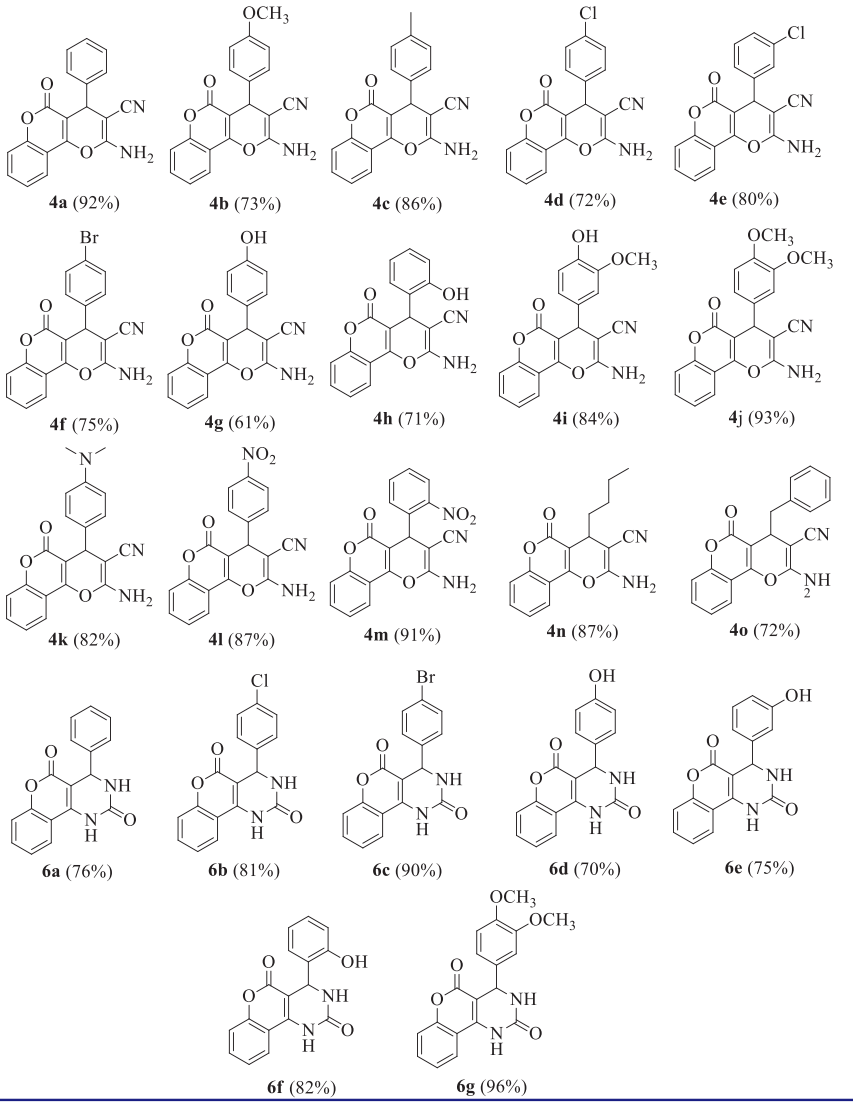
To test the recyclability of the catalyst, the aqueous β-CD was recovered after usual work-up with organic solvent and reused to afford **4a** without changing the degree of conversion significantly. The β-CD reused four times including the use of fresh catalyst for the synthesis of **4a**. The catalyst was almost quantitatively recovered and no significant loss in yield was observed (Fig. 2).

Under the optimized reaction conditions, we used various aromatic aldehydes to react with malononitrile and 4-hydroxy-2H-chromene-2-one and a series of functionalized pyranochromene derivatives were synthesized with excellent yield (Scheme 2). The results of the reactions are summarized in Table 3. As shown in Table 3, we found that all the reactions were carried out smoothly, and the aromatic aldehydes, with either electron withdrawing groups or electron-donating groups, could all be used for the synthesis of functionalized pyranochromene derivatives with excellent yields.

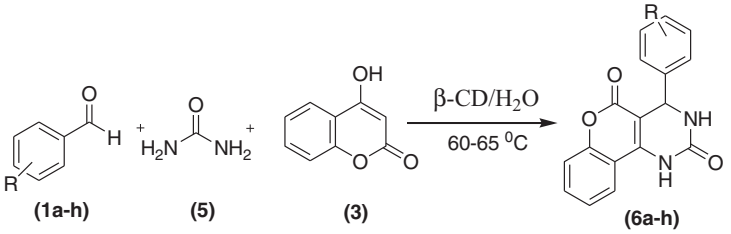
To further explore the scope of this protocol, we decided to investigate the condensation reaction of we next turned our attention to ascertain the scope and limitations of the reaction. We extended this process to other substrates. The scope of the reactions is

Table 3. Physical data of 2-amino-4,5-dihydro-5-oxo-4-(substitutedphenyl)pyrano[3,2-c]chromene-3-carbonitriles (**4a-o**) and 3,4-dihydro-4-(substituted phenyl)-1H-chromeno[4,3-d]pyrimidine-2,5-diones (**6a-g**)[a,b,c].

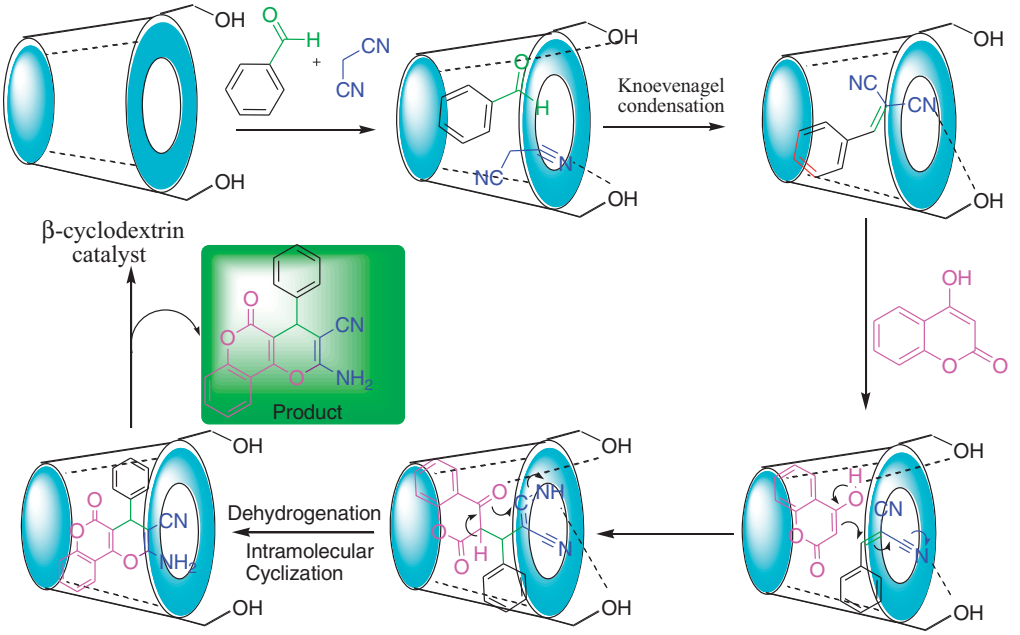




[a]Reaction conditions: substituted benzaldehyde (**1a-o**) (4 mmol); malononitrile (**2**) or urea (**5**) (4 mmol), 4-hydroxycoumarin (**3**) (4 mmol), β-cyclodextrin (10 mol%), at 60–65 °C.
[b]Isolated yield.
[c]Melting points are in good agreement with those reported in the literature.[45]



Scheme 3. Synthesis of 3,4-dihydro-4-(substituted phenyl)-1H-chromeno[4,3-d]pyrimidine-2,5-diones (**6a-h**).



Scheme 4. Plausible reaction mechanism for the synthesis of 4*H*,5*H*-pyrano[3,2 *c*][1]benzopyran-5-ones (**4a**).

embodied with respect to urea for synthesizing chromenopyrimidine-2,5-diones (**6a-g**) (Scheme 3) by employing number of aldehydes (**1a-h**), urea (**5**) and 4-hydroxycoumarin (**3**). The results are tabulated in Table 3.

Plausible reaction mechanism

The mechanism proposed for the reaction using β-CD as catalyst is also outlined in Scheme 4. The rate acceleration of this condensation can be endorsed as aqueous β-cyclodextrin catalyst appears to play an important role in increasing the efficiency of the reaction especially by activating the aldehyde carbonyl in addition to aiding the water solubility of all the reactants. A subsequent nucleophilic attack by the malanonitrile on the activated carbonyl carbon was also facilitated by β-CD leading to the generation of intermediate benzylidinemalononitrile *in situ*. Once formed, the benzylidinemalononitrile then subsequent Michael type of addition takes place by

Table 4. *In vitro* anticancer activity of 2-amino-4,5-dihydro-5-oxo-4 (substitutedphenyl) pyrano[3,2-c]chromene-3-carbonitriles (**4a-o**) and 3,4-dihydro-4-(substituted phenyl)-1H-chromeno[4,3-d]pyrimidine-2,5-diones (**6a-g**).

	IC_{50} μM	
Compound	HeLa	MCF-7
4a	92	76
4b	**35**	**20**
4c	62	50
4d	**19**	**7**
4e	**23**	**10**
4f	**22**	**12**
4g	70	60
4h	>100	88
4i	87	69
4j	42	31
4k	77	50
4l	38	22
4m	82	63
4n	>100	90
4o	>100	>100
6a	>100	>100
6b	46	33
6c	59	38
6d	86	71
6e	90	>100
6f	>100	>100
6g	73	52
ADR	<10	<10

The IC_{50} values are the concentrations in micromolar needed to inhibit cell growth by 50%.
ADR: adriamycin standard drug used; HeLa: human cervical cancer cell line; MCF-7: human breast cancer cell line.

sequential addition of 4-hydroxy coumarine resulting into intermediate, which is also stabilized by H-bonding with the secondary hydroxyl groups of β-CD. Then, followed by intramolecular cyclization and hydrolysis there is formation of desired product. After the formation of the product, the escape from the CD cavity occurs readily.

Anticancer activity

A series of 2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**4a-o)** and 3,4-dihydro-1H-chromeno[4,3-d]pyrimidine-2,5-dione (**6a-g)** were evaluated for their *in vitro* anticancer activity against MCF-7 (human breast cancer cell line) and HeLa (human cervical cancer cell line) by MTT assay using Adriamycin standard drug. The result obtained for *in vitro* anticancer activity is reported in Table 4. The IC_{50} (μM) value means concentration required to inhibit 50% of cancer cells growth.

From the close examination of IC_{50} values, it is observed that **4d**, **4e**, and **4f** were active and potent anticancer agents among the synthesized derivatives **4a-o**. The compound **4d** was found to be the most potent anticancer agents with IC_{50} value 19 and 7 μM against HeLa and MCF-7 respectively. The compound **4b** bearing para-methoxy group on the phenyl ring was found to be >2-folds less potent than the standard drug Adriamycin against HeLa and MCF-7 with IC_{50} value 35 and 20 μM, respectively.

Structure activity relationship (SAR) studies for these compounds demonstrated that the phenyl ring substituted at para position (**4b, 4c, 4d, 4f, 4g, 4k, 4l, 6b, 6c, 6d**) was more active than those substituted at ortho (**4h, 6f**). A series of 2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**4a-o)** were found to be more active than the 3,4-dihydro-1H-chromeno[4,3-d]pyrimidine-2,5-dione (**6a-g)** series.

Replacing the para-methoxy group **4b** by a para hydroxyl group **4g** resulted in two-fold reduction in anticancer activity, suggesting that the hydrophobic methoxy group is preferred over the hydrophilic hydroxyl group at the para-position on the phenyl ring. Compounds with para-position substitution on phenyl ring were more active than those with ortho-position substitution, suggesting that there might be a sterric hindrance effect due to ortho-position substitution on the phenyl ring. The compound **4g** bearing para-OH is more active than that of **4h** with ortho-OH group on the phenyl ring. Connecting the non-substituted phenyl ring via methyl linker to the chromone nucleus such as in **4o** resulted in decrease in anticancer activity, suggesting that the methyl linker between the phenyl ring and chromone nucleus does not all molecule to bind properly into the pocket. Compounds **4b**, **4d, 4e** and **4f** have shown significantly good *in vitro* anticancer activity against HeLa and MCF-7 cancer cell lines and can be developed as anticancer agents in the future.

Conclusions

This study describes the efficient, green synthesis of series of dihydropyranochromenes and chromenopyrimidine-2,5-diones, using β-cyclodextrin as a catalyst in aqueous medium. This multicomponent protocol is bestowed with merits like high yield, cost effectiveness, biomimetic, neutral aqueous phase conditions and environmentally benign nature. All the synthesized compounds screened for their *in vitro* anticancer activity on human cancer cell line MCF-7 and HeLa. In the series of dihydropyranochromenes derivatives, four compounds **4b**, **4d, 4e** and **4f** showed very potent activity and the data is helpful to further development of these compounds for anticancer activity mechanistic studies and also supportive to design and synthesize more such derivatives to be taken-up for further studies.

Experimental

General

All the chemicals used were of laboratory grade. Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance 400 spectrometer operating at 400 MHz using $CDCl_3$ solvent and tetramethylsilane (TMS) as the internal standard and chemical shift in δ ppm. Mass spectra were recorded on a Sciex, Model; API 3000 LCMS/MS Instrument. The purity of each compound was checked by TLC using silica-gel, $60F_{254}$ aluminum sheets as adsorbent and visualization was accomplished by iodine/ultraviolet light.

General procedure for the synthesis of 2-amino-4,5-dihydro-5-oxo-4-(substitutedphenyl)pyrano[3,2-c]chromene-3-carbonitriles (4a-o)

A mixture of substituted benzaldehydes (**1a-j**) (4 mmol), malononitrile (**2**) (4 mmol), 4-hydroxy coumarin (4 mmol) and β-cyclodextrin (10 mol%) in water (15 ml) was subjected to stir at 60–65 °C. Progress of the reaction was monitored by thin layer chromatography. After 60 min, reaction mixture was cooled to room temperature, filtered and washed with hot water. Obtained solid was crystallized by ethanol:DMF. Synthesized compounds characterized by IR, ^{1}H NMR and are in good agreement with those reported in the literature.[47]

General procedure for the synthesis of 3,4-dihydro-4-(substituted phenyl)-1H-chromeno[4,3-d]pyrimidine-2,5-dione (6a-g)

A mixture of substituted benzaldehydes (**1a-g**) (4 mmol), urea (**5**) (4 mmol), 4-hydroxy coumarin (**3**) (4 mmol) and β-cyclodextrin (10 mol%) in water (15 ml) was subjected to stir at 60–65 °C. Progress of the reaction was monitored by thin layer chromatography. After 2 h, reaction mixture was cooled to room temperature, filtered and washed with hot water. Obtained solid was crystallized by ethanol:DMF. Synthesized compounds characterized by IR, ^{1}H NMR and are in good agreement with those reported in the literature.[47]

Experimental procedure for MTT assays

The stock solutions of test compounds were prepared in DMSO. After 24 h incubation, different concentrations (2, 4, 6, 8 µM) of compounds, made by serial dilution in culture medium, were added in 48 h incubation. The final concentration of DMSO was 0.01% in each well. A separate well containing 0.01% DMSO only was run as DMSO control, which was found inactive under applied conditions. The cell growth was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma) reduction assay, which is based on ability of viable cells to reduce a soluble yellow tetrazolium salt to blue farmazan crystal.[48] Briefly, after 48 h of treatments, the 10 µl of MTT dye, prepared in phosphate-buffered saline (PBS) were added to all wells. The plates were then incubated for 4 h at 37 °C. Supernatant from each well was carefully removed, formazon crystals were dissolved in 100 µL of DMSO and absorbance at 540 nm wavelength was recorded and each concentration was tested in threefold. The IC_{50} values were determined as concentration of compounds that inhibited cancer cell growth by 50%.

Spectral analysis of representative compounds as follow[47]

2-Amino-4,5-dihydro-5-oxo-4-(4-methylphenyl)pyrano[3,2-c]chromene-3-carbonitrile (4c)

White solid mp: 258–259 °C (lit. 258–260 °C)[1,2]; IR (ATR υ cm^{-1}) characteristic absorptions: 3291 (N–H stretching), 2884 (C–H stretching), 1187 (C–O–C stretching),

1680 (C=O stretching). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.15 (s, 3H, CH3), 6.03 (s, 1H, CH), 7.09–7.37 (m, 6H, Ar-H), 7.49–7.54 (m, 2H, Ar-H), 11.21–11.49 (m, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-*d*6 δ ppm): 160.49, 158.37, 153.66, 152.57, 140.82, 136.74, 133.36, 129.47 (2C), 127.91 (2C), 125.08, 122.87, 119.68, 116.97, 113.36, 104.56, 58.54, 36.97, 21.04. Mass (LC-MS): *m/z* 331 $[M+H]^+$. Anal. calcd. For $C_{20}H_{14}N_2O_3$: N: 8.48; C: 72.72; H: 4.27; Found: N: 8.50; C: 72.69; H: 4.25%.

3,4-Dihydro-4-phenyl-1H-chromeno[4,3-d]pyrimidine-2,5-dione (6a)

White solid mp: 238–240 °C (lit. 237–239 °C)[47]; IR (ATR υ cm^{-1}) characteristic absorptions: 3282 (N–H stretching), 2882 (C–H stretching), 1181 and 1208 (C–O–C stretching); ^{1}H NMR (400 MHz, CDCl3, δ ppm): 6.04 (s, 1H, CH), 7.15–7.17 (d, 2H, *J* = 8 Hz, Ar-H), 7.26–7.30 (d, 2H, *J* = 8 Hz, Ar-H), 7.41–7.43 (m, 2H, Ar-H), 7.99–8.09 (m, 3H, Ar-H) , 11.31 (s, 1H, NH) and 11.54 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-*d*6 δ ppm): 162.87, 158.00, 152.86, 149.96, 149.48, 141.56, 131.79, 129.68 (2C), 128.66 (2C), 127.34, 125.69, 119.89, 119.46, 88.43, 58.67. Mass (LC-MS): *m/z* 292 $[M+H]^+$. Anal. calcd. For $C_{17}H_{12}N_2O_3$: N: 9.58; C: 69.86; H: 4.14; Found: N: 9.60; C: 70.00; H: 4.27%. Full experimental detail, IR, ^{1}H and ^{13}C NMR spectra, Mass and Elemental analysis can be found via the "Supplementary Content" section.

Acknowledgments

The authors are thankful to Professor Ramrao A. Mane for his invaluable discussions and guidance. The authors are also thankful to Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and Central Drug Research Institute (CDRI), Lucknow for providing necessary facilities and spectral analysis, respectively.

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