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Recyclable Amberlite IR-120 Catalyzed domino reaction: Synthesis, anticancer activity and molecular docking studies of biscoumarins

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ABSTRACT

Under mild conditions, an environmentally friendly method has been developed for the synthesis of bis-coumarins by using a reasonably lesser quantity of Amberlite IR-120 through Domino Knoevenagel–Michael reaction. In which, a series of aryl aldehydes have efficiently reacted with 4-hydroxycoumarin to give respective bis-coumarins through a short reaction time. The acidic Amberlite IR-120 catalyst used for a reaction was isolated and reused for successive four reactions. As per the observations made, the activity of the recycled catalyst was preserved. The *invitro* examination of bis-coumarins against two cancer cell lines was proved that, some of these have showed promising anti-proliferative properties. The molecular docking studies of bis-coumarin derivatives with GlcN-6-P synthase yielded potential conformations with prospective binding energy, docking energy, inhibition constant and electrostatic energy while comparing with the standard drug chloroquine.

1. Introduction

The enzyme, glucosamine-6-phosphatesynthase (EC 2.6.1.16), is a new target for antimicrobial studies. The enzyme catalytic pathway involved produce end product, uridine 5′diphospho-N-acetyl-D-glucosamine, giving rise to incorporation of *N*-acetylglucosamine into several macromolecules such as, chitin in fungi, insects and crustaceans; teichoic acids, lipopolysaccharides, and peptidoglycan in bacteria, and glycosaminoglycans mucopolysaccharides and glycoproteins in mammals. These molecules are building blocks in the assembly of the cell wall [1]. Presently, several natural and synthetic origin inhibitors have been reported on GlcN-6-P which exhibited antibacterial and antifungal activity.

Coumarin derivatives are widely distributed in nature [2,3] and exhibited broad spectrum of biological activities like anti-inflammatory [4,5], antitumor [6,7], analgesic [8], anticancer [9,11], antimutagenic [12], antibiotic [13,14] and anti-HIV activity [15,16]. In particular, 4–hydroxy-coumarins have showed important biological activities like antitumor, hypotoxicity, enzyme inhibition and anticoagulant activities [17, 18]. Above pharmacophores were isolated from natural sources [19,20], exhibited several useful biological and medicinal properties like anti-bacterial [21,22], anticancer [23], antioxidant, anti-inflammatory [24] and antiviral activity [25].

After identifying the considerable importance of bis-coumarins, several researchers have synthesized through diverse synthetic methods like Domino Knoevenagel–Michael reaction of 4–hydroxy coumarin and aromatic aldehydes in the presence of various catalysts viz., piperidine [26], $InCl_3$ [27], cyclic secondary amine [28], tetrabutylammonium bromide [29], molecular iodine [30], 1–butyl–3-methylimidazolium tetrafluoroborate [31], ionic liquids [32], nanoparticulate silica chloride [33], dodecylbenzenesulfonic acid [34], $Zn(proline)_2$ [35], ruthenium(III) chloride hydrate [36] and sodium dodecyl sulfate (SDS) [37] etc.

In the current study, we have successfully achieved the one pot synthesis of biscoumarins (**3a-l**) using Amberlite-IR 120 resin as an efficient heterogeneous catalyst. The catalytic activity of amberlite IR-120 acidic resin has gained importance in green chemistry due

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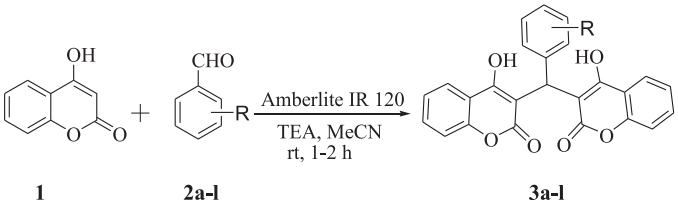


Scheme 1. one pot synthesis of biscoumarins (3a-l) through Knoevenagel condensation of 4-hydroxy coumarin (1) and aromatic aldehydes (2a-I) by using Amberlite-IR 120 resin as an efficient heterogeneous catalyst.

to several advantages such as an operational simplicity, non-toxic, low cost, high yield and efficient heterogeneous catalytic system for chemical transformations [38], ease of isolation after a reaction and reusability [39]. We have observed that, highly functionalized aromatic aldehydes and 4–hydroxy coumarin undergo domino reaction at room temperature in the presence of Amberlite IR-120 resin, and to give the corresponding symmetric and asymmetric biscoumarins in good to excellent yield (Scheme 1). Synthesized compounds were tested *invitro* for their anti-proliferative properties against leukemia (K-562) and breast (MD-AMB-231) cancer cell lines and the possible activity perhaps due to hydrogen bonding was investigated in detail using molecular docking studies.

2. Experimental

2.1. Apparatus and materials

Analytical thin layer chromatography was performed on precoated Aluminum-backed silica gel F254 plates with visualization under UV light. Melting points were obtained using a hot-stage apparatus and are uncorrected. ^{1}H NMR spectra, recorded at 400 MHz are referenced to the residual solvent peak at 7.26 ppm ($CDCl_3$) and 2.50 ppm (DMSO–d^6).^{13}C NMR spectra, recorded at 400 MHz, are referenced to the residual solvent peak at 77.0 ppm ($CDCl_3$) and 39.52 ppm (DMSO–d^6). All reactions were carried under room temperature. All other chemicals and solvents were of analytical grade.

2.2. General procedure for preparation of biscoumarin derivatives 3a-l

A mixture of 4–hydroxy coumarin (100 mg, 0.616 mmol), aldehydes (0.616 mmol), triethylamine (0.08 mL, 0.616 mmol) and Amberlite IR-120 (15 wt%) in acetonitrile (10 mL) was stirred at room temperature for about 1 to 2 h. The course of reaction was monitored by TLC. After completion of reaction, the mixture was filtered to recover the resin. The filtrate was evaporated under reduced pressure to remove acetonitrile and the residue obtained was washed with methyl tertiary butyl ether (MTBE) and dried under vacuum.

3,3′-((4-Chlorophenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3a): White solid; m.p 243.4–245.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 11.7 (s, 1H), 8.12 (d, j = 9.4 Hz, 1H), 7.90 (d, j = 4.0 Hz, 1H), 7.66–7.63 (m, 2H), 7.62 (m, 2H), 7.42–7.40 (m, 4H), 7.30 (m, 2H), 7.16 (m, 2H), 6.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$); δ (ppm) 165.9, 164.5, 152.4, 152.1, 133.7, 132.8, 132.5,128.6,127.8,124.8,124.3,116.5,116.2, 105.1, 103.5; Mass ESI calcd. $C_{25}H_{15}ClO_6$; ([M + H] +), 446.06; found: ([M + H] +), 446.8.

3,3′-((4-Bromophenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3b): Pale yellow solid; mp 277.3–279.7 °C ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 8.00 (d, J = 8.0 Hz, 2H), 7.43 (m, 2H), 7.35 (m, 2H), 7.28 −7.12 (m, 6H), 6.19 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$); δ (ppm) 170.9,167.9,152.4,139.4,131.1,130.8,128.5,124.9,123.3,119.8,119.0,115.4, 103.1; Mass ESI calcd. $C_{25}H_{15}BrO_6$: ([M + H] +), 490.01; found: ([M + 2] +) 492.6.

3,3′-((4-Fluorophenyl)methylene)bis (4–hydroxy-2*H*-chromen-2-one) (3c): White solid; mp 223.6–225.1 °C ^{1}H NMR (400 MHz, $CDCl_3$+DMSO–d^6); δ (ppm) 8.96 (s, 1H), 8.03 (d, j = 8.40 Hz, 2H), 7.46 (m, 2H), 7.35 (m, 5H), 6.88 (m, 2H), 6.22 (s, 1H); 13C NMR (400 MHz, $CDCl_3$); δ (ppm) 168.1, 164.9, 152.9, 138.6, 131.4, 128.8, 128.7, 124.5, 123.3, 120.2, 115.9, 114.8, 114.6, 103.8; Mass ESI calcd. $C_{25}H_{15}FO_6$: ([M + H] +), 430.09; found: ([M + 1] +) 430.8.

3,3′-((3-(Benzyloxy)−4-methoxyphenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3d): Light yellow solid; mp 217.8–219.0 °C; ^{1}H NMR (400 MHz, DMSO–d^6): δ (ppm) 7.83 (d, J = 7.2 Hz, 2H), 7.52 (m, 2H), 7.27 (m, 6H), 7.13 (m, 3H), 6.78 (m, 2H), 6.76 (s, 1H), 6.17 (s, 1H), 4.84 (s, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO–d^6); δ (ppm) 168.1, 165.0, 152.9, 147.7, 147.2, 137.7, 135.3, 131.2, 128.5, 128.0, 127.9, 124.5, 123.2, 120.4, 119.7, 115.8, 113.9, 112.2, 104.0, 70.7, 60.2, 56.0; Mass ESI calcd. $C_{33}H_{24}O_8$: ([M + H] +), 548.15; found: ([M + 1] +), 549.0

3,3′-((3-Chlorophenyl) methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3e): White solid; mp 249.8–251.0 °C; ^{1}H NMR (400 MHz, DMSO–d^6): δ (ppm) 7.83 (d, J = 6.8 Hz, 2H), 7.54 (m, 2H), 7.28 (m, 3H), 7.26 (m, 2H), 7.16 (s, 1H), 7.07 (m, 2H), 6.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO–d_6); δ (ppm) 167.8, 164.9, 152.9, 145.3, 133.0, 131.7, 130.1, 126.7, 126.0, 125.5, 124.5, 123.5, 119.8, 116.0, 103.4; Mass ESI calcd. $C_{25}H_{15}ClO_6$: ([M + H] +), 446.06; found: ([M + H] +), 446.8.

3,3′-((4-Nitrophenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3f): Yellow solid; mp 295–298.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, J = 9.2 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 7.49–7.48 (m, 4H), 7.23 (m, 4H), 6.43 (s,1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 164.7, 152.9, 151.9, 145.0, 131.6, 128.2, 124.6, 123.5, 123.5, 120.1, 116.0, 103.1; Mass ESI calcd. $C_{25}H_{15}NO_8$: ([M + H] +), 457.08; found: ([M-H] $^-$), 456.1.

3,3′-((3-Nitrophenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3 g): Yellow solid; mp 272–275 °C; ^{1}H NMR (400 MHz, DMSO–d^6); δ 8.46 (s, 2H), 7.76 (d, J = 6.8 Hz, 2H), 7.54–7.49 (m, 4H), 7.35 (m, 2H), 7.35 (m, 4H), 6.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO–d^6); δ 168.3, 163.9, 152.9, 150.0, 135.7, 131.8, 131.4, 130.0, 127.0, 124.5, 124.2, 123.3, 119.9, 115.9, 102.6; Mass ESI calcd. $C_{25}H_{15}NO_8$: ([M + H] +), 457.08; found: ([M + H] +), 457.9.

3,3′-((2-Hydroxyphenyl) methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3 h): White solid; mp 269–271 °C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.10 (d, J = 8.0 Hz, 2H), 7.53 (m, 3H), 7.30 (m, 4H), 7.07 (t, J = 6.8 Hz, 1H), 6.86 (t, J = 6.2 Hz, 1H), 6.77 (d, J = 6.8 Hz, 1H), 6.48 (s, 1H); ^{13}C NMR (100 MHz, DMSO–d^6); δ 164.5, 155.4, 152.7, 130.9, 129.4, 126.4, 124.3, 123.1, 120.5, 118.1, 115.7, 115.0, 110.0, 109.9, 104.2; Mass ESI calcd. $C_{25}H_{16}NO_7$: ([M + H] +), 428.09; found: ([M-H]$^-$), 427.7.

3,3′-((2-Fluoro-4-methoxyphenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one (3i): White solid; mp 248–251 °C; ^{1}H NMR (400 MHz, DMSO–d^6); δ 7.84 (d, J = 8.0 Hz, 2H), 7.53 (m, 2H), 7.29 (m, 4H), 6.99 (t, J = 17.6 Hz, 1H), 6.85 (m, 2H), 6.21 (s, 1H), 3.76 (s, 3H); δ ^{13}C NMR (100 MHz, DMSO–d6); δ 166.4, 165.0, 152.7, 145.3, 145.2, 132.1, 124.4, 123.9, 123.0, 118.9, 116.2, 114.8, 114.6, 113.8, 104.2, 56.3; Mass ESI calcd. $C_{26}H_{17}FO_7$: ([M + H] +), 460.10; found: ([M + H] +), 460.9.

3,3′-((4-(Dimethylamino)phenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3j): White solid; mp 187–194 °C; ^{1}H NMR (400 MHz, DMSO–d^6); δ 7.81 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.25 (m, 4H), 6.91 (d, J = 7.6 Hz, 2H), 6.57 (d, J = 8.0 Hz, 2H), 6.16 (s, 1H) 2.79 (s, 6H); ^{13}C NMR (100 MHz, DMSO–d6); δ 167.9, 165.0, 152.9, 131.1, 127.6, 123.2, 120.5, 115.8, 112.9, 104.2; Mass ESI calcd. $C_{27}H_{21}NO_6$: ([M + H] +), 455.14; found: ([M + H]$^+$), 456.0.

3,3′-((4-(Prop-2-ynyloxy)phenyl)methylene)bis(4–hydroxy-2*H*-chromen-2-one) (3k): White solid; mp 215–217 °C; ^{1}H NMR (400 MHz, DMSO–d^6); δ 7.80 (d, j = 1.2 Hz, 2H), 7.49 (t, j = 1.2 Hz, 2H), 7.23 (m, 5H), 7.10 (t, j = 7.2 Hz, 1H), 6.89 (d, j = 7.6 Hz, 1H), 6.81 (t, j = 7.2 Hz, 1H), 6.20 (s, 1H), 4.50 (s, 2H), 3.24 (s, 1H); ^{13}C NMR (100 MHz, DMSO–d^6); δ 167.2, 164.4, 155.7, 152.8, 131.0, 129.5, 126.7, 121.2, 120.3, 115.8, 112.7, 104.1, 79.6, 77.8, 60.2, 56.0; Mass ESI cald. $C_{28}H_{18}O_7$;:([M + H]+), 466.1; found: ([M + H] +), 467.1.

3,3′-(Phenylmethylene)bis(4–hydroxy-2*H*-chromen-2-one) (3l): White solid; mp 201.1–202.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 11.31 (s, 1H), 11.54 (s, 1H), 8.05 (dd, J = 27.92, 7.81 Hz, 2H), 7.68–7.58 (m, 2H), 7.42 (d, J = 8.14 Hz, 3H), 7.37–7.28 (m, 3H), 7.24 (m, 3H+$CDCl_3$) 6.11 (s, 1H); ^{13}C NMR (100 MHz, CDCl3); δ 165.7, 164.5, 164.5, 152.2, 135.1, 132.8, 128.6, 126.8, 126.4, 124.8, 124.3, 116.6, 105.5, 103.8, 103.8; Mass ESI calcd. $C_{25}H_{16}O_6$; ([M + H] +), 412.09; found: ([M + H] +), 412.9.

2.3. Molecular docking studies

In the present study, a Graphical User Interface AutoDockTools is used to prepare the files required for the docking of protein with ligand. The different conformations of ligand bound to the active site amino acids of GlcN-6-P synthase were analyzed [39]. The cif format of the compound is converted to 3D structure (.pdb) using Open able tool. The .pdb file is loaded on to PRODRG server [40] for energy minimization of inhibitor. The coordinate file of GlcN-6-P synthase (PDB ID: 1Jxa) is downloaded from www.rcsb.org/pdb, was edited by removing the heteroatoms, adding C-terminal oxygen [41]. For docking calculations, **Gasteiger-Marsili** empirical atomic partial charges [42] were assigned to the inhibitors and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations 10,000. The docking results for inhibitor against glucosamine-6-phosphate synthase [PDB Id: 1jxa], showed minimum docking energy, binding energy, inhibition constant, intermolecular energy with RMS as documented. The computer specification used- Operating system: Microsoft Windows XP, Processor: Intel Pentium 3.40 GHz, RAM: 2 GB, Hard disk: 500 GB, Python: 2.4.

2.4. Invitro anticancer activity

MTT assay: Cell viability was determined by (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5 × 10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 1, 10, and 100 mM concentration for 24 h in a final volume of 200 ml. After treatment, the medium was removed and 20 ml of MTT (5 mg/ml in PBS) was added to the fresh medium. After 2 h incubation at 37 °C, 100 ml of DMSO was added to each well and plates were agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader (Synergy Mx, Biotek Inc., USA). Percent inhibition of proliferation was calculated as a fraction of the control (without compound).

3. Result and discussion

Initially, the present study comprises an aim to discover an efficient heterogeneous solid catalyst for the synthesis of bis coumarins (**3**) through Domino Knoevenagel–Michael reaction. Accordingly, to prepare the compound **3a**, one pot reaction of 4–hydroxy coumarin (**1**) and 4–chloro benzaldehyde (**2a**) was carried as a probe model reaction with a series of solid catalysts like silica gel, silica sulfuric acid, Amberlyst-15 and Amberlite IR-120 resins in acetonitrile solvent at room temperature.

Table 1
Catalytic performance of solid catalysts on the synthesis of product **3a**.

Entry*	Catalyst	Time	Yield (%)**
1	Without Catalyst	24 h	Trace
2	Silica gel (1mole)	12 h	45
3	Silica sulfuric acid (1 mole)	12 h	54
4	Amberlyst-15(1mole)	12 h	76
5	Amberlite IR-120 (5 wt%)	2 h	86
6	Amberlite IR-120 (10 wt%)	1 h	90
7	Amberlite IR-120 (15 wt%)	1 h	94

* **Reaction condition**: 4-Chlorobenzaldehyde:4-hydroxycoumarin=0.5:1, catalyst, acetonitrile, rt.

Table 2
Effect of solvents in the synthesis of biscoumarins (**3a**) with Amberlite IR-120 resin catalyst.

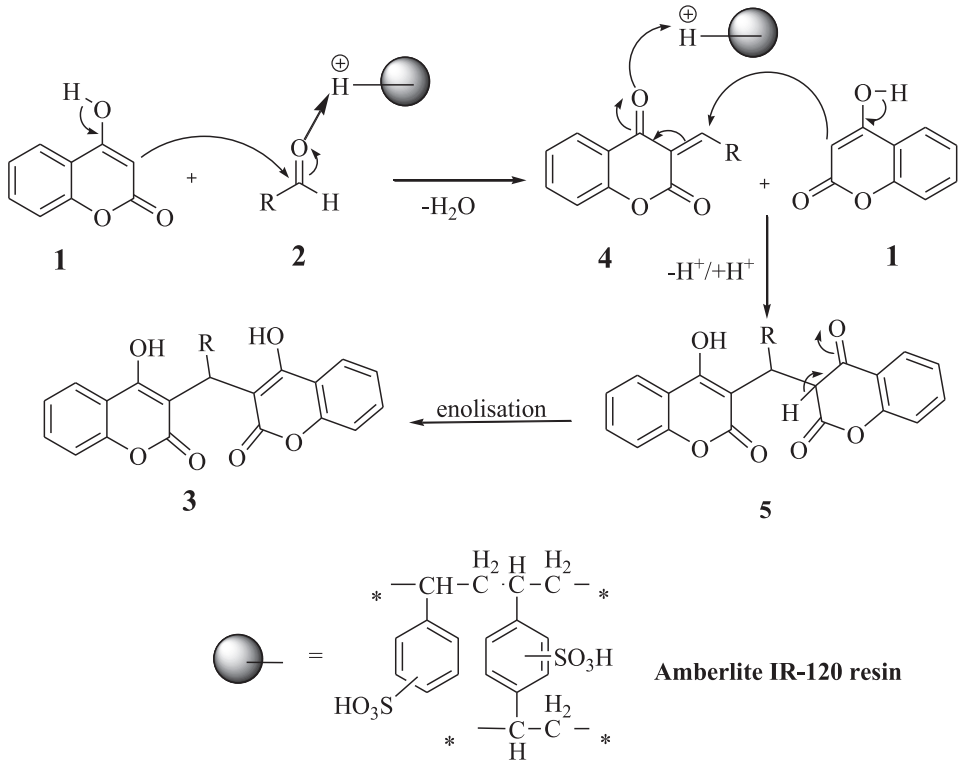
Entry*	Solvent	**Polarity Index**	Time	Yield (%)**
1	Dichloromethane	**3.4**	12 h	64
2	MTBE	**2.5**	12 h	52
3	Toluene	**2.4**	12 h	38
4	Ethanol	**5.1**	8 h	73
5	Acetonitrile	**5.8**	1 h	96
6	Water	**9.0**	6 h	80
7	Methanol	**6.6**	6 h	78

* **Reaction conditions**: 4-Chlorobenzaldehyde:4-hydroxycoumarin = 0.5:1, Amberlite IR-120 resin (15 wt%), solvents, room temperature.
** Isolated yields.

As per the results of current investigations Table 1, negligible amount of product was observed without the mentioned catalysts. Amberlyst-15, silica sulfuric acid and silica-gel have not shown the better catalytic activity as these offered a scope for longer reaction time, incomplete reaction and poorer yields. Further the study was continued with Amberlite IR-120 resin. With 15 wt% of Amberlite IR-120 resin and 1 h reaction time, 96% of the product (**3a**) was obtained, with 10 wt% of Amberlite IR-120 resin and 1 h reaction time, 90% of the product (**3a**) was obtained, and with 5 wt% of Amberlite IR-120 resin and 2 h reaction time, 86% of the product (**3a**) was obtained. So, 15 wt% of Amberlite IR-120 showed a better catalytic activity compared with the other selected acidic catalysts.

Further, the study is also focused to know a better solvent medium, for which a series of solvents like DCM, MTBE, toluene, ethanol, methanol and water were selected to run above probe model one pot Domino Knoevenagel–Michael reaction of **1** and **2a** by keeping 15 wt% of Amberlite IR-120 resin as standard. The results have been summarized in Table 2. Which suggested that, the reaction time and yield of the product obtained is also dependent of the nature of the solvent used. Here in general, the solvent with lower polarity like DCM, MTBE and toluene took longer reaction time and afforded the product (**3a**) of lower yield. Moderate yields were obtained with the polar and protic solvents like ethanol, water and methanol. The best result obtained was with acetonitrile i.e., 96% of biscoumarin (**3a**).

Trial results of the above procedures were encouraging as the biscoumarin (**3a**) obtained was 96% at room temperature. This optimized protocol has many advantages viz., highly efficient, cleanly working, isolation of product is easy and the catalyst is recyclable. Subsequently, with an optimized protocol, a series of biscoumarins (**3a-l**) were synthesized by using 4-hydroxycoumarin and a series of aldehydes (**2a-l**). The results have been summarized in Table 3. The developed procedure worked effectively with all the reactions. It was observed that the reaction is more compatible and tolerable with aromatic aldehydes, whether the nature of the substitu-



Scheme 2. Mechanism of Knoevenagel condensation of 4-hydroxycoumarin (1) with the aldehyde (2) towards biscoumarin (3).

Table 3
Synthesis of the biscoumarins (**3a-l**) by one pot condensation of 4-hydroxycoumarin and different aldehydes (**2a-l**) by using 15 wt% of Amberlite IR-120 resin.

Entry	Product	R	Time (min)	Yield (%)*
1	**3a**	4-Cl	60	96
2	**3b**	4-Br	60	95
3	**3c**	4-F	75	93
4	**3d**	5-OMe, 3-OBn	100	92
5	**3e**	3-Cl	60	95
6	**3f**	4-NO2	120	91
7	**3 g**	3-NO2	110	91
8	**3h**	2-OH	60	94
9	**3i**	2-F, 3-OMe	90	90
10	**3j**	4-N(Me)2	90	93
11	**3k**	4-O(propargyl)	110	92
12	**3l**	H	60	95

* Isolated yield.

tion was electron withdrawing or electron donating which arrive at good to excellent isolated yield at rt within 60 min to 120 min. the obtained products were recrystallized from ethanol to obtain pure biscoumarins (**3a-l**). Identification of the synthesized compounds with a new procedure was attained by means of their ^{1}H NMR, ^{13}C NMR and mass spectral data. The spectral results are summarized in the experimental section.

The proposed reaction mechanism is shown in Scheme 2. Which involves a Knoevenagel condensation of 4-hydroxycoumarin (**1**) with the aldehyde (**2**) gives **4**, which underwent Michael addition with another molecule of **1** gives **5**, and which upon enolization gives **3**.

Recyclability of the Amberlite IR-120 resin catalyst was also very important part of this study and was examined cautiously. So, the catalyst used was recovered from the reaction of 4-hydroxycoumarin (**1**) and 4-chlorobenzaldehyde (**2a**) via filtration. The filtered triethylammonium salt of resin from the reaction mixture was stirred with sufficient quantity of 0.1 N HCl for about

Table 4
Recyclability of Amberlite IR-120 resin.

Cycle	Isolated Amberlite IR-120 (mg)	Isolated Yield (%)
1	15	96
2	14	94
3	13	92
4	12	90

15 min to regenerate free -SO_3H group, filtered, washed with distilled water then with acetone and dried and that resin was reused for next successive reactions. This way it was recycled for four different model reactions. The results are summarized in Table 4. In the same way, one 15 wt% of Amberlite IR-120 resin was used for the synthesis of succeeding four different bis coumarins (**3**) by using a respective aromatic aldehyde (**2**). Hence, it is determined that, the process of synthesis of several biscoumarins (**3**) was succeeded efficiently with the recycling of Amberlite IR-120 resin without the significant loss of catalytic activity. During the successive reactions, the observed weight of the isolated Amberlite IR-120 resin was declined by 1 mg (15 mg, 14 mg, 13 mg and 12 mg). And accordingly, the observed yield of the isolated product was declined slightly by 2%, i.e., from 96%, 94%, 92% and 90%.

3.1. Biological activity

3.1.1. Molecular docking studies

The newer targets for better antimicrobials acting through novel mechanisms are the important developments of research in recent years. One such enzyme present in microbial cells is Glucosamine-6-Phosphate synthase. The dormant of G-6-P synthase may give out as a novel approach to find improved antimicrobials. The molecular docking studies of biscoumarins derivatives and the standard drug chloroquine with GlcN-6-P synthase yielded all potential conformations with the binding energy, docking energy, in-

Table 5
Molecular docking results of biscoumarins (**3a-l**) with glucosamine-6-phosphatesynthase protein receptor.

Ligands	Binding Energy (kcal/mol)	Docking Energy(kcal/mol)	Inhibition Constant(μM)	Electrostatic Energy (kcal/Mol)
3a	−6.80	−7.14	1.03×10^{-5}	−7.12
3b	−7.58	−7.97	2.78×10^{-6}	−7.89
3c	**−15.83**	**−16.19**	**2.5×10^{-12}**	**−16.14**
3d	**−15.93**	**−16.18**	**2.1×10^{-12}**	**−16.24**
3e	**−18.37**	**−18.73**	**3.4×10^{-14}**	**−18.68**
3f	−7.92	−8.32	1.56×10^{-6}	−8.23
3 g	−7.38	−7.69	3.9×10^{-6}	−7.69
3h	−7.99	−8.79	1.39×10^{-6}	−8.93
3i	−8.94	−9.73	2.79×10^{-7}	−9.87
3j	**−17.05**	**−17.88**	**3.17×10^{-13}**	**−17.98**
3k	−8.92	−9.12	1.58×10^{-6}	−9.30
3l	−7.92	−8.39	1.86×10^{-6}	−8.11
Chloroquine	−6.79	−9.42	1.05×10^{-5}	−9.28

Table 5a
The% inhibition of growth of cancer cell lines by compounds **3***.

Compounds	K-562 (leukemia)			MDA-MB-231 (breast)		
	100 M	10 mM	1mM	100 mM	10 mM	1 mM
3b	37.6	36.2	30.9	56.5	54.8	45.8
3c	33.4	32.6	28.9	67.8	57.5	43.7
3d	55.6	50.9	46.8	65.9	61.3	48.8
3f	46.9	45.7	44.6	54.8	50.8	47.8
3i	42.5	37.8	33.6	45.6	43.6	39.8
3k	43.8	34.5	32.1	51.3	45.6	41.7

*Data presented are the average of three experiments.

hibition constant and electrostatic energy (Table 5). The entire set of molecules docked against the enzyme showed significant docking energy ranging from −7.14 kcal/mol to −18.73 kcal/mol with an estimated inhibition constant of 1.03×10^{-5} to 3.4×10^{-14} respectivley. Whereas, docked energy of the standard drug chloroquine was 9.42 kcal/mol with an inhibition constant of 1.05×10^{-5}. The molecules **3c, 3d, 3e** and **3j** showed significant inhibition against all compounds docked. The compound **3e** showed minimum docking energy −18.73 kcal/mol with an inhibition constant of 3.4×10^{-14}. Theoretically, the activity may be due to inhibition of enzyme GlcN-6-Psynthase, which catalyzes a complex reaction involving ammonia transfer from L-glutamine to Fru-6-P followed by isomerization of the formed fructosamine-6-phosphate to glucosamine-6-phosphate. In the present study, the derivatives with chlorine as a halogen have not formed any hydrogen bond with an active site of amino acids of GlcN-6-P synthase, but showed excellent docking energy, since the halogens are endowed with an ability to establish intermolecular bonds in a fashion that resembles the H-bonds. But the compounds containing halogens will improve oral absorption, skin penetration and membrane permeability [43]. It is generally accepted that halogen atoms are not capable of significant hydrogen bonding.

3.1.2. Invitro *anticancer activity*

Some of the compounds (**3**) synthesized [44] were tested for their *invitro* anti-proliferative properties against leukemia (K-562) and breast (MD-AMB-231) cancer cell lines in a MTT assay. Harmine, a member of β-carboline family of compounds showed cytotoxicity against HL60 and K562 cell lines [45] was used as a reference compound in this assay. The results of active molecules identified by this assay are presented in Table 5. While **3i** and **3k** showed good activity against leukemia cells (Table 5a), most of the compounds, for example, **3b, 3c, 3d, 3f, 3i** and **3k** were found to be effective against breast cancer and **3i** being the best among them (Table 5). The compound **3i** showed significant anti-proliferative properties at all the concentrations tested and maintained the same at low concentrations. Notably, IC_{50} value of Harmine was found to be 46 and 52 mM when tested against K-562 and MDAMB231 cell lines in our MTT assay. The compound **3i** therefore appeared to be a promising and potential anticancer agent of further interest.

4. Conclusions

In conclusion, the present method demonstrates an operationally simple and clean procedure for the synthesis of biscoumarins using a catalytic amount of Amberlite IR-120 resin. Moreover, the catalyst used is of low cost, recyclable, less toxic and moisture compatible. The yields of products (**3**) were excellent within short reaction time. Therefore, this methodology is one of the valid contributions to the field of biscoumarin synthesis.

Most of the synthesized biscoumarins (**3**) have displayed potent biological activities. The data of molecular docking studies clearly showed that, most of these docked with the enzyme GlcN-6-P synthase with significant docking energy. The IC_{50} value of the compounds **3b, 3c, 3d, 3f, 3i** and **3k** shows that, these have effective anti-proliferative properties against leukemia (K-562) and breast (MD-AMB-231) cancer cell lines.

Declaration of Competing Interest

"Recyclable Amberlite IR-120 Catalyzed domino reaction: Synthesis, anticancer activity and molecular docking studies of biscoumarins" declare that, we don't have any conflict of interest among the authors/corresponding author about the manuscript submission to the Journal of Molecular Structure"

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131093.

References

[1] S. Milewski, Glucosamine-6-phosphate synthase-the multi-facets enzyme, Biochim. Biophys. Acta 1597 (2002) 173–192.
[2] R.O. Kennedy, R.D. Thornes, Coumarins: Biology, Applications and Mode of Action, Wiley: Chichester, U.K, 1997.
[3] W.L. Stanley, L. Jurd, Citrus Coumarins, Agric. Food Chem. 19 (1971) 1106–1110.
[4] C.A. Kontogiorgis, D.J. Hadjipavlou-Litina, Synthesis and antiinflammatory activity of coumarin derivatives, J. Med. Chem. 48 (2005) 6400–6408.
[5] G. Melagraki, A. Afantitis, O.I. Markopoulou, A. Detsi, M. Koufaki, C. Kontogiorgis, D.J. Hadjipavlou-Litina, Synthesis and evaluation of the antioxidant and anti-inflammatory activity of novel coumarin-3-aminoamides and their alpha-lipoic acid adducts, Eur. J. Med. Chem. 44 (2009) 3020–3026.

[6] M.A. Gouda, M.A. Berghot, E.A. Baz, W.S. Hamama, Synthesis, antitumor and antioxidant evaluation of some new thiazole and thiophene derivatives incorporated coumarin moiety, Med. Chem. Res. 21 (2012) 1062–1070.

[7] L. Raev, E. Voinova, I. Ivanov, D. Popov, Antitumor activity of some coumarin derivatives, Pharmazie 45 (1990) 696–702.

[8] S. Khode, V. Maddi, P. Aragade, M. Palkar, P.K. Ronad, S. Mamledesai, A. Thippeswamy, D. Satyanarayana, Synthesis and pharmacological evaluation of a novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents, Eur. J. Med. Chem. 44 (2009) 1682–1688.

[9] C. Zwergel, S. Valente, A. Salvato, Z. Xu, O. Talhi, A. Mai, A. Silva, L. Altucci, G. Kirsch, Novel benzofuran–chromone and –coumarin derivatives: synthesis and biological activity in K562 human leukemia cells, Med. Chem. Commun. 4 (2013) 1571–1578.

[10] F. Belluti, G. Fontana, L.D. Bo, N. Carenini, C. Giommarelli, F. Zunino, Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: identification of novel proapoptotic agents, Bioorg. Med. Chem. 18 (2010) 3543–3550.

[11] D.M. Manidhar, R.K. Kesharwani, N.B. Reddy, C.S. Reddy, K. Misra, Designing, synthesis, and characterization of some novel coumarin derivatives as probable anticancer drugs, Med. Chem. Res. 22 (2013) 4146–4157.

[12] C.A. Kontogiorgis, D.J. Hadjipavlou-Litina, Synthesis and antiinflammatory activity of coumarin derivatives, J. Med. Chem. 48 (2005) 6400–6408.

[13] N. Hamdi, M. Saoud, A. Romerosa, 4-Hydroxy coumarine: a versatile reagent for the synthesis of heterocyclic and vanillin ether coumarins with biological activities, Top. Heterocycl. Chem. 11 (2007) 283–301.

[14] K.M. Khan, Z.S. Saify, M.Z. Khan, Zia-Ullah, M.I. Choudhary, A.U. Rahman, S. Parveen, Z.H. Chohan, C.T. Supuran, Synthesis of coumarin derivatives with cytotoxic, antibacterial and antifungal activity, J. Enzyme Inhib. Med. Chem. 9 (2004) 373–379.

[15] D. Bhavsar, J. Trivedi, S. Parekh, M. Savant, S. Thakrar, A. Bavishi, A. Radadiya, H. Vala, J. Lunagariya, M. Parmar, Synthesis and in vitro anti-HIV activity of N-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2H-chromen-4-yl)acetamide derivatives using MTT method, Bioorg. Med. Chem. Lett. 21 (2011) 3443–3446.

[16] C. Spino, M. Dodier, S. Sotheeswaran, Anti-HIV coumarins from Calophyllum seed oil, Bioorg. Med. Chem. Lett 8 (24) (1998) 3475–3478.

[17] A. Dolmella, S. Gatto, E. Girardi, G. Bandoli, X-ray structures of the anticoagulants coumatetralyl and chlorophacinone. Theoretical calculations and SAR investigations on thirteen anticoagulant rodenticides, J. Mol. Struct. 513 (1999) 177–199.

[18] F. Rosskopf, J. Kraus, G. Franz, Immunological and antitumor effects of coumarin and some derivatives, Die Pharmazie 47 (2) (1992) 139–142.

[19] P. Sengupta, M. Sen, P. Karuri, Structure of gerberinol novel dimethyldicoumarol from Gerbera lanuginose benth, J. Indian Chem.Soc. 62 (1985) 916–919.

[20] K.P. Link, The anticoagulant 3,3-methyl-bis-4-hydroxycoumarin, Fed. Proc 4 (1945) 176–182.

[21] M. Gersch, J. Kreuzer, S.A. Sieber, Electrophilic natural products and their biological targets, Nat. Prod. Rep. 29 (2012) 659–682.

[22] K. Petnapapun, W. Chavasiri, P. Sompornpisut, Structure-activity relationships of 3,3′-Phenylmethylene-bis-4-hydroxycoumarins: selective and potent inhibitors of gram-positive bacteria, The Sci. World J. 2 (2013) 178649.

[23] [a] N.H. Kim, S.N. Kim, J.S. Oh, Y.K.Kim S.Lee, Anti-mitotic potential of 7-diethylamino-3(2′-benzoxazolyl)-coumarin in 5-fluorouracil-resistant human gastric cancer cell line SNU620/5-FU, Biochem. Biophys. Res. Commun. 418 (2012) 616–621; [b] S.N. Kim, N.H. Kim, Y.S. Park, H. Kim, S. Lee, Q. Wang, Y.K. Kim, 7-Diethylamino-3(2′-benzoxazolyl)-coumarin is a novel microtubule inhibitor with antimitotic activity in multidrug resistant cancer cells, Biochem. Pharmacol 77 (2009) 1773–1779.

[24] N. Au, A.E. Rettie, Pharmacogenomics of 4-hydroxycoumarin anticoagulants drug, Metab. Rev. 40 (2008) 355–375.

[25] D.K. Mayank, N. Kaur, R. Giri, N. Singh, A biscoumarin scaffold as an efficient anti-Zika virus lead with NS3-helicase inhibitory potential: in vitro and *in silico* investigations, New J. Chem. 44 (2020) 1872–1880.

[26] [a] J.J. Xin, J. Li, Z. Zhang, X. Hu, M. Li, Biscoumarin derivatives: synthesis, crystal structure, theoretical studies and induced apoptosis activity on bladder urothelial cancer cell, J. Mole. Str. 1084 (2015) 200–206; [b] Fen Li, Chang-wei Lv, Zi-dan Zhang, Jing Li, Zheng Hou, Xiao-hui Yang, Jiang-tao Li, Xiao-xing Luo, Ming-kai Li, Crystal structure and activities of three biscoumarin derivatives against *Staphylococcus aureus*, J. Mole. Stru 1097 (2015) 117–123.

[27] S. Kolita, L. Dutta, P. Das, P.J. Bhuyan, One-pot synthesis of unsymmetrical Bis(4-Hydroxycoumarin-3-yl)methanes, Synlett 28 (2017) 2291–2294.

[28] F.A. El-Samahy, H.A. Abd El Salam, N.F. El-Sayed, E.M. Shalaby, M.F. Dondeti, Synthesis of unexpected novel bis-coumarin derivatives via three component reactions of 4-hydroxycoumarin, aldehydes and cyclic secondary amines. Conformation in the solid state and pharmacological evaluation, Naturforsch 72 (10) (2017) 705–716 b.

[29] J.M. Khurana, S. Kumar, Tetrabutylammonium bromide (TBAB): a neutral and efficient catalyst for the synthesis of biscoumarin and 3,4-dihydropyrano[*c*]chromene derivatives in water and solvent-free conditions, Tetrahedron Lett. 50 (2009) 4125–4127.

[30] M. Kidwai, V. Bansal, P. Mothsra, S. Saxena, R.K. Somvanshi, S. Day, T.P. Singh, Molecular iodine: a versatile catalyst for the synthesis of bis(4-hydroxycoumarin) methanes in water, J. Mol. Catal. A: Chem 268 (2007) 76–81.

[31] J.M. Khurana, S. Kumar, Ionic liquid: an efficient and recyclable medium for the synthesis of octahydroquinazolinone and biscoumarin derivatives, Monatsh. Chem. 141 (5) (2010) 561–564.

[32] [a] A. Tzani, A. Douka, A. Papadopoulos, E.A. Pavlatou, E. Voutsas, A. Detsi, Synthesis of biscoumarins using recyclable and biodegradable task-specific ionic liquids, ACS Sustain. Chem. Eng. 1 (2013) 1180–1185; [b] W. Li, Y. Wang, Z. Wang, L. Dai, Y. Wang, Novel SO_3H-functionalized ionic liquids based on benzimidazolium cation: efficient and recyclable catalysts for one-pot synthesis of biscoumarin derivatives, Catal. Lett. 141 (2011) 1651–1658.

[33] R. Karimian, F. Piri, A.A. Safari, S.J. Davarpanah, One-pot and chemoselective synthesis of bis(4-hydroxycoumarin) derivatives catalyzed by nano silica chloride, J. Nanostruct. Chem 3 (2013) 52–57.

[34] B. Pawar, V. Shinde, A. Chaskar, n-Dodecylbenzene Sulfonic Acid (DBSA) as a novel brønsted acid catalyst for the synthesis of Bis(indolyl)methanes and Bis(4-hydroxycoumarin-3-yl)methanes in water, Green Sustain. Chem 3 (2013) 56–60.

[35] Z.N. Siddiqui, T.N. Mohammed Musthafa, S. Praveen, F. Farooq, $Zn(Proline)_2$-catalyzed Knoevenagel condensation under solvent-free/aqueous conditions and biological evaluation of products, Med. Chem. Res. 20 (2011) 1438–1444.

[36] K. Tabatabaeian, H. Heidari, A. Khorshidi, M. Mamaghani, N.O. Mahmoodi, Synthesis of biscoumarin derivatives by the reaction of aldehydes and 4-hydroxycoumarin using ruthenium (III) chloride hydrate as a versatile homogeneous catalyst, J. Serb. Chem. Soc. 77 (4) (2012) 407–413.

[37] H. Mehrabi, H. Abusaidi, Synthesis of biscoumarin and 3,4-dihydropyrano[c]chromene derivatives catalysed by sodium dodecyl sulfate (SDS) in neat water, J. Iran. Chem. Soc. 7 (2010) 890-594.

[38] [a] N. Tewari, D. Katiyar, V.K. Tiwari, R.P. Tripathi, Amberlite IR-120 catalysed efficient synthesis of glycosyl enamines and their application, Tetrahedron Lett. 44 (2003) 6639–6642; [b] K. Akagawa, S. Sakamoto, K. Kudo, Resin-supported acid- and base-catalyzed one-pot sequential reaction including an enantioselective step, Tetrahedron Lett. 48 (2007) 985–987.

[39] N. Mulakayala, K.M.K. Ismail, R.K. Rapolu, B. Kandagatla, P. Rao, S. Oruganti, M. Pal, Catalysis by Amberlite IR-120 resin: a rapid and green method for the synthesis of phenols from arylboronic acids under metal, ligand, and base-free conditions, Tetrahedron Lett. 53 (2012) 6004–6007.

[40] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Computational Chem. 16 (2009) 2785–2791.

[41] A.K. Ghose, G.M. Crippen, Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions, J. Chem. Inf. Comput. Sci. 27 (1987) 21–35.

[42] T.A. Binkowski, S. Naghibzadeg, J. Liang, Computed atlas of surface topography of proteins, Nucleic Acids Res. 319 (2003) 3352–3355.

[43] J. Gasteiger, M. Marsili, Iterative partial equalization of orbital electronegativity, Tetrahedron 36 (1980) 3219–3228.

[44] For identification of novel anticancer agents, see:

[a] N. Mulakayala, B. Kandagatla, Ismail, R.K. Rapolu, P. Rao, C. Mulakayala, C.S. Kumar, J. Iqbal, S. Oruganti, $InCl_3$-catalysed synthesis of 2-aryl quinazolin-4(3*H*)-ones and 5-aryl pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones and their evaluation as potential anticancer agents, Bioorg. Med. Chem. Lett. 22 (2012) 5063–5066.

[b] N. Mulakayala, D. Rambabu, M.R. Raja, M. Chaitanya, C.S. Kumar, A.M. Kalle, G.R. Krishna, C.M. Reddy, M.V.B. Rao, M. Pal, Ultrasound mediated catalyst free synthesis of 6H-1-benzopyrano[4,3-b]quinolin-6-ones leading to novel quinoline derivatives: their evaluation as potential anti-cancer agents, Bioorg. Med. Chem. 20 (2012) 759–768.

[c] R.M. Rao, C.H.U. Reddy, A. Nakhi, N. Mulakayala, M. Alvala, M.K. Arunasree, R.R. Poondra, J. Iqbal, M. Pal, Sequential coupling/desilylation–coupling/cyclization in a single pot under Pd/C–Cu catalysis: synthesis of 2-(hetero)aryl indoles, Org. Biomol. Chem. 9 (2011) 3808–3816.

[45] F. Jahaniani, S.A. Ebrahimi, N. Rahbar-Roshandel, M. Mahmoudian, Xanthomicrol is the main cytotoxic component of Dracocephalum kotschyii and a potential anti-cancer agent, Phytochemistry 66 (2005) 1581–1592.