Triethyl ammonium sulphate catalyst one pot, Solvent free synthesis of novel Coumarin derivatives as antimicrobial agents.

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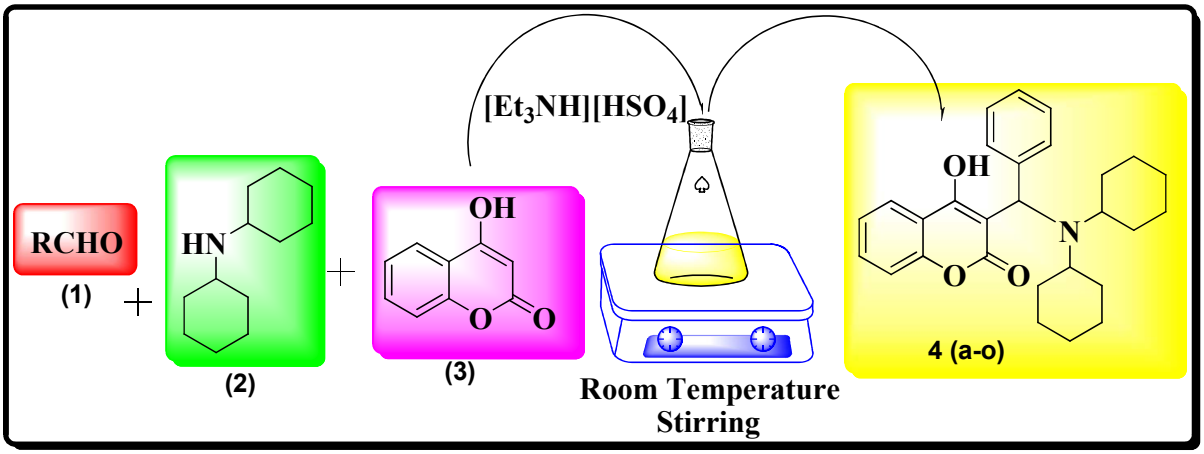
GRAPHICAL ABSTRACT



ABSTRACT

The work reports synthesis of 15 novel 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives **4 (a-o)** as potential antimicrobial agents in solvent-free condition using Triethyl ammonium sulphate $[Et_3NH][HSO_4]$ as an efficient, eco-friendly and reusable catalyst. Compared to other methods, this new method consistently has advantages, including excellent yields, a short reaction time, mild reaction conditions and catalyst reusability. The heterocyclic compound Coumarin, is associated with diverse biological activities of immense importance. Due to the presence of coumarin moiety in various pharmaceutically active compounds, we planned the green synthesis of a series of 15 novel compounds containing coumarin moiety coupled with dicyclohexyl rings by an eco-friendly ionic-liquid mediated protocol at room temperature by stirring. The structures of the synthesized compounds were confirmed by spectral characterization such as IR, 1H NMR, 13CNMR and Mass spectral studies. All the synthesized compounds **4 (a-o)** were evaluated for anti-fungal and antibacterial activities and have exhibited promising antimicrobial activity.

Keywords: Triethyl ammonium sulphate, Coumarin, anti-fungal activity, antibacterial activity.

1. INTRODUCTION

Many drug-resistant human pathogenic microbes have been observed in the past few decades [1] and it is serious public health problem in a wide range of infectious disease [2,3]. These resistant pathogenic microbes' strains cause failure in antimicrobial treatment and enhance the mortality risks, and sometimes contribute to complications. To overcome this problem the best way is the development of new bioactive compounds effective against resistant strains is highly needed. In spite of a large number of antibiotics and chemotherapeutics available for medical use, antimicrobial resistance created substantial medical need for new classes of antimicrobial agents. Design and synthesis of newer antimicrobials will always remain an area of immense significance [4-5]. The novel and potent antimicrobial agents can be obtained by modifying the structure of a well known antimicrobial agent or the second strategy is to combine together two or more different antimicrobial pharmacophores in one molecule.

Coumarin derivatives are an important class of natural, synthetic compounds and pharmacologically active substances displaying a broad range of biological activities including cytotoxicity [6], antioxidant [7], antiplasmodial [8], antimalarial [9], antirhinovirus [10], antifungal [11] and antibacterial [12].

The Mannich reaction is one of the most important carbon-carbon bond forming reactions in organic synthesis because of its atom economy and potential application in the synthesis of biologically active molecules. In this reaction, an amine, two carbonyl compounds, and acid (or base) catalysts are used to produce β-amino carbonyl compounds, which constitute various pharmaceuticals, natural products and versatile synthetic intermediates [13,14]. Conventional catalyst of the classic Mannich reaction involves inorganic and organic acids like HCl [15], proline [16], p-dodecybenzenesulfonic acid [17]. Reaction using these catalysts, however, often suffers drawbacks including long reaction times, harsh reaction condition, and difficult product separation.

Considering the focus on green synthesis in recent years, ionic liquid have attracted attention many of researchers. Ionic liquid have been referred as "designer solvents/ green solvents" because their physical and chemical properties can be adjusted by varying the cation and anion. Mannich reaction have been performed using various ionic liquid such as [BMIM][PF_6] [18], [emim][OTf] [19], [CMMIM][BF_4] [20], [Hmim][PF_6] [21] and some other bronsted ionic

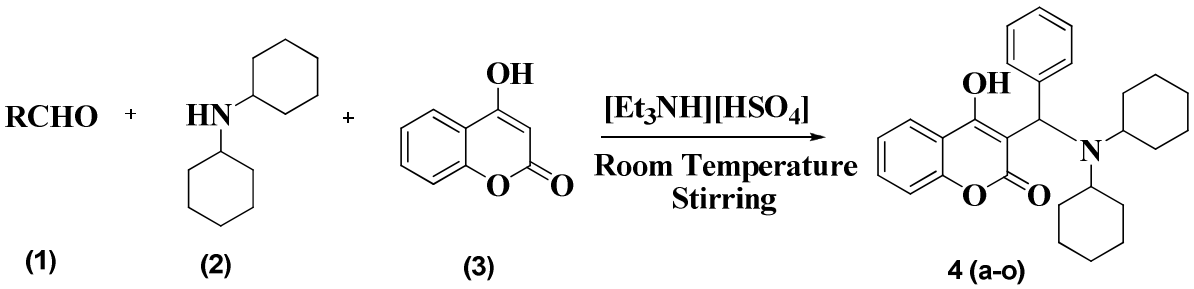
liquids (22-23). Although extensive work has been done in this area, the disadvantage of the above mentioned catalytic systems, are large amount of catalyst required, the necessity of an organic co-solvent, cost, the ionic liquids contain halogen, which in some ways, limits their "greenness". Thus synthesizing halogen free, water soluble, economic, reusable and easy to prepare ionic liquid was the main aim of our research team. Taking in consideration the above mentioned points we have carried out the synthesis of coumarin-dicyclohexyl coupled hybrid derivatives **4(a-o)** using $[Et_3NH][HSO_4]$ as an solvent and easily recoverable green catalyst (Scheme 1).

All the synthesized compounds **4(a-o)** were screened for *in vitro* antifungal and antibacterial activity. Minimum inhibitory concentration (MIC) values were determined using the standard agar method as per CLSI guidelines [24-27].

2. RESULTS AND DISCUSSION

2.1 Chemistry:

Herein we report the one-pot synthesis of 15 novel 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives **4 (a-o)** from three component reactions of an suitable aldehydes **(1),** dicyclohexylamine **(2)** and 4-hydroxy coumarin **(3)** in presence of $[Et_3NH][HSO_4]$ as an solvent and catalyst as shown in Scheme 1.



Scheme 1. One-Pot, three component synthesis of novel 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives **4 (a-o)**

In search of an efficient catalyst and the best experimental reaction conditions, the reaction of benzaldehyde **(1a),** dicyclohexylamine **(2)** and 4-hydroxy coumarin **(3)** at room temperature was considered as a standard model reaction to obtain **4a**.

Initially, the reaction was carried out in the absence of the catalyst; the product formed in a trace/negligible amount Table 1, entry 1. To determine the appropriate concentration of the catalyst $[Et_3NH][HSO_4]$, we investigated the model reaction at different concentrations of

$[Et_3NH][HSO_4]$, such as 5, 10, 15, 20 and 25 mol%. The product **4a** formed in 72, 85, 90, 92 and 92 % yields, respectively.

Table 1 Effect of $[Et_3NH][HSO_4]$ catalyst concentration on model reaction **4a**

Entry	$[Et_3NH][HSO_4]$ mol%	Time (min)	Yield (%)
1.	No catalyst	90	Trace
2.	5	85	72
3.	10	60	85
4.	15	50	90
5.	20	30	92
6.	25	30	92

The increase in concentration of catalyst from 20 to 25 mol% does not increase the yield of product. This indicates that 20 mol% of $[Et_3NH][HSO_4]$ is sufficient for the reaction by considering the product yield.

The re-usability of the ionic liquid $[Et_3NH][HSO_4]$ was also studied and the results obtained are as shown in Table 3. After the completion of the reaction, the reaction mixture was quenched with ice crystals and filtered recrystallized using ethanol. The residual ionic liquid was washed with diethyl ether, dried under vacuum at 60 ^{0}C and reused for subsequent reactions. The recovered ionic liquid could be used for five times without obvious loss of catalytic activity.

Table 3 Reusability of $[Et_3NH][HSO_4]$ catalyst for model reaction **4a**

Entry	Run	Time	Yield
1.	1	30	92
2.	2	30	92
3.	3	30	90
4.	4	30	88
5.	5	30	88

With these optimized reaction conditions for the model reaction **4a**, i.e. 20 mol% $[Et_3NH][HSO_4]$ catalyst, room temperature and solvent-free conditions, we have synthesized 15 novel 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives **4 (a-o).** The physical characterization data of the synthesized compounds **4 (a-o)** are as shown in Table 4 All the synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and IR.

Table 4 Physical characterization of synthesized compounds **4 (a-o).**

Compound	R	Molecular weight	Molecular formula	Melting point ^{0}C	Yield %
4a	Phenyl	431.25	$C_{28}H_{33}NO_3$	112-114	92
4b	4-chlorophenyl	465.21	$C_{28}H_{32}ClNO_3$	120-122	95
4c	2,6-dichlorophenyl	500.17	$C_{28}H_{31}Cl_2NO_3$	126-128	90
4d	4-flurophenyl	449.56	$C_{28}H_{32}FNO_3$	122-124	92
4e	2,4-diflurophenyl	467.55	$C_{28}H_{31}F_2NO_3$	122-124	89
4f	4-methoxyphenyl	461.59	$C_{29}H_{35}NO_4$	133-135	86
4g	3,4 dimethoxyphenyl	491.62	$C_{30}H_{37}NO_5$	138-140	84
4h	3,4,5 trimethoxyphenyl	521.64	$C_{31}H_{39}NO_6$	136-138	82
4i	4-hydroxyphenyl	447.57	$C_{28}H_{33}NO_4$	128-130	90
4j	2-hydroxyphenyl	447.57	$C_{28}H_{33}NO_4$	130-132	88
4k	4-hydroxy-3-methoxyphenyl	477.59	$C_{29}H_{35}NO_5$	144-146	86
4l	4-hydroxy-3-ethoxyphenyl	491.27	$C_{30}H_{37}NO_5$	140-142	86
4m	Pyridine-2-yl	432.55	$C_{27}H_{32}N_2O_3$	148-150	84
4n	Thiophene-2-yl	437.59	$C_{26}H_{31}NO_3S$	140-142	88
4o	Furan-2-yl	421.53	$C_{26}H_{31}NO_4$	148-150	86

2.2 *In-vitro* antifungal activity

The newly synthesized compounds **4(a–o)** were screened for *in vitro* antifungal activity against different yeast and filamentous fungal pathogens. All the compounds have shown good to moderate antifungal activity as shown in Table 5. The compound **4b, 4c, 4d** and **4e** having electron withdrawing groups exhibited good antifungal activity against these three fungal strains *Aspergillus fumigates* (NCIM 902), *Aspergillus flavus* (NCIM539) and *Aspergillus niger* (NCIM1196). The compound **4l** bearing 4-hydroxy-3-ethoxy was found to be the most active compound among the synthesized series having MIC values 25 µg/ml for *C. albicans*, 28 µg/ml for *C. glabrata*, 28 µg/ml for *F. oxysporum*, 36 µg/ml for *Asp. fumigates*, 15 µg/ml for *Asp. flavus*, 12 µg/ml for *Asp. niger*, 12 µg/ml for *Crypt. Neoformans***.**

Table 5. *In-vitro* antifungal activity of synthesized compounds **4 (a-o)**

Compound	MIC[a] µg/ml						
	Candida albicans	***Candida glabrata***	***Fusarium oxysporum***	***Aspergillus fumigates***	***Aspergillus flavus***	***Aspergillus niger***	***Cryptococcus neoformans***
4a	66	58	55	84	38	43	54
4b	30	32	34	30	**14**	**15**	**15**
4c	30	28	35	28	**15**	**18**	**14**
4d	28	30	30	28	**15**	**20**	**18**
4e	28	26	30	28	**12**	**15**	**14**
4f	43	57	39	44	20	22	20
4g	50	57	35	52	24	20	26
4h	48	64	45	50	38	34	34
4i	32	35	35	42	25	28	24
4j	46	47	38	55	32	30	35
4k	**25**	**30**	**28**	**38**	**12**	**15**	**15**
4l	**25**	**28**	**28**	**36**	**15**	**12**	**12**
4m	48	46	40	45	25	22	28
4n	55	53	58	67	32	38	33
4o	56	55	55	65	46	49	48
Miconazole	25	25	25	35	12	12	12

[a]Values are the average of three readings

2.2 *In-vitro* antibacterial activity

The newly synthesized compounds **4(a–o)** were screened for *in vitro* antibacterial activity against different bacterial strains. All the compounds have shown good to moderate antibacterial activity as shown in Table 6 The compound **4b** bearing 2,4 difluro was found to be the most active compound among the synthesized series having MIC values 48 µg/ml for *E. coli*, 50 µg/ml for *B. subtilis* and 52 µg/ml for *S. aureus*.

Table 6: *In-vitro* Antibacterial activity of the synthesized compounds **4 (a-0)**.

Compounds	**MIC[a]µg/ml**		
	E. coli	***B. subtilus***	***S. aureus***
5a	70	68	65
5b	**52**	**50**	**54**
5c	**50**	**52**	**52**
5d	**50**	**49**	**50**
5e	**48**	**50**	**52**
5f	64	58	55
5g	62	60	62
5h	68	68	66
5i	64	66	67
5j	68	72	72
5k	55	55	54
5l	56	54	54
5m	68	74	78
5n	65	74	72
5o	66	74	70
Ampicillin	50	50	50

[a]Values are the average of three readings

3. MATERIALS AND METHODS

3.1. General Information

All the reactions were performed in oven-dried glass-wares. All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled unless otherwise noted. The purity of the synthesized compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck, Darmstadt, Germany) coated aluminum plates, visualized by iodine vapor and melting points were determined in open capillary tubes. The FTIR spectra were obtained using Jasco FTIR-4000 and peaks were expressed in terms of wave number (cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra of synthesized compounds were recorded on Bruker Avance II 400 NMR Spectrometer at 400 MHz Frequency in $CDCl_3$ and using TMS as internal standard (chemical shift δ in ppm), Mass spectra were scanned on Water's Micromass Q-Tof system Elemental analyses (C, H, and N) were done with a FLASHEA 112 Shimadzu' analyzer

(Mumbai, Maharashtra, India) and all analyses were consistent (within 0.4%) with theoretical values.

3.2 Synthesis of 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives 4 (a-o)

A 25 mL a beaker was charged with a mixture of a suitable aldehyde (1.25mmol), dicyclohexyamine (1.25mmol), 4-hydroxy coumarin (1.25mmol), and 20 mol % of $[Et_3NH][HSO_4]$ as catalyst and the reaction mixture was stirred at room temperature. After completion of the reaction (monitored by TLC), the mixture was poured into ice cold water. The product obtained, was filtered and dried. The corresponding product was obtained in high purity after recrystallization of the crude product from ethanol. The authenticity of compounds was established by ^{1}H-NMR, ^{13}C-NMR, IR and Mass spectra.

3-((dicyclohexylamino)(phenyl)methyl)-4-hydroxy-2H-chromen-2-one **4a**

Yield 92%; M. P.: 112-114 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3160.41(CH stretching of aromatic), 1708.62 (C-O Stretch), 1646.91 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.11-1.58 (m, 20 H, cyclohexyl ring), 2.57 (m, 2H, C-N), 4.60 (s, 1H, CH), 7.22-7.35 (m, 5H, aromatic ring), 7.44-7.87 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.49 (CH_2), 25.79 (CH_2), 32.33 (CH_2), 33.11 (CH_2), 67.89 (CH_2-N), 77.89 (CH-N), 93.34 (C), 117.72 (C), 118.00 (CH), 123.45 (CH), 125.43 (CH), 127.23 (CH), 128.12 (CH), 128.99 (CH), 129.56 (CH), 137.23 (C), 158.99 (C), 161.89 (C-OH), 162.35 (C=O); m/z: 431.25 (100.0%), 432.25 (30.8%), 433.25 (5.2%); Molecular formula: $C_{28}H_{33}NO_3$; Elemental Analysis: Calculated (C, H, N, O): 77.93, 7.71, 3.25, 11.12, Found: 77.95, 7.70, 3.22, 11.15.

3-((4-chlorophenyl)(dicyclohexylamino)methyl)-4-hydroxy-2H-chromen-2-one **4b**

Yield 95%; M. P.: 120-122 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3162.41(CH stretching of aromatic), 1700.62 (C-O Stretch), 1646.77 (C=O Stretch), 740.55 (C-Cl of aromatic ring); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.11-1.58 (m, 20 H, cyclohexyl ring), 2.55 (m, 2H, C-N), 4.65 (s, 1H, CH), 7.32-7.39 (m, 4H, aromatic ring), 7.43-7.87 (m, 4H, coumarin ring), 15.74 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.55 (CH_2), 25.79 (CH_2), 32.33 (CH_2), 33.15 (CH_2), 67.80 (CH_2-N), 77.88 (CH-N), 93.44 (C), 116.72 (C), 117.99 (CH), 123.34 (CH), 125.56 (CH), 128.00 (CH), 128.39 (CH), 128.55 (CH), 129.34 (CH), 132.87 (C-Cl), 135.3 (C), 158.89 (C), 161.79 (C-OH), 162.55 (C=O); m/z: 465.21 (100.0%), 467.20 (32.0%), 466.21 (30.8%), 468.21 (10.0%), 467.21

(5.2%), 469.21 (1.7%); Molecular formula: $C_{28}H_{32}ClNO_3$; Elemental Analysis: Calculated (C, H, Cl, N, O): 72.17, 6.92, 7.61, 3.01, 10.30, Found: 72.15, 6.90, 7.65, 3.00, 10.33.

3-((2,6-dichlorophenyl)(dicyclohexylamino)methyl)-4-hydroxy-2H-chromen-2-one **4c**

Yield 90%; M. P.: 126-128 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3162.33(CH stretching of aromatic), 1707.12 (C-O Stretch), 1645.77 (C=O Stretch), 744.55 (C-Cl of aromatic ring); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.12-1.59 (m, 20 H, cyclohexyl ring), 2.59 (m, 2H, C-N), 4.67 (s, 1H, CH), 7.40-7.50 (m, 3H, aromatic ring), 7.49-7.88 (m, 4H, coumarin ring), 15.74 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.55 (CH_2), 25.79 (CH_2), 32.33 (CH_2), 33.15 (CH_2), 67.80 (CH_2-N), 77.88 (CH-N), 93.44 (C), 116.72 (C), 117.99 (CH), 123.38 (CH), 125.66 (CH), 126.78 (CH), 128.56 (CH), 129.00 (CH), 135.55 (C-Cl), 158.99 (C), 161.59 (C-OH), 162.55 (C=O); m/z: 499.17 (100.0%), 501.17 (69.1%), 500.17 (31.1%), 502.17 (19.7%), 503.16 (10.2%), 503.17 (3.4%), 504.17 (3.3%); Molecular formula: $C_{28}H_{31}Cl_2NO_3$; Elemental Analysis: Calculated (C, H, Cl, N, O): 67.20, 6.24, 14.17, 2.80, 9.59, Found: 67.18, 6.22, 14.19, 2.78, 9.56.

3-((dicyclohexylamino)(4-fluorophenyl)methyl)-4-hydroxy-2H-chromen-2-one **4d**

Yield 92%; M. P.: 122-124 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3160.33(CH stretching of aromatic), 1708.32 (C-O Stretch), 1648.67 (C=O Stretch), 1053.44 (C-F of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.13-1.60 (m, 20 H, cyclohexyl ring), 2.59 (m, 2H, C-N), 4.60 (s, 1H, CH), 7.13-7.25 (m, 4H, aromatic ring), 7.49-7.88 (m, 4H, coumarin ring), 15.74 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.55 (CH_2), 25.79 (CH_2), 32.33 (CH_2), 33.15 (CH_2), 67.80 (CH_2-N), 77.88 (CH-N), 93.44 (C), 115.55 (C), 116.72 (C), 117.99 (CH), 123.35 (CH), 125.66 (CH), 128.06 (CH), 129.99 (CH), 132.55 (C), 158.99 (C), 160.00 (C-F), 161.59 (C-OH), 162.55 (C=O); m/z: 449.24 (100.0%), 450.24 (30.8%), 451.24 (5.2%); Molecular formula: $C_{28}H_{32}FNO_3$; Elemental Analysis: Calculated (C, H, F, N, O): 74.81, 7.17, 4.23, 3.12, 10.68, Found: 74.80, 7.14, 4.25, 3.10, 10.67.

3-((dicyclohexylamino)(2,4-difluorophenyl)methyl)-4-hydroxy-2H-chromen-2-one **4e**

Yield 89%; M. P.: 122-124 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3166.33(CH stretching of aromatic), 1708.32 (C-O Stretch), 1648.67 (C=O Stretch), 1055.64 (C-F of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.12-1.60 (m, 20 H, cyclohexyl ring), 2.60 (m, 2H, C-N), 4.60 (s, 1H, CH),

6.63-7.15 (m, 3H, aromatic ring), 7.45-7.86 (m, 4H, coumarin ring), 15.75 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.45 (CH_2), 26.49 (CH_2), 31.33 (CH_2), 33.15 (CH_2), 66.40 (CH_2-N), 70.86 (CH-N), 93.44 (C), 105.56 (CH), 111.11 (CH), 115.55 (C), 116.82 (C), 117.90 (CH), 124.15 (CH), 125.96 (CH), 128.00 (CH), 132.00 (CH), 158.19 (C), 159.90 (C-F), 161.07 (C-F), 161.59 (C-OH), 162.55 (C=O); m/z: 467.23 (100.0%), 468.23 (30.8%), 469.23 (5.2%); Molecular formula: $C_{28}H_{31}F_2NO_3$; Elemental Analysis: Calculated (C, H, F, N, O): 71.93, 6.68, 8.13, 3.00, 10.27, Found: 71.90, 6.64, 8.15, 3.27, 10.28.

3-((dicyclohexylamino)(4-methoxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4f**

Yield 86%; M. P.: 133-135 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3162.33(CH stretching of aromatic), 1707.32 (C-O Stretch), 1650.67 (C=O Stretch), 1230.23 (C-OCH_3 of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.12-1.58 (m, 20 H, cyclohexyl ring), 2.62 (m, 2H, C-N), 3.53 (s, 3H, OCH_3), 4.57 (s, 1H, CH), 6.68-7.17 (m, 4H, aromatic ring), 7.42-7.86 (m, 4H, coumarin ring), 15.75 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.45 (CH_2), 26.49 (CH_2), 31.33 (CH_2), 33.15 (CH_2), 56.65 (OCH_3), 66.40 (CH_2-N), 70.86 (CH-N), 93.44 (C), 114.77 (CH), 116.72 (C), 117.59 (CH), 123.95 (CH), 125.96 (CH), 127.56 (CH), 128.00 (CH), 129.77 (C), 158.19 (C), 159.99 (C-OCH_3), 161.59 (C-OH), 162.55 (C=O); m/z: 461.26 (100.0%), 462.26 (31.9%), 463.26 (5.7%); Molecular formula: $C_{29}H_{35}NO_4$; Elemental Analysis: Calculated (C, H, N, O): 75.46, 7.64, 3.03, 13.86, Found: 75.44, 7.61, 3.00, 13.88.

3-((dicyclohexylamino)(3,4-dimethoxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4g**

Yield 84%; M. P.: 138-140 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3160.53(CH stretching of aromatic), 1709.52 (C-O Stretch), 1655.17 (C=O Stretch), 1235.03 (C-OCH_3 of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.13-1.59 (m, 20 H, cyclohexyl ring), 2.58 (m, 2H, C-N), 3.55 (s, 6H, OCH_3), 4.57 (s, 1H, CH), 6.68-7.00 (m, 3H, aromatic ring), 7.45-7.86 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.55 (CH_2), 24.99 (CH_2), 33.23 (CH_2), 34.15 (CH_2), 69.40 (CH_2-N), 77.86 (CH-N), 56.66 (OCH_3), 94.34 (C), 112.99 (CH), 113.79 (CH), 117.02 (C), 117.89 (CH), 120.09 (CH), 123.95 (CH), 125.96 (CH), 128.00 (CH), 130.57 (C), 148.45 (C-OCH_3), 149.39 (C-OCH_3), 158.10 (C), 162.19 (C-OH), 162.55 (C=O); m/z: 491.27 (100.0%), 492.27 (33.1%), 493.27 (6.3%); Molecular formula: $C_{30}H_{37}NO_5$; Elemental Analysis: Calculated (C, H, N, O): 73.29, 7.59, 2.85, 16.27, Found: 73.25, 7.57, 2.82, 16.29.

3-((dicyclohexylamino)(3,4,5-trimethoxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4h**

Yield 82%; M. P.: 136-138 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3168.03(CH stretching of aromatic), 1710.02 (C-O Stretch), 1658.07 (C=O Stretch), 1234.93 (C-OCH_3 of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.60 (m, 20 H, cyclohexyl ring), 2.59 (m, 2H, C-N), 3.56 (s, 9H, OCH_3), 4.59 (s, 1H, CH), 6.29-6.90 (m, 2H, aromatic ring), 7.42-7.89 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.39 (CH_2), 25.66 (CH_2), 32.73 (CH_2), 34.15 (CH_2), 56.65 (OCH_3), 62.56 (OCH_3), 70.40 (CH_2-N), 78.46 (CH-N), 93.44 (C), 106.22 (CH), 116.92 (C), 117.59 (CH), 124.15 (CH), 126.06 (CH), 128.10 (CH), 131.77 (C), 137.78 (C-OCH_3), 155.66 (C-OCH_3), 158.11 (C), 162.09 (C-OH), 162.75 (C=O); m/z: 521.28 (100.0%), 522.28 (34.2%), 523.28 (6.8%), 524.29 (1.0%); Molecular formula: $C_{31}H_{39}NO_6$; Elemental Analysis: Calculated (C, H, N, O): 71.38, 7.54, 2.69, 18.40, Found: 71.35, 7.52, 2.65, 18.42.

3-((dicyclohexylamino)(4-hydroxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4i**

Yield 90%; M. P.: 128-130 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3333.56 (C-OH of aromatic ring), 3170.03(CH stretching of aromatic), 1715.02 (C-O Stretch), 1660.07 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.60 (m, 20 H, cyclohexyl ring), 2.59 (m, 2H, C-N), 4.59 (s, 1H, CH), 6.29-6.90 (m, 4H, aromatic ring), 7.42-7.89 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.39 (CH_2), 25.66 (CH_2), 32.73 (CH_2), 34.15 (CH_2), 66.54 (C), 78.46 (CH-N), 92.64 (C), 116.22 (CH), 116.92 (C), 117.59 (CH), 123.95 (CH), 124.86 (CH), 128.10 (CH), 129.17 (C), 131.77 (C), 158.11 (C), 159.00 (C-OH), 161.09 (C-OH), 162.95 (C=O); m/z: 447.24 (100.0%), 448.24 (30.7%), 449.25 (5.4%); Molecular formula: $C_{28}H_{33}NO_4$; Elemental Analysis: Calculated (C, H, N, O): 75.14, 7.43, 3.13, 14.30, Found: 75.12, 7.41, 3.10, 14.32.

3-((dicyclohexylamino)(2-hydroxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4j**

Yield 88%; M. P.: 130-132 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3333.86 (C-OH of aromatic ring), 3172.03(CH stretching of aromatic), 1720.02 (C-O Stretch), 1665.00 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.62 (m, 20 H, cyclohexyl ring), 2.55 (m, 2H, C-N), 4.59 (s, 1H, CH), 6.29-6.90 (m, 4H, aromatic ring), 7.43-7.88 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.33 (CH_2), 25.65 (CH_2), 32.72 (CH_2), 34.25 (CH_2), 66.64 (C), 79.36 (CH-N), 93.54 (C), 116.21 (CH), 116.91 (C), 117.48 (CH), 122.95 (CH), 124.86 (CH), 128.19 (CH), 129.07 (C), 131.87 (C), 158.91 (C), 159.20 (C-OH), 161.19 (C-OH), 162.96 (C=O); m/z:

447.24 (100.0%), 448.24 (30.7%), 449.25 (5.4%); Molecular formula: $C_{28}H_{33}NO_4$; Elemental Analysis: Calculated (C, H, N, O): 75.14, 7.43, 3.13, 14.30, Found: 75.13, 7.40, 3.11, 14.31.

3-((dicyclohexylamino)(4-hydroxy-3-methoxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4k**

Yield 86%; M. P.: 144-146 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3334.56 (C-OH of aromatic ring), 3170.03(CH stretching of aromatic), 1725.02 (C-O Stretch), 1665.10 (C=O Stretch), 1234.95 (C-OCH_3 of aromatic rings); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.62 (m, 20 H, cyclohexyl ring), 2.55 (m, 2H, C-N), 3.57 (s, 3H, OCH_3), 4.59 (s, 1H, CH), 6.29-6.97 (m, 3H, aromatic ring), 7.43-7.88 (m, 4H, coumarin ring), 15.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.33 (CH_2), 25.65 (CH_2), 32.72 (CH_2), 34.25 (CH_2), 56.65 (OCH_3), 66.64 (C), 79.36 (CH-N), 93.54 (C), 114.48 (CH), 115.78 (CH), 116.61 (CH), 116.91 (C), 120.95 (CH), 123.86 (CH), 125.19 (CH), 129.07 (C), 131.87 (C), 147.77 (C-OCH_3), 148.99 (C-OH), 155.91 (C), 161.19 (C-OH), 162.96 (C=O); m/z: 477.25 (100.0%), 478.25 (31.7%), 479.26 (6.0%); Molecular formula: $C_{29}H_{35}NO_5$; Elemental Analysis: Calculated (C, H, N, O): 72.93, 7.39, 2.93, 16.75, Found: 72.91, 7.36, 2.90, 16.77.

3-((dicyclohexylamino)(3-ethoxy-4-hydroxyphenyl)methyl)-4-hydroxy-2H-chromen-2-one **4l**

Yield 86%; M. P.: 140-142 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3333.66 (C-OH of aromatic ring), 3170.03(CH stretching of aromatic), 1725.02 (C-O Stretch), 1665.10 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.62 (m, 20 H, cyclohexyl ring), 1.65 (t, 3H, OCH_2OCH_3), 2.55 (m, 2H, C-N), 3.57 (s, 3H, OCH_3), 4.09 (q, 2H, OCH_2OCH_3), 4.59 (s, 1H, CH), 5.35 (s, 1H, OH), 6.69-6.87 (m, 3H, aromatic ring), 7.43-7.88 (m, 4H, coumarin ring), 16.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 14.88 (CH_3), 25.30 (CH_2), 25.65 (CH_2), 32.72 (CH_2), 34.25 (CH_2), 65.10 (OCH_2), 66.65 (C), 77.86 (CH-N), 92.54 (C), 114.58 (CH), 114.78 (CH), 116.51 (CH), 116.91 (C), 119.95 (CH), 123.76 (CH), 125.39 (CH), 128.07 (CH), 130.47 (C), 147.87 (C-OH), 148.99 (C-OCH_2CH_3), 153.91 (C), 162.19 (C-OH), 163.06 (C=O); m/z: 491.27 (100.0%), 492.27 (33.1%), 493.27 (6.3%); Molecular formula: $C_{30}H_{37}NO_5$; Elemental Analysis: Calculated (C, H, N, O): 73.29, 7.59, 2.85, 16.27, Found: 73.24, 7.55, 2.81, 16.28.

3-((dicyclohexylamino)(pyridin-2-yl)methyl)-4-hydroxy-2H-chromen-2-one **4m**

Yield 84%; M. P.: 148-150 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3170.03(CH stretching of aromatic), 1725.02 (C-O Stretch), 1665.10 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.62 (m, 20 H, cyclohexyl ring), 2.55 (m, 2H, C-N), 4.59 (s, 1H, CH), 7.31-7.46 (m, 2H, pyridine ring), 7.43-7.80 (m, 4H, coumarin ring), 7.73 (d, 2H, CH_2), 8.46 (d, 2H, CH_2), 16.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.30 (CH_2), 25.65 (CH_2), 32.72 (CH_2), 34.25 (CH_2), 54.33 (CH-N), 66.22 (C), 92.65 (C), 116.89 (C), 117. 45 (CH), 121.21 (CH), 123.34 (CH), 126.87 (CH), 128.78 (CH), 136.77 (CH), 148.67 (CH), 152.33 (C), 155.22 (C), 161.18 (C=O), 163.17 (C-OH); m/z: 432.24 (100.0%), 433.24 (29.9%), 434.25 (4.9%); Molecular formula: $C_{27}H_{32}N_2O_3$; Elemental Analysis: Calculated (C, H, N, O): 74.97, 7.46, 6.48, 11.10, Found: 74.95, 7.44, 6.44, 11.11.

3-((dicyclohexylamino)(thiophen-2-yl)methyl)-4-hydroxy-2H-chromen-2-one **4n**

Yield 88%; M. P.: 140-142 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3170.03(CH stretching of aromatic), 1725.02 (C-O Stretch), 1665.10 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.14-1.62 (m, 20 H, cyclohexyl ring), 2.57 (m, 2H, C-N), 4.49 (s, 1H, CH), 6.77-7.40 (m, 3H, thiophene ring), 7.43-7.80 (m, 4H, coumarin ring), 16.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.30 (CH_2), 25.65 (CH_2), 32.72 (CH_2), 34.25 (CH_2), 54.77 (CH-N), 66.29 (C), 92.55 (C), 116.69 (C), 117. 15 (CH), 123.21 (CH), 125.56 (CH), 125.99 (CH), 126.75 (CH), 127.78 (CH), 128.76 (CH), 139.89 (C), 153.45 (C), 162.21 (C-OH), 163.00 (C=O); 437.20 (100.0%), 438.21 (28.6%), 439.21 (4.8%), 439.20 (4.6%), 440.20 (1.3%), 438.20 (1.2%); Molecular formula: $C_{26}H_{31}NO_3S$; Elemental Analysis: Calculated (C, H, N, O, S): 71.36, 7.14, 3.20, 10.97, 7.33; Found: 71.34, 7.12, 3.18, 10.98, 7.32.

3-((dicyclohexylamino)(furan-2-yl)methyl)-4-hydroxy-2H-chromen-2-one **4o**

Yield 86%; M. P.: 148-150 ^{0}C; IR (KBr $\nu_{max\ in}$ cm^{-1}): 3170.03(CH stretching of aromatic), 1725.02 (C-O Stretch), 1665.10 (C=O Stretch); ^{1}HNMR: ($CDCl_3$) δ ppm: 1.17-1.72 (m, 20 H, cyclohexyl ring), 2.58 (m, 2H, C-N), 4.89 (s, 1H, CH), 6.27-6.50 (m, 2H, thiophene ring), 7.40-7.89 (m, 4H, coumarin ring), 16.79 (s, 1H, OH); ^{13}C NMR: ($CDCl_3$) δ ppm: 25.38 (CH_2), 25.75 (CH_2), 32.79 (CH_2), 34.85 (CH_2), 55.37 (CH-N), 63.89 (C), 93.35 (C), 106.77 (CH), 110.78 (CH), 116.79 (CH), 116.99 (C), 123.55 (CH), 125.78 (CH), 128.87 (CH), 143.44 (CH), 152.36 (C), 154.00 (C), 161.99 (C-OH), 162.67 (C=O); m/z: 421.23 (100.0%), 422.23 (28.6%), 423.23

(4.9%); Molecular formula: $C_{26}H_{31}NO_4$; Elemental Analysis: Calculated (C, H, N, O): 74.08, 7.41, 3.32, 15.18, Found: 74.08, 7.41, 3.32, 15.18.

3.3 In-vitro antimicrobial activity

All the synthesized compounds were screened for *in vitro* antifungal and antibacterial activity. The antibacterial activity was evaluated against three human pathogenic bacterial strains, such as *Escherichia coli* (NCIM-2256), *Bacillus subtilis* (NCIM-2063) and *Staphylococcus aureus* (NCIM-2901). The antifungal activity was evaluated against seven human pathogenic fungal strains, such as *Candida albicans* (NCIM3471), *Candida glabrata* (NCYC 388), *Fusarium oxysporum* (NCIM1332), *Aspergillus fumigates* (NCIM 902), *Aspergillus flavus* (NCIM539), *Aspergillus niger* (NCIM1196), *Cryptococcus neoformans* (NCIM576), which are often encountered clinically, and were compared with standard drug, miconazole (Table 4). Minimum inhibitory concentration (MIC) values were determined using the standard agar method as per CLSI guidelines [24-27].

4. CONCLUSION

In conclusion, a novel series of 3-((dicyclohexylamino)(substituted phenyl/heteryl)methyl)-4-hydroxy-2H-chromen-2-one derivatives **4 (a-o)** have been synthesized using Green protocol. The synthesized compounds were evaluated for their antifungal activity. Use of green catalyst, i.e. triethyl ammonium sulphate as an ionic liquid helped us in the synthesis of expected derivatives in good yields and is advantageous being an eco-friendly method. The mild reaction conditions, excellent yields in shorter reaction time and evasion of cumbersome work-up procedures make this process economically lucrative for industrial application with the advantage of reusability of catalyst. In the present series the compound **4e** with 2,4-di Fluro substituent on phenyl group found to be most potent antibacterial agent. The compound **4k** with 4-hydroxy-3-methoxy on phenyl group found to be most potent antibacterial agent.

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