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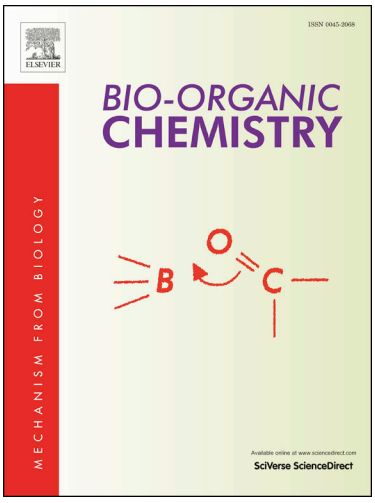

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PII: S0045-2068(19)31651-7
DOI: https://doi.org/10.1016/j.bioorg.2020.103700
Reference: YBIOO 103700

To appear in: *Bioorganic Chemistry*

Received Date: 3 October 2019
Revised Date: 30 January 2020
Accepted Date: 24 February 2020

Please cite this article as: R. Kumar Sharma, V. Singh, N. Tiwari, R.J. Butcher, D. Katiyar, Synthesis, antimicrobial and chitinase inhibitory activities of 3-amidocoumarins, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103700

Synthesis, antimicrobial and chitinase inhibitory activities of 3-amidocoumarins

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Abstract

A series of 3-amido coumarins has been synthesized and tested in vitro for their anitimicrobial and chitinase inhibitory activities. Among these, compounds **5k**, **5l**, **8b-8d, 8f** and **8g** exhibited good antibacterial activity with MIC values in the range of 6.25-25 μg/mL against some of the tested strains while compounds **5l, 8c** and **8f** showed good activity against at least one or two fungal strains. Some of the assayed compounds **5d, 5k, 5l, 8b** and **8c** displayed significant chitinase inhibitory activity with IC_{50} values in the range of 3.74-5.6 μM. Among them, **5l** proved to be potent chitinase inhibitor with IC_{50} value of 3.74 μM. To better understand the enzyme-inhibitor interactions molecular docking study of all the synthesized compounds was carried out on *Aspergillus fumigatus* chitinase 1W9U. The compound **5l** showed high binding affinity with the receptor with binding energy -8.44 Kcal/mol. This study also provides structure activity relationship (SAR) of synthesized compounds.

Keywords: Coumarin, 3-amidocoumarin, Antimicrobial, Antifungal, Chitinase inhibitors, Molecular docking.

1. Introduction

Microbial diseases, especially bacterial and fungal, are plaguing the world's population since ancient times.[1] In spite of new treatment approaches and development of novel effective drugs from both natural and synthetic sources, these diseases continue to affect billions of people worldwide leading to enormous loss of human life to the tune of millions of deaths annually.[2-4] The mortality rate is significantly higher in immunosuppressed patients, those undergoing organ transplants, recipients of cancer chemotherapy and patients undergoing AIDS therapy. Current drugs of choice for the treatment of these infections are associated with toxicity, low efficacy and emerging antimicrobial resistance.[5-8] Therefore, development of newer drugs involving new targets is of priority in antimicrobial drug research.

Chitin is N-acetylglucosamine polymer present as main structural component in exoskeletons of arthropods and fungal cell walls. Chitinase and chitin synthase are the two major enzymes which are involved in chitin biosynthesis. Chitinases play crucial role in exogenous chitin decomposition, fungal cell wall degradation and remodeling.[9-11] Chitin is essential for development and survival of fungi. Because of the absence in humans, chitin is considered as important target for antifungal drug development. Therefore, inhibition of fungal chitinases has been proposed as important approach for the development of novel antifungals. Over recent years, several natural and non-natural molecules have been identified and characterized as chitinase inhibitors (Fig. 1). The most important natural inhibitors are pseudotrisaccharide allosamidin[12,13] and the cyclic peptides such as argifin[14] and argadin.[15] Allosamidine was isolated from the mycelia extract of *Streptomyces* spp. Argifin and argadin were isolated from fungi. The former was extracted from mycelia of Gliocladium sp., while the latter was extracted from mycelia of Clonostachys sp. However, because of complex chemical structures and cumbersome synthesis these molecules are not considered suitable antifungal therapeutic candidates.[16-18] Several small designed and/or synthesized molecules such as xanthine[19] and purine[20] derivatives as the fungal chitinase *Af*ChiB1 inhibitors and acetazolamide[21] as an *Af*ChiA1 inhibitor, have also been reported. In a quest to discover potent chitinase inhibitors, Jiang *et al.* had also recently screened a library of over 4 million chemicals by two rounds structure-based virtual screening and identified seventeen 1-thia-7-aza-2-indene based carboxamides.[22] Recently, the chitinase inhibitory activity of closantel,[23] belonging to salicylanilide class of antihelmintic drugs, has also

been reported. Albeit low potency of the molecules all these researches led to renewed interest in the rationale design of more efficient chitinase inhibitors.[24]

Coumarins constitute a biologically active class of oxygen-containing 2*H*-1-benzopyran-2-one derivatives. They are found widely as secondary metabolite in several plant families, bacteria and fungi.[25] The nature and pattern of substitution on coumarin moiety play important role in the pharmacological and therapeutic profile of coumarin compounds.[26] Coumarins substituted with amido group at C-3 position are known to display a wide range of biological and pharmacological properties.[27-31] The 3-benzamidocoumarin derivatives, novobiocin and clorobiocin, are the coumarin based antibiotics which target DNA gyrase.[32] Inspired by the bioactivities of coumarin derivatives especially their anti-chitin synthase activity, Batran et al. recently synthesized 4-hydroxycoumarins containing Schiff's base motif at the C-3 position as antifungal agents and studied their binding interactions with modelled structures of some fungal chitinases.[33] Exploiting these ongoing development and in continuation of our work on the development of antimicrobial agents,[34,35] we envisaged to develop some novel bioactive coumarin derivatives. Inspired by the importance of amide linkage at C-3 position of coumarin nucleus for biological activity and also by the fact that most of the known fungal chitinase inhibitors bear amide linkage in their chemical structures, we synthesized a series of 3-amido coumarin derivatives and examined their antibacterial, antifungal and anti-chitinase activities. The structure activity relationship (SAR) study of amidocoumarins was also performed to explore the influence of length, lipophilic or electronic characteristics of the amide moiety with various substituents. Molecular docking study was also performed to investigate the binding interactions of synthetic compounds with the active site of *A. fumigates* chitinase 1W9U.

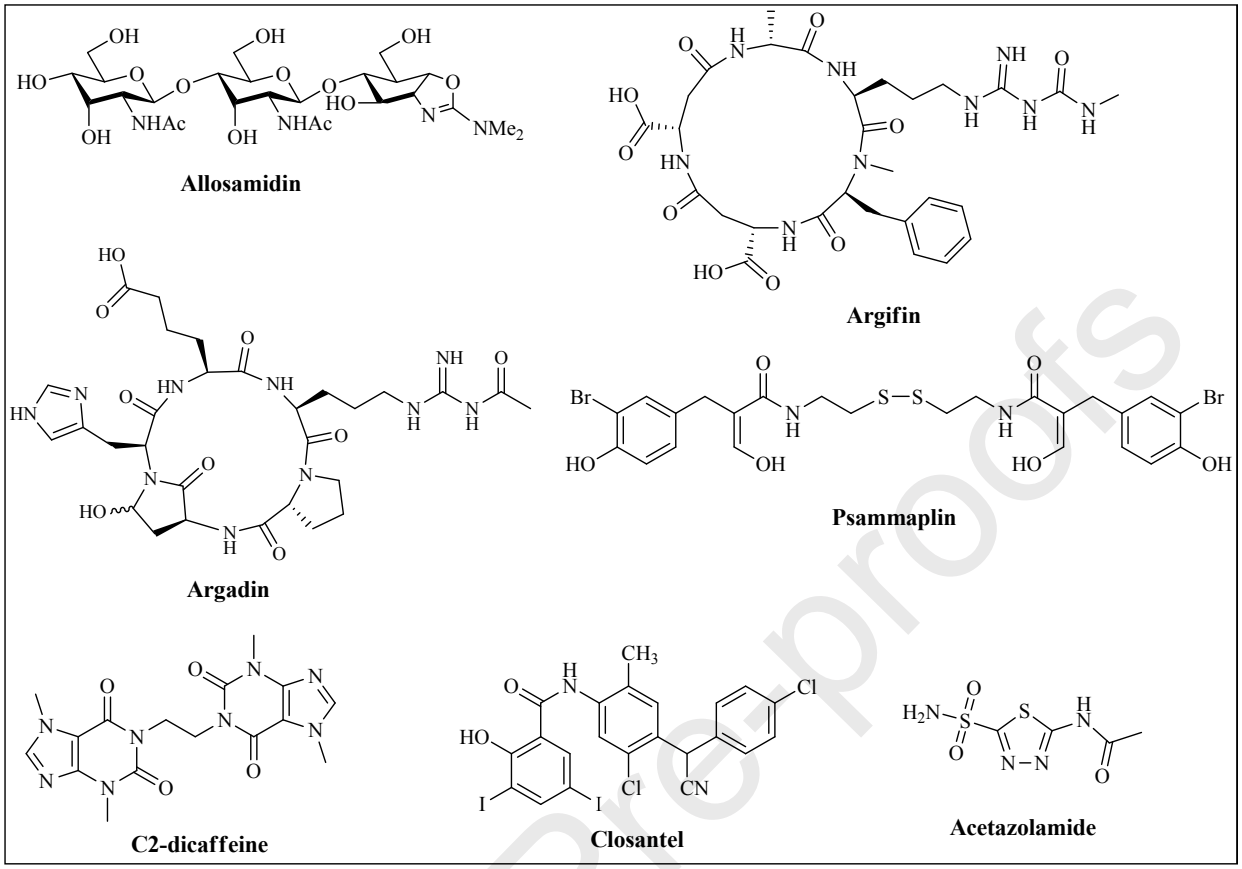


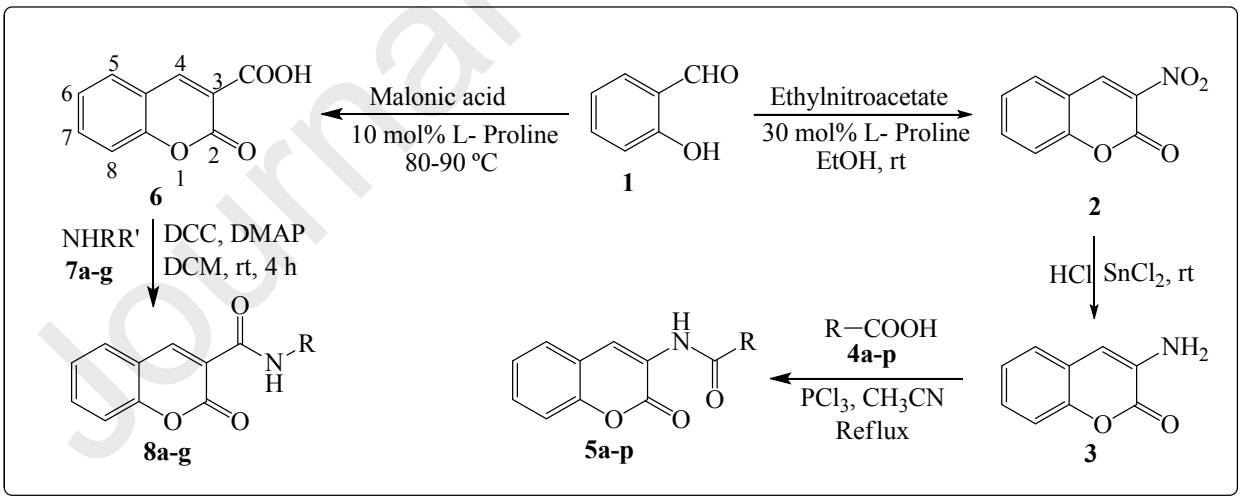
Fig.1. Chemical structures of some chitinase inhibitors

2. Results and dicussion

2.1. Chemistry

The synthetic routes to target coumarin compounds are depicted in **Scheme 1**. 3-nitrocoumarin **2** was prepared via L-proline catalyzed condensation of salicylaldehyde **1** and ethyl nitroacetate according to our previously reported method.[36] Reduction of 3-nitrocoumarin to 3-aminocoumarin **3** using acidified $SnCl_2$ followed by acylation reaction with suitable carboxylic acids **4a-p** using PCl_3 in acetonitrile under reflux for 5-6 h afforded the 3-amidocoumarins **5a-p** in good yields. It is worth mentioning that acylation of 3-aminocoumarin with acid chloride, obtained by treatment of **4a** with $SOCl_2$ in dry DCM under reflux condition for 6 hours, in presence of triethylamine in DCM at room temperature for more than 6 hours was also attempted. But the former method proved more efficient in respect of yield, reaction time and work up procedure and more importantly afforded 3-amidocoumarins in one step.

For the preparation of coumarins derivatives **8a-g**, first the commercially available salicylaldehyde was converted into the coumarin-3-carboxylic acid **6** by knoevenagel condensation with malonic acid in the presence of catalytic amount of L-proline. The condensation of carboxylic acid **6** with suitable amines **7a-g** using *N*, *N*'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dry DCM at room temperature afforded corresponding 3-carboxamide-coumarins **8a-g** in good yields (ranging from 68% to 88%). The final step was also tried with PCl_3 in acetonitrile but this route gave low yield. The chemical structures of synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and MS spectral data. Suitable crystals of compound **5e** could be obtained by slow evaporation of acetone solvent.[37] The crystallographic data of compound **5e** is summarized in **Table 1** and ORTEP plot is shown in **Fig. 2**. The 2D packing arrangement of crystal has shown intra and inter molecular H-bonding interactions between N(1)-H(1) and O(1) with bond length of 2.63 Å (blue) and between O(1)H(10) and O(2) with bond length of 2.65 Å (pink), respectively (**Fig. 3**). Crystallographic data for the structure reported in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-1950376. The data can be obtained free of charge from http://www.ccdc.ac.uk/structures.



Scheme 1. Synthesis of 3-amidocoumarins

Table 1. Crystallographic data collection and structure refinement information of compound **5e**

	5e
Empirical formula	$C_{16}H_{11}NO_4$
Fw	281.26
Temp [K]	100(2)
λ [Å]	0.71073
Crystal system	Orthorhombic
Space group	Pbca
a [Å]	12.7766(3)
b [Å]	10.8204(2)
c [Å]	17.8796(4)
α [°]	90
β [°]	90
γ [°]	90
V [Å^3]	2471.82(9)
Z	8
Density [mg/m^3]	1.512
F(000)	1168
Goodness-of-fit on F^2	1.045
R indices (all data)	R1 = 0.0710, wR2 = 0.1327

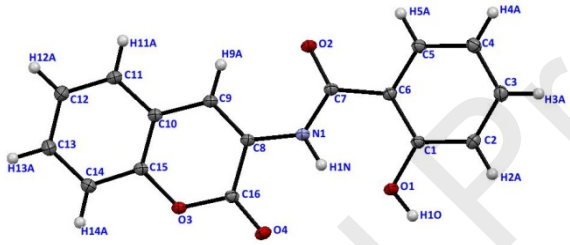


Fig. 2. ORTEP plot of the X-ray structure of **5e**

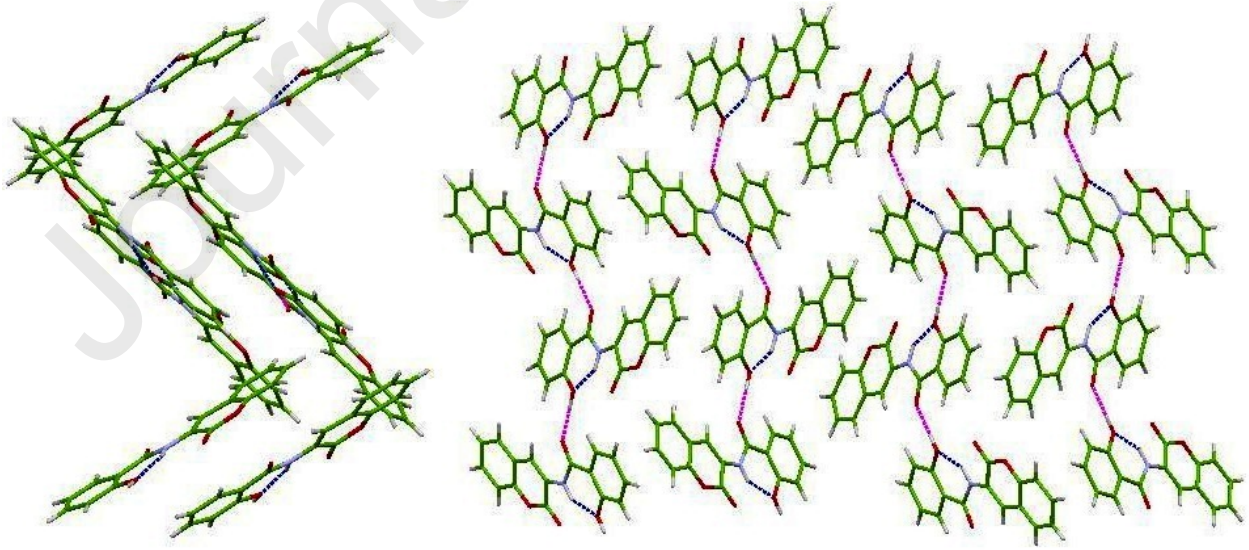
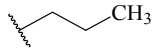
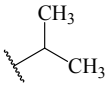
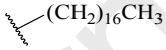
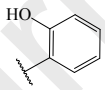
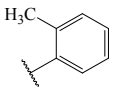
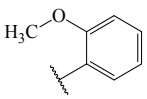
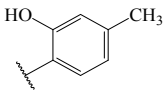
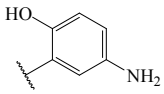


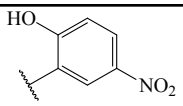
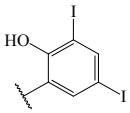
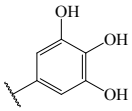
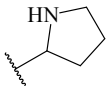
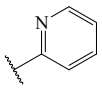
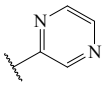
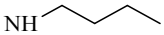
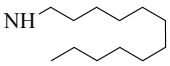
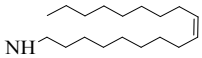
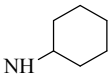
Fig. 3. 2D packing diagram of **5e** viewed along a axis (left) and b axis (right), showing hydrogen-bonding interactions (dotted lines).

2.2. Biology

The minimum inhibitory concentrations (MICs) of amido-coumarins **5a-p** and **8a-g** against four Gram-negative bacterial strains *Klebsiella pneumoniae* MTCC 661, *Salmonella typhi* MTCC 537, *Pseudomonas aeruginosa* MTCC 424 and *Escherichia coli* MTCC 64 and two Gram-positive bacterial strains *Staphylococcus aureus* MTCC 96, *Bacillus pumilus* MTCC 1607, and three fungi *Candida albicans* MTCC 3017, *A. fumigatus* MTCC 2483 and *C. tropicalis* MTCC 184 were determined by two-fold serial dilution method using antibacterial drugs novobiocin and chloramphenicol and antifungal drug amphotericin B and fluconazole as standards.[38] The results are summarized in **Table 2.**

Table 2: Antimicrobial activity and chitinase inhibitory (expressed in IC_{50} μM) activities of synthetic compounds

Compound	R (5a-5p) and NRR' (8a-8g)	Minimum Inhibitory Concentration (MIC in μg/mL)[a]									IC_{50} μM
		Kp	St	Pa	Ec	Sa	Bp	Ca	Af	Ct	
5a		200	50	200		100	100	200	200	>200	8.6
5b		100	50	200	50	100	200	100	100	100	8.6
5c	$-C_{11}H_{23}$	25	50	100	25	100	200	100	100	100	6.2
5d		25	50	100	50	100	200	50	50	200	4.68
5e		50	50	>200	50	100	200	100	200	200	7.0
5f		100	50	>200	50	100	100	200	>200	>200	6.68
5g		>200	>200	>200	100	>200	>200	100	200	100	7.2
5h		50	200	200	50	200	100	200	>200	>200	6.78
5i		200	12.5	25	100	100	200	50	100	200	6.76
5j		200	25	>200	100	25	50	200	>200	200	10.14

5k		100	50	12.5	12.5	25	200	25	50	200	5.38
5l		200	12.5	50	6.25	12.5	25	12.5	25	100	3.74
5m		200	25	100	25	50	200	50	100	100	6.4
5n		200	100	>200	50	100	200	25	50	100	11.61
5o		25	100	200	50	200	200	200	200	200	7.6
5p		100	25	200	25	50	100	25	50	100	7.5
8a		200	100	200	25	>200	200	100	100	100	8.2
8b		200	6.25	100	12.5	12.5	>200	12.5	50	200	5.6
8c		200	25	200	12.5	25	>200	25	12.5	100	4.6
8d		>200	12.5	>200	25	12.5	>200	>200	>200	200	8.8
8e		>200	>200	200	25	>200	25	25	50	100	7.4
8f		>200	6.25	6.25	12.5	25	200	6.25	12.5	50	11.7
8g		>200	50	25	6.25	12.5	>200	25	50	100	7.4
Novobiocin		25	>25	Rt	30	0.15	<0.78	-	-	-	-
Chloramphenicol		12.5	12.5	12.5	12.5	12.5	12.5	-	-	-	-
Amphotericin B		-	-	-	-	-	-	12.5	12.5	6.25	-
Fluconazole		-	-	-	-	-	-	12.5	12.5	12.5	-

'–': not determined; 'Rt': resistant; [a]*K. pneumoniae* (Kp), *S. typhi* (St), *P. aeruginosa* (Pa), *E. coli* (Ec), *S. aureus* (Sa), *B. pumilus* (Bp), *C. albicans* (Ca), *A. fumigates* (af), *C. tropicalis* (Ct).

By analyzing the data, it can be seen that substitution of small acyclic or cyclic aliphatic side chain at amidic nitrogen appears to be unfavorable as compounds **5a**, **5b** and **5n** bearing butyl isopropyl and pyrrolidene substituents, respectively, displayed poor activity toward the tested strains. However, the presence of a bulky aliphatic group such as dodecyl **5c** and stearyl **5d** led to some enhancement in activity. These compounds exhibited activity in the range of 25-50 μg/mL against three bacterial strains. Interesting results were obtained with the introduction of aromatic group in place of aliphatic group. Compounds **5e**, **5f**, **5g** and **5h** bearing hydroxyl,

chloro, methyl and methoxy substituted phenyl ring, respectively, displayed high MIC values in the range of 50 to >200 μg/mL. Some enhancement in activity was observed upon introduction of disubstituted phenyl ring in compounds **5i, 5j** and **5k.** Compound **5i** inhibited the growth of *S. typhi* and *P. aeruginosa* at 12.5 and 25μg/mL concentration, respectively, and compound **5j** having amino and hydroxyl substituted phenyl ring resulted in activity against bacterial strains *S. typhi* and *S. aureus* with MIC values 25 μg/mL. The activity increases with the introduction of electron withdrawing nitro group in place of amino group as compound **5k** showed activity against three bacterial strains with MIC 12.5-25 μg/mL. Whereas compound **5l** having diiodo and hydroxyl groups exhibited significant activity against *S. typhi*, *E. coli*, *S. aureus* and *B. pumilus* at lower MIC (6.25-25 μg/mL). However, introduction of three hydroxyl groups at the phenyl ring led to compound (**5m)** showing decreased bactericidal activity. Furthermore, introduction of heteroaromatic substitutents in compounds **5o** and **5p** also resulted in reduction in activity profile. Among the 3-carboxycoumarin derivatives, long alkyl chain containing compounds **8b** (dodecyl group) and **8c** (oleyl goup) showed better activity against three (*S. typhi*, *E. coli* and *S. aureus*) bacterial strains with MIC in range of 6.25-25 μg/mL, respectively, than the short alkyl chain containing compound **8a**. Compounds **8d** and **8e** bearing cyclopropyl and cyclohexyl residues displayed activity against three and one bacterial strains, respectively. Interestingly, compound **8f** bearing piperidinyl ring displayed very good activity against four bacterial strains *S. typhi*, *P. aeruginosa*, *E. coli* and *S. aureus* (MIC = 6.25-25 μg/mL) and **8g** with piperazinyl ring showed activity against two bacterial stains (*E. coli* and *S. aureus*) at concentration 6.25 μg/mL and 12.5 μg/mL, respectively.

As indicated in **Table 2**, compounds **5l, 5n, 5p, 8b, 8c, 8f** and **8g** showed activity at concentrations 6.25-25 μg/mL at least against one fungal strains. Among these, compounds **5l, 8c** and **8f** displayed good antifungal activity against *C. albicans* and *A. fumigatus* with MIC's (6.25-25 μg/mL) comparable to that of standard drugs. However, the other compounds of the series showed poor activity with MIC's in the range of 50 to >200 μg/mL against all the fungal strains tested.

Altogether, in this series of compounds, SAR study revealed that the antimicrobial activity of compounds depends on the nature of substituents present at the amido component. The synthesized compounds bearing phenyl ring substituted with hydroxyl group along with electron donating methyl group **5i**, amino group **5j**, and electron withdrawing nitro **5k** and diiodo **5l** substitutents, showed

higher antibacterial activity with MIC values ranging from 6.25-25 μg/mL than compounds bearing monosubstituted phenyl group (**5i-5l** vs **5e-5h**). This may be due to the reason that the di or trisubstituted phenyl ring probably induces additional intermolecular interactions leading to the better penetration of the compounds through the bacterial membrane. Next, comparison of activity profile of compounds **5c** and **8b** bearing the dodecyl side chain indicated that 3-carboxy coumarin derivative showed superior activity than 3-amino coumarin derivative. Furthermore, the higher activity of compounds bearing long alkyl chains (**5c**, **5d**, **8b** and **8c**) than those bearing short alkyl chains (**5a,b** and **8a**) also supports the results of previous studies reporting relationship between lipophilicity and antimicrobial activity of compounds.[35,39,40] Among all the tested compounds, compounds **5l, 8b** and **8f** were the most promising and showed broad spectrum antimicrobial activity.

2.3. Chitinase inhibition effect

All synthesized coumarins were further assessed for their ability to inhibit chitinase *in vitro* by Dinitorsalicylic acid (DNS) method as mentioned by Miller[41] using colloidal chitin as a substrate. The activity results indicated that compounds inhibited the chitinase activity with IC_{50} values ranging from 3.74 μM to 11.7 μM. It can be seen that compound **5l** with diiodo hydroxyl substituent was the most potent chitinase inhibitor with IC_{50} value of 3.74 μM. Further, substitution of amidic nitrogens with long alkyl chain (compounds **5d**, **8b** and **8c**) appears to be favorable. These compounds exhibited IC_{50} values at 4.68, 5.6 and 4.6 μM, respectively. While substitution of nitrogen with short alkyl chain and cycloalkyl group resulted in decrease in anti-chitinase activity (compounds **5a, 5b, 8a, 8d** and **8e**). It can be seen that the presence of an electron withdrawing nitro group at N-phenyl moiety in compound **5k** improves activity compared to compounds **5i**, **5j** and **5m** containing electron donating groups. The mono substitution in N-phenyl moiety (compounds **5e-5h**) and introduction of heterocyclic group as amidic substituents (compounds **5n, 5o, 5p, 8f** and **8g**) leads to inferior activity towards chitinase. Interestingly, these amido coumarins showed superior activity compared to recently reported chitinase inhibitors.[33] Sakuda *et al.* reported that the allosamidin at 10μM or above this concentration is able to inhibit the chitinase.[42]

2.4 Molecular docking study

The docking studies of amidocoumarins were performed using the AutoDock Tools (ADT),[43] to rationalize their chitinase inhibitory activity. A well categorized chitinase of *Aspergillus fumigatus* (PDB code: 1W9U) was used as the template. The binding energies of docked compounds are shown in Table 3. The active pocket consisted of 16 amino acid residues including Ala244, Tyr245, Asp246, Phe251, Trp384, Tyr48, Trp137, Phe76, Gly136, Asp175, Glu177, Tyr176, Ala217, Met243, Met382, Tyr178. The docked molecules showed binding energy towards the target receptor ranging from -5.54 to -8.44 Kcal/mol. For comparison purpose, drug allosamidine was also docked into the same binding site and interestingly out of twenty three docked molecules, twelve showed higher binding affinity i.e. lower binging energy (-6.52 to -8.44 Kcal/mol) with the receptor than the allosamidine (B. E. = -6.46 Kcal/mol). Here, we discuss the binding modes of top five ranked amidocoumarins in the binding site of chitinase. Among the docked molecules, **5l** showed the minimum binding energy value of -8.44 Kcal/mol. This compound exhibited van der Waals interactions with both hydrophobic and polar residues in the binding pocket. The hydroxyl group of amido substituent showed hydrogen bond with carboxyl group of Asp246 of length 2.92Å. The amido substituent was stabilized by Trp137, Gly136, Asp175, Phe 76, Tyr48, Trp384. The binding is further augmented by π stacking interactions between benzene group of coumarin and Tyr245. Next compound **5n** showed binding energy value of -8.21 Kcal/mol. The coumarin ring of this compound showed hydrophobic interactions with Pro219, Ala220 and Gly 221. This compound displayed three hydrogen bonds between: (i) NH of amido group and Asp246 (ii) carbonyl of amido group with Tyr178 (iii) NH of proline ring with carboxylic group of Glu177. In compound **5e,** coumarin ring was involved in π stacking with Trp137 and the hydroxyl group of amide substituent formed hydrogen bond with Ala220 with bond length of 2.82Å. Whereas compound **8e**, bearing cyclohexyl substitution at amide showed slightly less effective binding with the receptor (binding energy=-7.57 kcal/mol) as compared to compound **5e**. The NH of the amide linkage of this compound formed a hydrogen bond with the hydroxyl group of the Tyr178 with the bond length of 2.10Å. Benzene ring of coumarin moiety formed stacking contacts to Trp384 and Trp137. The coumarin ring also showed hydrophobic interactions with Trp137, Gly136 and Phe76 and the substituent cyclohexane formed hydrophobic interactions with Pro219, Ala220, Gly221, Phe 273 and Tyr247. Compounds **5i** showed one hydrogen bond interaction between hydroxyl oxygen of

amide substituent and Ala220 residue with bond length of 3.07Å. This compound also showed hydrophobic interactions with Trp137, Ala217, Tyr245, Glu177, Ala244, Gly136 and Tyr48 residues. Coumarin ring showed π stacking with Trp384 residue. Overall, the docking results showed that the compound **5l** which found to be the best in vitro chitinase assay also showed lowest binding energy. However, it seems that the other compounds **5c, 5d, 5k, 8b** and **8c** which displayed good to moderate in vitro chitinase activity did not exactly fit in the active site and fail to achieve good in silico score.

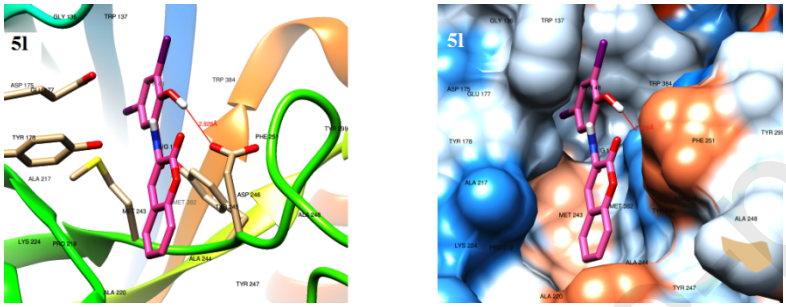


Fig. 4. Compound **5l** docked with in the active site of 1W9U.

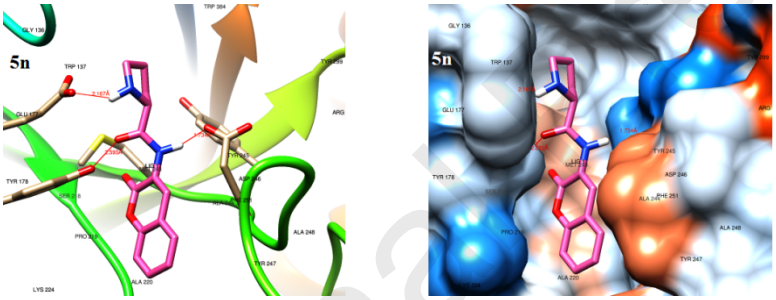


Fig. 5. Compound **5n** docked with in the active site of 1W9U.

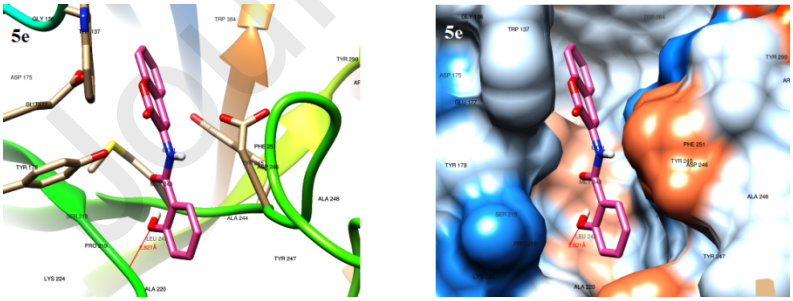


Fig. 6. Compound **5e** docked with in the active site of 1W9U.

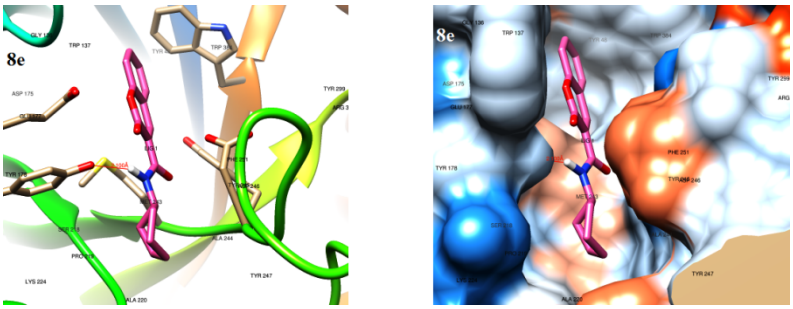


Fig. 7. Compound **8e** docked with in the active site of 1W9U.

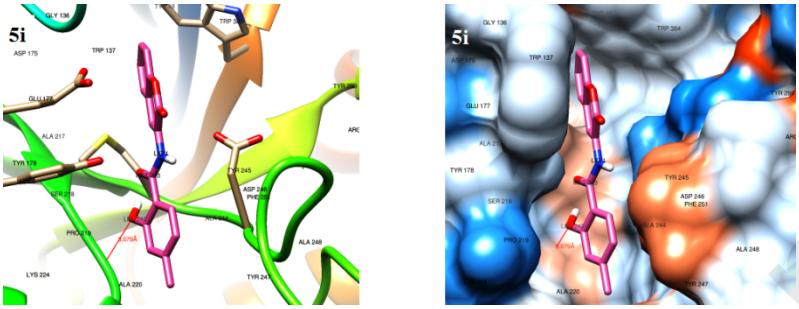


Fig. 8. Compound **5i** docked with in the active site of 1W9U.

Table 3. Docking results of coumarin derivatives with *A. fumigatus* chitinase 1W9U

Ligand	Binding Energy (Kcal/mol)	No. of H-Bonds	Ligand	Binding Energy (Kcal/mol)	No. of H-Bonds
5l	-8.44	1	**8a**	-6.42	1
5n	-8.21	3	**5h**	-6.38	2
5e	-7.90	1	**5p**	-6.36	2
8e	-7.57	1	**5m**	-6.35	2
5i	-7.36	1	**8f**	-6.30	2
8b	-7.15	3	**8d**	-6.08	1
8g	-7.10	2	**5c**	-6.05	2
5f	-6.8	1	**5b**	-5.91	0
5j	-6.79	6	**5a**	-5.57	1
5g	-6.79	1	**8c**	-5.56	1
5o	-6.74	1	**5d**	-5.54	1
5k	-6.52	3	**Allosamidine**	-6.46	3

3. Conclusion

In summary, we designed and synthesized a new series of 3-amidocoumarins and evaluated the compounds for their in vitro antimicrobial and chitinase inhibitory properties. The results show that the 3-amidocoumarin derivatives **5k, 5l**, **8b-8d, 8f** and **8g** exhibited superior bactericidal activity among all the tested compounds. These compounds were found to be equipotent or even displayed better antibacterial activity against some of the bacterial strains than the standard drug chloramphenicol. Compounds **5k** and **8f** (MIC = 12.5 µg/ml and 6.25 µg/ml, respectively) displayed significant activity against novobiocin resistant *P. aeruginosa.* In case of antifungal activity, compounds **5l** and **8b** were equipotent to standard drugs fluconazole and amphotericin B against *C. albicans* while compound **8f** was two-fold more active. Compound **8f** and **8c** exhibited activity at concentration of 12.5 µg/mL against *A. fumigatus*, which was camparable to fluconazole. Some of the compounds from this new series such as **5d, 5k, 5l, 8b** and **8c** also exhibited good in vitro chitinase inhibitory activity in which compound **5l** showed the highest potency for chitinase inhibition with IC_{50} value of 3.74 µM. Compound **5l** was stabilized into the active site of receptor through both hydrophobic and polar interactions and also exhibited lowest binding energy (-8.44 Kcal/mol) with the receptor among all the docked compounds. Overall, from this study compounds **5l** and **8f** emerged as promising antimicrobial agents and further optimization of these compounds is in progress. This work also provides a new structural type for the design of chitinase inhibitors.

4. Experimental Section

4.1 Chemistry

Unless otherwise stated, all common reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich and Spectrochem Pvt. Ltd.) and were used without further purification. Melting points were determined on a Buchi 510 apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) over basic alumina coated TLC plates and the spots were visualized by UV absorbance (254 nm) and by iodine vapors. The pure compounds were isolated by column chromatography using silica gel of mesh size 60-120. IR spectra were recorded on a JASCO FTIR 5300 in KBr from 400 to 4000 cm-1. Electron spray ionization Mass Spectra (ESIMS) were obtained on Micromass quadro II spectrometer. ^{1}H and

^{13}C NMR spectra were recorded on JEOL ECZ 500 MHz spectrometer using the residual solvent peaks of $CDCl_3$ (^{1}H δ 7.26; ^{13}C δ 77.2), and DMSO-d_6 (^{1}H δ 2.50; ^{13}C δ 39.5), as an indirect reference to TMS (δ = 0 ppm). The chemical shifts (δ scale) are reported in parts per million (ppm). ^{1}H NMR spectra are reported in the order: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet and brs = broad singlet), coupling constant (J value) in hertz (Hz), and integration.

3-nitrocoumarin **2**

Salicylaldehyde (1 mmol) and ethyl nitroacetate (1 mmol) were dissolved in 3 ml ethanol and L-proline (30 mol %) was added. The reaction mixture was stirred for four hours at room temperature and the status of the reaction was monitored by TLC. After completion of the reaction the solvent was evaporated and the crude product was dissolved in 12 mL $CHCl_3$ and washed with water (3 x 10 mL). The organic layer was separated and washed with brine (2 x 10 mL), dried over anhydrous $NaSO_4$, and evaporated under reduced pressure. The residue was recrystallized from ethanol. Yield: 84%, mp 142-143 °C (lit.[36] 141-142 °C); ^{1}H NMR ($CDCl_3$): 8.78 (s, 1H, H-4), 7.76 (m, 2H, H-5 and H-7), 7.46 (m, 2H, H-6 and H-8).

3-aminocoumarin **3**

To a stirred suspension of 37.4 % HCl (8 mL) and stannous chloride dihydrate (7.5 mmol) compound **3** (1.0 mmol) was added at room temperature in small portions. The resulting mixture was stirred for 5 h and then poured onto 20 g of ice. The solution was made alkaline by adding NaOH solution (5 M) in an ice bath. The resulting suspension was then extracted with ethyl acetate (2 x 25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated.[44] The pale yellow solid obtained was recrystallized from methanol. Pale yellow; Yield: 69%, mp 136-138 °C. MS (ESI): *m/z* 162.05 $[M + H]^+$; ^{1}H NMR ($CDCl_3$): 7.28 (m, 4H, H-5, H-6, H-7 and H-8), 6.69 (s, 1H, H-4), 4.27 (brs, 2H, NH_2).

General Procedure for the synthesis of compounds 5a-p

The 3-aminocompound **4** (1 mmol) and appropriate carboxylic acid **4a-p** (1.1 mmol) were dissolved in 3 mL CH_3CN and the mixture was stirred at room temperature for half an hour. The

PCl_3 (1 mmol) was added dropwise in the reaction mixture and the reaction mixture was again refluxed for 5-6 h but in case of compounds **5c** and **5p** 8 h refluxing was required. After completion the reaction was quenched by adding ice cold water (5 mL) and the solvent was evaporated under reduced pressure. The residue was dissolved in chloroform (10 mL) and washed twice with water (2 x 20 mL) and then with saturated $NaHCO_3$ solution (10 mL) and finally with brine (20 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated under vacuum. The solid products obtained in case of compounds **5a, 5b, 5d**, **5f, 5h,** and **5p** were recrystallized from acetone while those in case of compounds **5c, 5i** and **5l** were recrystallized in acetone and DMSO mixture (3:1). The crude products obtained in case of compounds **5e**, **5g**, **5j, 5k** and **5m-5o** were purified by silica gel column using chloroform:methanol (9:1) as eluent.

N-(2-Oxo-2*H*-chromen-3-yl)butyramide **5a:**

Yellow solid; Yield: 91%; mp 116-118 °C; R_f = 0.23 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3333, 3089, 2930, 1714, 1680, 1604, 1535, 752; MS (ESI): *m/z* 232 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 8.69 (s, 1H, H-4), 8.09 (bs, 1H, N*H*), 7.49 (d, *J* = 7.5 Hz, 1H, H-5), 7.44 (m, 1H, H-7), 7.30 (m, 2H, H-6 and H-8), 2.40 (t, *J* = 7.5 Hz, 2H, -C$H_2$$CH_2$), 1.77 (m, 2H, -C$H_2$$CH_3$), 1.01 (t, *J* = 6.5 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.4, 158.9, 149.9, 129.6, 127.8, 125.2, 124.0, 123.2, 119.9, 116.4, 39.6, 18.8, 13.7.

N-(2-Oxo-2*H*-chromen-3-yl)isobutyramide **5b:**

Orange solid; Yield: 90%; mp 125-126 °C; R_f = 0.35 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3336, 3083, 2931, 1717, 1681, 1606, 1527, 1361, 1176, 756; MS (ESI): *m/z* 232.09 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 8.69 (s, 1H, H-4), 8.03 (brs, 1H, NH), 7.47 (d, *J* = 6.5 Hz, 1H, H-5), 7.42 (m, 1H, H-7), 7.32 (m, 2H, H-6 and H-8), 2.59 (m, 1H, -CH), 1.26 (d, *J* = 6.5 Hz, 6H, $2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.5, 159.0, 149.9, 129.6, 127.8, 125.2, 124.1, 123.3, 120.0, 116.4, 36.8, 19.4.

N-(2s-Oxo-2*H*-chromen-3-yl)dodecanamide **5c:**

White solid; Yield: 72%; mp143-144 °C; R_f = 0.53 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3339, 3087, 1715, 1685, 1523, 1367, 1171, 753 cm^{-1}; MS (ESI): *m/z* 344.21 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 8.50 (s, 1H, H-4), 7.80 (brs, 1H, NH), 7.45 (d, *J* = 7.5 Hz, 1H, H-5), 7.42

(m, 1H, H-7), 7.32 (m, 2H, H-6 and H-8), 2.43 (m, 2H, -C*H*$_2$CH$_2$), 1.68 (m, 2H, -CH$_2$C*H*$_2$), 1.28 (m, 16H, 8 x CH$_2$), 0.86 (t, *J* = 6.5 Hz, 3H, CH$_3$); ^{13}C NMR (125 MHz, DMSO-*d*$_6$): *δ* 173.5, 157.9, 150.0, 129.5, 127.8, 125.1, 123.7, 120.0, 116.0, 110.0, 36.8, 31.8, 29.9, 29.7, 29.7, 29.6, 29.6, 25.5, 22.4, 14.3.

N-(2-Oxo-2*H*-chromen-3-yl)stearamide **5d:**

White solid; Yield: 89%; mp 85-86 °C; R$_f$ = 0.59 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3331, 3083, 1716, 1687, 1529, 1361, 1179, 757 cm^{-1}; MS (ESI): *m/z* 428.31 [M + H]$^+$; ^{1}H NMR (500 MHz, CDCl$_3$): *δ* 8.69 (s, 1H, H-4), 8.01 (brs, 1H, NH), 7.48 (d, *J* = 7.5 Hz, 1H, H-5), 7.42 (m, 1H, H-7), 7.32 (m, 2H, H-6 and H-8), 2.45 (m, 2H, COC*H*$_2$), 1.69 (m, 2H, COCH$_2$C*H*$_2$), 1.54 (m, 2H, COCH$_2$CH$_2$C*H*$_2$), 1.29 (m, 26H, 13 x CH$_2$), 0.87 (t, *J* = 6.5 Hz, 3H, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): *δ* 172.8, 157.8, 149.9, 129.6, 127.8, 125.2, 124.0, 123.2, 119.9, 116.4, 36.8, 32.6, 32.0, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.2, 27.2, 22.7, 14.1.

2-Hydroxy-N-(2-oxo-2H-chromen-3-yl)benzamide **5e:**

Yellow solid; Yield: 68%; mp 196-197 °C; R$_f$ = 0.65 (Hexane-Ethyl acetate, 7:3); (KBr) ν cm^{-1}: 3105, 3078, 1716, 1629, 1556, 1229, 1167, 757 cm^{-1}; MS (ESI): *m/z* 282.07 [M + H]$^+$; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): *δ* 11.16 (brs, 1H, OH), 8.79 (s, 1H, H-4), 7.98 (dd, *J* = 8.5 and 1.5 Hz, 1H, ArH), 7.56 (d, *J* = 7.0 Hz, 1H, H-5), 7.41 (m, 1H, H-7), 7.33 (m, 3H, H-6, H-8 and NH), 7.24 (m, 1H, ArH), 6.92 (d, *J* = 8.5 Hz, 1H, ArH), 6.91 (m, 1H, ArH); ^{13}C NMR (125 MHz, DMSO-*d*$_6$): *δ* 164.6, 157.9, 155.9, 149.3, 133.3, 130.7, 128.9, 127.4, 125.4, 124.7, 122.3, 119.4, 118.0, 117.3, 116.7, 115.6.

2-Chloro-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5f:**

White solid; Yield: 65%; mp 150-151 °C; R$_f$ = 0.46 (Hexane-Ethyl acetate, 7:3); IR (KBr): V = 3379, 3079, 3039, 1714, 1672, 1594, 1368, 740 cm^{-1}; MS (ESI): *m/z* 300.04 [M + H]$^+$; ^{1}H NMR (500 MHz, CDCl$_3$): *δ* 8.93 (brs, 1H, NH), 8.87 (s, 1H, H-4), 7.77 (d, *J* = 7.5 Hz, 1H, H-5), 7.57 (d, *J* = 7.0 Hz, 1H, ArH), 7.48 (m, 3H, ArH and H-7), 7.44 (m, 1H, ArH), 7.33 (m, 2H, H-6 and H-8); ^{13}C NMR (125 MHz, CDCl$_3$): *δ* 165.3, 158.7, 150.1, 133.9, 132.4, 131.1, 130.8, 130.3, 130.0, 128.0, 127.4, 125.3, 124.1, 124.0, 119.8, 116.5.

2-Methyl-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5g:**

White solid; Yield: 86%; mp 149-150 °C; R_f = 0.64 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3380, 3075, 1722, 1683, 1542, 1372, 745 cm^{-1}; MS (ESI): *m/z* 280.0 $[M + H]^+$; ^1H NMR (500 MHz, DMSO-d_6): δ 8.92 (brs, 1H, NH), 8.85 (s, 1H, H-4), 7.98 (d, *J* = 8.5 Hz, 1H, ArH), 7.76 (d, *J* = 8.5 Hz, 1H, H-5), 7.41 (m, 2H, H-7, ArH), 7.36 (m, 2H, H-6 and H-8), 7.22 (m, 2H, Ar-H), 2.44 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.2, 159.1, 156.0, 147.7, 139.4, 132.6, 128.6, 126.6, 126.3, 125.6, 125.2, 124.5, 121.7, 119.7, 117.1, 116.0, 21.4.

2-Methoxy-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5h:**

White solid; Yield: 56%; mp 178-179 °C; R_f = 0.56 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3294, 1716, 1665, 1602, 1539, 1363, 1163, 750 cm^{-1}; MS (ESI): *m/z* 296.0 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 10.93 (brs, 1H, NH), 8.88 (s, 1H, H-4), 8.26 (dd, *J* = 7.5 and 2.0 Hz, 1H, ArH), 7.53 (m, 2H, H-5 and ArH), 7.42 (m, 1H, H-7), 7.32 (m, 2H, H-6 and H-8), 7.13 (m, 1H, ArH), 7.05 (d, *J* = 8.5 Hz, 1H, ArH), 4.11 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.2, 158.9, 157.6, 149.9, 133.8, 132.2, 129.2, 127.7, 124.9, 123.3, 121.4, 120.7, 120.3, 120.2, 116.2, 112.1, 56.2.

2-Hydroxy-4-methyl-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5i**

Off white solid; Yield: 58%; mp 142-144 °C; R_f = 0.62 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3110, 2830, 1746, 1619, 1558, 1222, 1049, 761 cm^{-1}; MS (ESI): *m/z* 318.07 $[M + Na]^+$; ^1H NMR (300 MHz, DMSO-d_6): δ 11.08 (brs, 1H, OH), 8.77 (s, 1H, H-4), 7.86 (m, 1H, ArH), 7.52 (m, 1H, H-5), 7.37 (m, 1H, H-7), 7.25 (m, 2H, H-6 and H-8), 6.75 (m, 2H, ArH), 2.23 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.2, 157.9, 155.8, 149.2, 144.0, 132.1, 130.5, 128.8, 127.2, 125.4, 124.7, 122.1, 121.2, 120.6, 119.6, 116.9, 20.9.

5-Amino-2-hydroxy-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5j:**

Yellow solid; Yield: 80%; mp 217-218 °C; R_f = 0.64 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3286, 1705, 1658, 1549, 1365, 1219, 754 cm^{-1}; MS (ESI): *m/z* 296.08 $[M + H]^+$; ^1H NMR (500 MHz, DMSO-d_6): δ 11.27 (brs, 1H, OH), 8.81 (s, 1H, H-4), 7.73 (d, *J* = 7.0 Hz, 1H, H-5), 7.52 (m, 2H, ArH and H-7), 7.38 (m, 2H, H-6 and H-8), 6.97 (dd, *J* = 8.5 and 3.0 Hz, 1H, ArH), 6.90

(d, J = 9.0 Hz, 1H, ArH), 3.5 (brs, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.8, 158.0, 150.0, 149.4, 135.4, 128.6, 127.9, 125.0, 124.9, 123.5, 122.2, 119.8, 118.4, 117.8, 115.9.

2-Hydroxy-5-nitro-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5k:**

Sticky solid; Yield: 72%; R_f = 0.52 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3352, 3016, 2745, 1716, 1672, 1529, 1265, 1170, 756 cm^{-1}; MS (ESI): *m/z* 327.03 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.4 (brs, 1H, OH), 8.79 (m, 2H, ArH), 8.53 (s, 1H, H-4), 7.66 (d, J = 7.5 Hz, 1H, H-5), 7.45 (m, 1H, H-7), 7.29 (m, 3H, H-6, H-8 and ArH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.1, 164.4, 158.1, 150.0, 141.4, 130.5, 129.8, 128.4, 127.4, 125.7, 125.5, 125.4, 123.1, 122.7, 120.4, 116.3.

2-Hydroxy-3,5-diiodo-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5l:**

Yellow solid; Yield: 75%; mp 190-191 °C; R_f = 0.43 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3258, 3021, 2755, 1719, 1685, 1535, 1269, 1168, 759 cm^{-1}; MS (ESI): *m/z* 533.87 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.0 (brs, 1H), 8.57 (s, 1H, ArH), 8.24 (s, 1H, ArH), 8.20 (s, 1H, H-4), 7.73 (d, J = 7.5 Hz, 1H, H-5), 7.53 (m, 1H, H-7), 7.41 (d, J = 7.5 Hz, 1H, H-8), 7.34 (m, 1H, H-6); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.3, 158.2, 157.9, 150.8, 150.4, 138.2, 131.0, 128.8, 128.3, 125.6, 124.2, 120.8, 119.7, 116.5, 92.0.

3,4,5-Trihydroxy-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide **5m:**

Off white solid; Yield: 55%; mp 209-211 °C; R_f = 0.32 (Hexane-Ethyl acetate, 1:1); IR (KBr) ν cm^{-1}: 3345, 3018, 1716, 1688, 1533, 1266, 1165, 756 cm^{-1}; MS (ESI): *m/z* 314 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.0 (brs, 1H, OH), 8.56 (s, 1H, H-4), 7.71 (d, J = 7.5 Hz, 1H, H-5), 7.49 (m, 1H, H-7), 7.39 (m, 3H, H-6, H-8 and OH), 7.34 (bs, 1H, OH), 6.89 (m, 2H, ArH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.8, 158.3, 150.2, 146.3, 132.5, 128.4, 125.6, 124.6, 120.1, 118.7, 116.5, 107.2.

N-(2-Oxo-2*H*-chromen-3-yl)pyrrolidine-2-carboxamide **5n:**

Yellow solid; Yield: 63%; mp 115-117 °C; R_f = 0.23 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3334, 3081, 1715, 1679, 1525, 1359, 1174, 754 cm^{-1}; MS (ESI): *m/z* 259.1 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (s, 1H, H-4), 7.90 (brs, 1H, NH), 7.46 (d, J = 7.5 Hz, 1H, H-5),

7.41 (m, 1H, H-7), 7.29 (m, 2H, H-6 and H-8), 4.24 (m, 1H, NCH), 3.47 (m, 2H, pyrrolidene CH_2), 2.12 (m, 2H, pyrrolidene CH_2), 1.82 (m, 2H, pyrrolidene CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2, 159.5, 149.1, 129.3, 127.9, 125.1, 124.7, 124.5, 121.2, 119.8, 117.0, 116.2, 61.8, 45.6, 31.5, 24.7.

N-(2-Oxo-2*H*-chromen-3-yl)picolinamide **5o:**

White solid; Yield: 88%; mp 168-169 °C; R_f = 0.36 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3335, 3085, 1714, 1682, 1525, 1364, 1175, 758 cm^{-1}; MS (ESI): *m/z* 267 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.7 (bs, 1H, NH), 8.87 (s, 1H, H-4), 8.68 (d, *J* = 5.0 Hz, 1H, pyridinyl proton), 8.25 (d, *J* = 8.5 Hz, 1H, pyridinyl proton), 7.91 (m, 1H, pyridinyl proton), 7.54 (m, 2H, H-5 and pyridinyl proton), 7.45 (m, 1H, H-7), 7.33 (m, 2H, H-6 and H-8); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6, 158.2, 150.3, 149.0, 148.6, 137.6, 129.7, 127.9, 127.0, 125.1, 123.5, 122.3, 120.0, 116.5.

N-(2-Oxo-2*H*-chromen-3-yl)pyrazine-2-carboxamide **5p:**

Coffee brown solid; Yield: 92%; mp 198-200°C; R_f = 0.60 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3340, 3078, 1712, 1683, 1537, 1371, 1171, 1020, 757 cm^{-1}; MS (ESI): *m/z* 268 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.47 (brs, 1H, NH), 9.48 (s, 1H, pyrazinyl proton), 8.89 (s, 1H, H-4), 8.85 (d, *J* = 3.0 Hz, 1H, Pyrazinyl proton), 8.67 (d, *J* = 2.0 Hz, 1H, pyrazinyl proton), 7.59 (dd, *J* = 7.5 and 2.0 Hz, 1H, H-5), 7.51 (m, 1H, H-7), 7.38 (m, 2H, H-6 and H-8); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.1, 158.5, 150.4, 148.1, 144.5, 143.7, 143.0, 130.1, 128.0, 125.3, 124.2, 123.7, 119.7, 116.5.

3-Carboxycoumarin **6**

To the stirred reaction mixture of malonic acid (1 mmol) and salicylaldehyde (1 mmol), L-proline (10 mol %) was added under neat conditions. The reaction mixture was refluxed for 6 h and the completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled and solid obtained was recrystallized from ethanol. Yield: 89%, mp 189-191 °C (lit.[45] 190-191 °C); 1H NMR ($CDCl_3$): 7.48 (s, 1H, H-4), 6.54 (d, J = 8.0 Hz, 1H, H-5), 6.45 (m, 1H, H-7), 6.13 (m, 2H, H-6 and H-8).

General Procedure for the synthesis of coumarin derivatives **8a-g**

3-Carboxycoumarin **6** (1 mmol) was dissolved in 10 mL of dichloromethane and DCC (1 mmol) was added to the mixture. The resulting reaction mixture was stirred for 1 h at room temperature. Then appropriate amine **7a-g** (1 mmol) and DMAP (10 mol%) were added sand stirring continued at room temperature for 3-4 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was diluted with DCM (5 mL) and solid formed was separated by filtration. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel (60–120 mesh) column chromatography using 1% MeOH in $CHCl_3$ as eluent.

N-(Butyl)-2-oxo-2*H*-chromene-3-carboxamide **8a**

White solid; Yield: 70%; mp 70-71 °C; R_f = 0.75 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3337, 3084, 1718, 1682, 1528, 1362, 1177, 757 cm^{-1}; MS (ESI): *m/z* 246.11 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 8.89 (s, 1H, H-4), 8.78 (bs, 1H, NH), 7.65 (m, 2H, H-5 and H-7), 7.36 (m, 2H, H-6 and H-8), 3.45 (m, 2H, NCH_2), 1.61 (m, 2H, NCH_2CH_2), 1.41 (m, 2H, CH_2CH_3), 0.94 (t, *J* = 7.5 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 161.4, 154.4, 148.2, 134.0, 129.8, 125.3, 118.7, 118.6, 116.6, 39.7, 31.5, 20.2, 13.8.

N-(Dodecyl)-2-oxo-2*H*-chromene-3-carboxamide **8b**

White solid; Yield: 75%; mp 168-170 °C; R_f = 0.43 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3331, 3077, 1715, 1677, 1522, 1356, 1171, 751 cm^{-1}; MS (ESI): *m/z* 358.23 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 7.79 (s, 1H, H-4), 7.55 (m, 2H, H-5 and H-7), 7.34 (m, 2H, H-6 and H-8), 3.35 (t, *J* = 6.5 Hz, 2H, NCH_2), 1.62 (m, 2H, NCH_2CH_2), 1.29 (m, 18H, 9 x CH_2), 0.87 (t, *J* = 6.5 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.3, 159.2, 153.2, 140.9, 132.9, 128.8, 125.8, 125.2, 118.1, 116.8, 39.7, 34.0, 31.9, 29.8, 29.7, 29.6, 29.6, 29.4, 29.3, 26.0, 22.7, 14.1.

N-(Octadec-9-en-1-yl)-2-oxo-2*H*-chromene-3-carboxamide **8c**

White solid; Yield: 68%; mp 55-56°C; R_f = 0.67 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3338, 3085, 1722, 1684, 1529, 1363, 1178, 758 cm^{-1}; MS (ESI): *m/z* 440 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 8.89 (s, 1H, H-4), 8.79 (brs, 1H, NH), 7.66 (m, 2H, H-5 and H-7), 7.37 (m, 2H, H-6 and H-8), 5.32 (m, 2H, CH=CH), 3.43 (t, J = 6.5 Hz, 2H, NCH_2), 1.97 (m, 4H,

CH_2CH=CHCH_2), 1.61 (m, 2H, NCH$_2$CH_2), 1.30 (m, 22H, 11 x CH$_2$), 0.87 (t, J = 6.5, 3H, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 161.57, 161.4, 154.4, 148.2, 133.9, 129.8, 125.3, 118.7, 118.6, 116.6, 40.0, 32.6, 32.0, 31.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.2, 29.1, 27.2, 27.1, 22.7, 14.1.

N-(Cyclopropyl)-2-oxo-2*H*-chromene-3-carboxamide **8d**

White solid; Yield: 82%; mp 170-172 °C; R_f = 0.46 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3329, 3074, 1712, 1674, 1519, 1353, 1168, 748 cm^{-1}; MS (ESI): *m/z* 230 [M + H]$^+$; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.90 (s, 1H, H-4), 8.80 (brs, 1H, NH), 7.64 (d, J = 7.5 Hz, 1H, H-5), 7.55 (m, 1H, H-7), 7.36 (m, 2H, H-6 and H-8), 2.19 (m, 1H, NCH), 0.45 (m, 2H, Cyclopropyl CH$_2$), 0.31 (m, 2H, Cyclopropyl CH$_2$); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 163.5, 162.8, 153.2, 148.2, 134.1, 129.9, 125.2, 118.7, 118.1, 116.8, 30.7, 6.7.

N-(Cyclohexyl)-2-oxo-2*H*-chromene-3-carboxamide **8e**

White solid; Yield: 88%; mp 170-171 °C (lit.[30] 176.2–177.3 °C); R_f = 0.52 (Hexane-Ethyl acetate, 7:3); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.89 (s, 1H, H-4), 8.75 (brs, 1H, NH), 7.89 (d, J = 8.0, Hz, 1H, H-5), 7.72 (m, 1H, H-7), 7.42 (m, 2H, H-6 and H-8), 3.76 (m, 1H, NCH), 1.72 (m, 4H, 2 x NCH$_2$), 1.24 (m, 6H, cyclohexyl CH$_2$).

3-(Piperidine-1-carbonyl)-2H-chromen-2-one **8f**

Brown solid; Yield: 80%; mp 139-140 °C; R_f = 0.23 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3337, 3784, 1718, 1682, 1528, 1362, 1177, 757 cm^{-1}; MS (ESI): *m/z* 258 [M + H]$^+$; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.80 (s, 1H, H-4), 7.51 (m, 2H, H-5 and H-7), 7.27 (m, 2H, H-6 and H-8), 3.44 (m, 4H, 2 x NCH$_2$), 1.63 (m, 4H, piperidinyl CH$_2$), 1.52 (m, 2H, piperidinyl CH$_2$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 163.3, 158.1, 154.0, 142.3, 132.9, 128.5, 125.9, 118.6, 118.4, 116.8, 61.6, 49.1, 24.4.

3-(4-Methylpiperazine-1-carbonyl)-2*H*-chromen-2-one **8g**

Light brown solid; Yield: 85%; mp 175-176 °C; R_f = 0.25 (Hexane-Ethyl acetate, 7:3); IR (KBr) ν cm^{-1}: 3339, 3517, 3786, 1720, 1684, 1530, 1364, 1179, 759 cm^{-1}; MS (ESI): *m/z* 273.12 [M + H]$^+$; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.79 (s, 1H, H-4), 7.56 (m, 2H, H-5 and H-7), 7.34

(m, 2H, H-6 and H-8), 3.49 (m, 4H, piperazinyl ring protons), 2.42 (m, 7H, NCH_3 and piperazinyl ring protons); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4, 159.1, 153.1, 140.8, 132.8, 128.7, 125.7, 125.0, 117.9, 116.7, 60.0, 59.6, 51.2, 49.9, 47.1.

4.2 Biological Studies

4.2.1. *In vitro* evaluation of antimicrobial activity

All the prepared coumarin derivatives **5a-5p and 8a -8g** were evaluated for their antibacterial activity against *K.pneumoniae* MTCC 661, *S. typhi* MTCC 537, *P. aeruginosa* MTCC 424 and *E. coli* MTCC 64 and two Gram-positive bacterial strains *Staphylococcus aureus* MTCC 96, *Bacillus pumilus* MTCC 1607 and antifungal activity against *Candida albicans* MTCC 3017, *A. fumigatus* MTCC and *Candida tropicalis* MTCC 184. All the test cultures were retrieved from Institute of Microbial Technology, Chandigarh, India. Antibacterial drugs novobiocin and chloramphenicol and antifungal drugs amphotericin B and fluconazole were used as standards. Antimicrobial activities of the coumarins were quantitatively determined in terms of minimum inhibitory concentration (MIC) values depicting the lowest concentration of the tested compound at which no growth of the strain was observed in a period of time and under specified experimental conditions. MIC of compounds was tested by micro broth dilution method using 96 well plates using nutrient broth for bacterial test strain and *sabouraud dextrose broth for fungal test strains* as per guidelines of National Committee for Clinical Laboratory Standards. Test organisms were inoculated in sterile NB/SDB and incubated at 30°C until the solution turbidity achieved to 0.5 McFarland standards. Compounds of concentration 1 mg/mL were prepared. In the first well 100 µL of blank (SDB/NB without test inoculum) as negative control, was added whereas in the second well 180 µL of SDB/NB with test culture was added. In the rest of the well of a row 100 µL of the test culture were added. 20 µL of the compound from the 1mg/mL stock was added in the second well and mixed. From this well, 100 µL of the suspension was transferred to the third well and the so on upto eleventh well. 100 µL of suspension was discarded from eleventh well. Last well was taken as positive control. For fungi, the plates were incubated for 48-72 h at 28 ˚C and for bacteria the plates were incubated for 24 h at 37 ˚C. All the experiments regarding the activity were performed in triplicate and average value of results were considered. MIC values were visually read as the lowest concentration of compound that prevented the microbial growth completely.

4.2.2 Chitinase assay for coumarin derivatives

Chitinase inhibitory activity of the coumarin compounds was determined by Dinitorsalicylic acid (DNS) method as mentioned by Miller using colloidal chitin as a substrate.[46] Chitin from crab shell was used to prepare colloidal chitin. Five grams of chitin powder was gradually mixed with 6 mL of concentrated HCl and the mixture was incubated at 27 ^{o}C for 2 h on the orbital shaker. Chitin was precipitated by adding ice-cold water to the mixture and was further incubated for overnight. After incubation, the solution was centrifuged at 10000 rpm for 20 min at 4 ^{o}C. The pellet was washed repeatedly with distilled water until the pH of the colloidal chitin reached the pH 7. Finally, the colloidal chitin solution was autoclaved and stored at 4 ^{o}C.

The stock solutions of coumarin compounds of 1 mg/mL concentrations were prepared in DMSO. To examine the anti-chitinase activity in term of IC_{50} of the coumarin derivatives, in a reaction mixture different concentration of the coumarin compounds and 20 mU chitinase were taken in test tubes and incubated for 30 min at 37 ^{o}C in shaking water bath. After that 1ml colloidal chitin was added to the and further incubated for 30 min at 37^{o}C in shaking water bath. The reaction was stopped by adding 2 mL DNS reagent and kept in a boiling water bath for 5 min to develop the color. After cooling the test tubes for 10 min; color and optical density were observed against control and IC_{50} was calculated. The color concept lies in DNS reaction is the change in color from yellowish orange to brownish orange.

4.2.3. Molecular docking study

The high resolution crystal structure of *A. fumigatus* chitinase with PDB ID: 1w9u was retrieved from the Protein Data Bank (www.rcsb.org). Active site was predicted by supercomputing facility for bioinformatics & computational biology, IIT Delhi through the link *http://www.scfbio-iitd.res.in/dock/ActiveSite_new.jsp*. The model showed 16 active site pockets/cavities. The cavity 10 which was the most prominent (152 Å^3 with Grid X-Y-Z coordinates: 78.669, 70.021, -3.316 Å) was selected as active site. Molecular docking was compound **5l** and allosamidine performed using the Auto- Dock Tools (ADT) version 1.5.6 and AutoDock version 4.0 docking program. The graphical user interface AUTODOCKTOOLS was employed to setup the protein: all polar hydrogens were added, Kollman charges and nonpolar hydrogens were merged to protein structure. The distance between donor and acceptor atoms that

form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å and the acceptor–hydrogen– donor angle was not less than 120°. The structures were then saved in .pdbqt file format for further studies in ADT. The structure of ligand and allosamidine were drawn using ACD/ChemSketch and converted into 3D structures, optimized and saved in MDL Molfiles [V2000] format. These partial charges of MDL Molfiles were further modified by using the Open Babel GUI to .pdb format for input to ADT. Further the generated files were saved in .pdbqt file format to carry out docking in ADT. All docking were carried out with 1.000 Å spacing and centered on grid box size of 78.669, 70.021, -3.316 points in x, y, and z directions was built. Ten runs were generated by using Lamarckian genetic algorithm searches. Results differing by less than 0.5 Å in positional root–mean– square deviation (RMSD) were clustered together and the results of the most satisfactory free energy of binding were selected as the resultant complex structures. Docked ligand conformations were evaluated in terms of energy, hydrogen bonding, and hydrophobic interaction between ligand and receptor protein. Detailed analysis of the ligand–receptor interactions were carried out and final coordinates of the ligand and receptor were saved. For display of the receptor with the ligand binding site, Chimera 1.8.1 software was used.

Acknowledgement

DK thanks Department of Science and Technology (DST), New Delhi (Grant No. EMR/2016/001396) for the funding and RKS thanks Council of Scientific and Industrial Research (CSIR), New Delhi for Senior Research Fellowship.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Baker SJ, Payne DJ, Rappuoli R, De Gregorio E. Technologies to address antimicrobial resistance. Proc. Natl. Acad. Sci. USA **2018**, 115, 12887–12895.
2. Belete TM. Novel targets to develop new antibacterial agents and novel alternatives to antibacterial agents. Human Microbiome Journal **2019**, 11, 100052.
3. Cole ST. Who will develop new antibacterial agents? Phil. Trans. R. Soc. B **2014**, 369, 20130430.
4. Gatadi S, Madhavi YV, Chopra S, Nanduri S. Promising antibacterial agents against multidrug resistant *Staphylococcus aureus*. Bioorg. Chem. 2019, 92, 103252.
5. Lewis K. Antibiotics: recover the lost art of drug discovery. Nature **2012**, 485, 439–440.

6. Denning DW, Bromley MJ. How to bloster the antifungal pipeline. Science **2015**, 347, 1414-1416.
7. Hwang AY, Gums JG. The emergence and evolution of antimicrobial resistance: Impact on a global scale. Bioorg. Med. Chem. 2016, 15, 6440-6445.
8. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* 2015, 109, 309–318.
9. Hartl L, Zach S, Seidl-Seiboth V. Fungal chitinases: diversity, mechanistic properties and biotechnological potential. Appl. Microbiol. Biotechnol. **2012**, 93, 533-543.
10. Kuranda MJ, Robbins PW. Chitinase is required for cell separation during growth of Saccharomyces cerevisiae. J. Biol. Chem. **1991**, 266, 19758−19767.
11. Mazu TK, Bricker BA, Flores-Rozas H, Ablordeppey SY, The mechanistic targets of antifungal agents: an overview. Mini Rev. Med. Chem. **2016**, 16, 555-578.
12. Huang G, Huang H. Synthesis, antiasthmatic, insecticidal/antifungal activities of allosamidins, J. Enzym. Inhib. Med. Chem. 2019, 34, 1226-1232.
13. Huang G. Recent progress on synthesis and activities of allosamidin and its analogues. Mini-Rev. Med. Chem. 2012, 12, 665-670.
14. Hirose T, Sunazuka T, Omura S. Recent development of two chitinase inhibitors, argifin and argadin, produced by soil microorganisms. Proc. Jpn. Acad., Ser. B **2010**, 86, 85.
15. Arai N, Shiomi K, Yamaguchi Y, Masuma R, Iwai Y, Turberg A, Kölbl H, Ōmura S. Argadin, a new chitinase inhibitor, produced by Clonostachys sp. FO-7314. Chem. Pharm. Bull. **2000**, 48, 1442−1446.
16. Huang G, Dai YP. Solid phase synthesis of allosamidin. Synlett, 2010, 21, 1554-1556.
17. Huang G, Tang Q. Synthesis of allosamidin and its analogues. Curr. Org. Syn. 2014, 11, 696-700.
18. Huang G, Shu S. An efficient synthesis of allosamidin. Synlett, 2012, 23, 1829-1831.
19. Tsirilakis K, Kim C, Vicencio AG, Andrade C, Casadevall A, Goldman DL. Methylxanthine inhibit fungal chitinases and exhibit antifungal activity, Mycopathologia **2012**, 173, 83-91.
20. Schuttelkopf AW, Andersen OA, Rao FV, Allwood M, Lloyd C, Eggleston IM, van Aalten DM. Screening-based discovery and structural dissection of a novel family 18 chitinase inhibitor. J. Biol. Chem. **2006**, 281, 27278-27285.
21. Schuttelkopf AW, Gros L, Blair DE, Frearson JA, van Aalten DM, Gilbert IH. Acetazolamide based fungal chitinase inhibitors. Bioorg. Med. Chem. **2010**, 18, 8334-8340.
22. Jiang X, Kumar A, Liu T, Zhang KYJ, Yang Q. A Novel Scaffold for Developing Specific or Broad-Spectrum Chitinase Inhibitors. J. Chem. Inf. Model. **2016**, 56, 2413−2420.
23. Neelabh, Tirkey NN, Singh K. *In-silico* and *in-vitro* studies on fungal chitinase as a target enzyme for antifungal activity of closantel. J Microbiol Biotech Food Sci, **2018**, 7, 449-453.
24. Dong Y, Jiang X, Liu T, Ling Y, Yang Q, Zhang L, He X. Structure-Based Virtual screening, compound synthesis, and bioassay for the design of chitinase inhibitors. J. Agric. Food Chem. **2018**, 66, 3351−3357.
25. Singh H, Singh JV, Bhagat K, Gulati HK, Sanduja M, Kumar N, Kinarivala N, Sharma S. Rational approaches, design strategies, structure activity relationship and mechanistic insights for therapeutic coumarin hybrids. Bioorg. Med. Chem. 2019, 27, 3477-3510.

26. Hoult JRS, Paydt M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen. Pharmac. 1996, 27, 713-722.
27. Al-Majedv YK, Kadhum AAH, Al-Amiery AA, Mohamad AB. Coumarins: The antimicrobial agents. Sys. Rev. Pharm. **2017**, 8, 62-70.
28. Robert S, Bertolla C, Masereel B, Dogné J-M, Pochet L. Novel 3-carboxamide-coumarins as potent and selective FXIIa inhibitors. J. Med. Chem. **2008**, 51, 3077–3080.
29. Grover J, Jachak SM. Coumarins as privileged scaffold for anti-inflammatory drug development. RSC Adv. **2015**, 5, 38892–38905.
30. Zhang T, Yan Z, Li YF, Wang N. Simplified aminocoumarin analogues as anticancer agents: Amino isosteric replacement in the noviose moiety resulted in substantial enhancement of antiproliferative activity, Chinese Chem. Lett. **2013**, 24, 719-726.
31. Yu X, Teng P, Zhang Y-L, Xu Z-J, Zhang M-Z, Zhang W-H. Design, synthesis and antifungal activity evaluation of coumarin-3-carboxamide derivatives. Fitoterapia **2018**, 127, 387-395.
32. Maxwell A. DNA gyrase as a drug target. Trends Microbiol. **1997**, 5, 102-109.
33. Batran RZ, Khedr MA, Latif NAA, Abeer A. Abd El Aty, Abeer N. Shehata. Synthesis, homology modeling, molecular docking, dynamics, and antifungal screening of new 4-hydroxycoumarin derivatives as potential chitinase inhibitors. J. Mol. Struct. 2019, 1180, 260-271.
34. Priyanka, Singh V, Ekta, Katiyar D. Synthesis, antimicrobial, cytotoxic and *E. coli* DNA gyrase inhibitory activities of coumarinyl amino alcohols. Bioorg. Chem. **2017**, 71, 120-127.
35. Singh LK, Singh V, Katiyar D, Design, Synthesis and biological evaluation of some coumarin derivatives as potential antimicrobial agents. Med. Chem. 2015, 11, 128-134.
36. Sharma RK, Priyanka, Katiyar D, L-Proline catalyzed condensation of salicylaldehydes with ethyl nitroacetate: an efficient access to 3-nitrocoumarins. Monatsh. Chem. 2016, 147, 2157-2161.
37. CCDC 1950376 contains the supplementary crystallographic data for compound **5e**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
38. National Committee for Clinical Laboratory Standards approved standard document, M27–A2, Reference method for broth dilution antifungal susceptibility testing of yeasts, National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.
39. Muroi H, Nihei K-I, Tsujimoto K, Kubo I. Synergistic effects of anacardic acids and methicillin against methicillin resistant *Staphylococcus aureus*. Bioorg. Med. Chem, 2004, 12, 583-587.
40. Yao B, Ji H, Cao Y, Zhou Y, Zhu, J, Lü J, Li Y, Chen J, Zheng C, Jiang Y, Liang R, Tang H. Synthesis and antifungal activities of novel 2-aminotetralin derivatives. J. Med. Chem. 50, 5293-5300.
41. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **1959**, 31, 426-428.
42. Sakuda S, Inoue H, Nagasawa H. Novel biological activities of allosamidins. Molecules **2013**, 18, 6952-6968.
43. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJJ. Comput. Chem. 2009, 30, 2785–2791.

44. Sivakumar K, Xie F, Cash BM, Long S, Barnhill HN, Wang Q. A fluorogenic 1,3-dipolar cycloaddition reaction of 3-azidocoumarins and acetylenes. Org. Lett. 2004, 6, 4603–4606.
45. Karade NN, Gampawar SV, Shinde SV, Jadhav WN. L-Proline catalyzed solvent-free Knoevenagel condensation for the Synthesis of 3-substituted coumarins. Chin. J. Chem. **2007**, 25, 1686-1689.
46. Gangwar M, Singh V, Pandey AK, Tripathi CK, Mishra BN. Purification and characterization of chitinase from Streptomyces violascens NRRL B2700. Ind. J. Exp. Biol. 2016, 54, 64-71.

Highlights

- Novel 3-amidocoumarins were designed and synthesized as antimicrobial and anti-chitinase agents.
- Antimicrobial activity of the compounds was evaluated against six bacterial and three fungal strains.
- Compounds **5l** and **8f** showed significant antimicrobial activity with MIC values ranging between 6.25 and 25 μg/mL.
- Compound **5l** with IC_{50} value of 3.74 μM was the most potent chitinase inhibitor.
- Compound **5l** also exhibited lowest binding energy (-8.44 Kcal/mol) with the active site of docked receptor chitinase (1W9U) among all the docked compounds.

Graphical abstract