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Developing new hybrid scaffold for urease inhibition based on carbazole-chalcone conjugates: Synthesis, assessment of therapeutic potential and computational docking analysis

Madiha Kazmi,[a,b,†] Imtiaz Khan,[c,d, †] Ajmal Khan,[e] Sobia Ahsan Halim,[e] Aamer Saeed,[a,*] Saifullah Mehsud,[f] Ahmed Al-Harrasi,[e,*] Aliya Ibrar,[a,f,*]

[a]Department of Chemistry, Quaid–i–Azam University, Islamabad 45320, Pakistan

[b]Department of Chemistry, University of Gujrat, Rawalpindi Sub–campus, Satellite Town, Rawalpindi, Pakistan

[c]School of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom

[d]Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom

[e]Natural and Medical Sciences Research Center, University of Nizwa, P.O Box 33, Postal Code 616, Birkat Al Mauz, Nizwa, Sultanate of Oman

[f]Department of Chemistry, Abbottabad University of Science and Technology, Havelian, Abbottabad, Pakistan

[†]These authors contributed equally to this work.

Correspondence: *jadoonquaidian@gmail.com* (A. Ibrar); *aharrasi@unizwa.edu.om* (A. Al-Harrasi); *aamersaeed@yahoo.com* (A. Saeed)

Abstract

Although a diverse range of chemical entities offering striking therapeutic potential against urease enzyme has been reported, the key challenges (toxicity and safety) associated with these inhibitors create a large unmet medical need to unveil new, potent and safe inhibitors of urease enzyme. In this pursuit, the present study demonstrates the successful synthesis of carbazole-chalcone hybrids (**4a-n**) in good yields. The evaluation of the preliminary *in vitro* biological results showed that selected members of the investigated library of hybrid compounds possess excellent urease inhibitory efficacy. In particular, compounds **4c** and **4k** were the most potent inhibitors with lowest IC_{50} values of 8.93 ± 0.21 and 6.88 ± 0.42 μM, respectively. Molecular docking analysis of the most potent inhibitor **4k** suggests that the compound is fitted neatly at the

active site interface and mediates interaction with both nickel atoms present in the active site. Several other obvious interactions including metal-carbonyl contact, hydrogen bonding and hydrophobic interactions were also observed, playing a crucial part in the stabilization of **4k** in the active site of urease.

Keywords: Carbazole; Chalcone; Hybrid scaffold; Urease; Structural diversity; Enzyme inhibition.

1. Introduction

Despite many technological advances, the identification, optimization and selection of a candidate drug molecule with desirable properties have remained a challenging task, thus appealing medicinal chemists to explore the wider chemical space in the hunt for effective therapeutics. Notably, this drug discovery compaign is well-driven by nitrogen-containing heterocycles which make the significant structural components of numerous pharmaceuticals and natural products [1-4]. Among *N*-heterocycles, carbazoles constitute an important class of fused aromatics with established profile of biological significance. They are widely encountered as privileged motifs in numerous bioactive natural products and pharmaceuticals [5-10]. Carbazole and its derivatives have successfully demonstrated their biological potential in terms of their antitumor, antimicrobial, antihistaminic, antioxidative, anti-inflammatory, and psychotropic [7,11-15] activities in addition to their application as useful building blocks for the synthesis of functional organic materials [16,17].

In a similar fashion to carbazoles, chalcones (α,β,-unsaturated ketones) represent an important class of open chain flavonoids with a diverse range of applications in synthetic and medicinal chemistry [18]. The easy accessibility of these molecules offers a convenient platform to exploit their potential to serve as new leads in drug discovery [19,20]. The enriching literature successfully demonstrate the diverse use of natural and synthetic chalcones in the form of various biological activities such as anticancer [21], anti-inflammatory [22], antioxidant [23], antimicrobial [24], antimalarial [25], anti-HIV [26], anti-arrhythmic [27], antiplatelet [28], anti-diabetic [29], anti-neoplastic [30], anti-angiogenic [31], anti-retroviral [32], and monoamine oxidase inhibitors [33], among many others [34-37]. In addition, several hybrid chalcones incorporating heterocyclic systems [38,39], biphenyl functionality [40] or other substitutions [41-43] have also displayed

anticancer and antiviral activities [44,45]. Figure 1 represents the illustrative bioactive potential of carbazole and chalcone scaffolds [46,47].

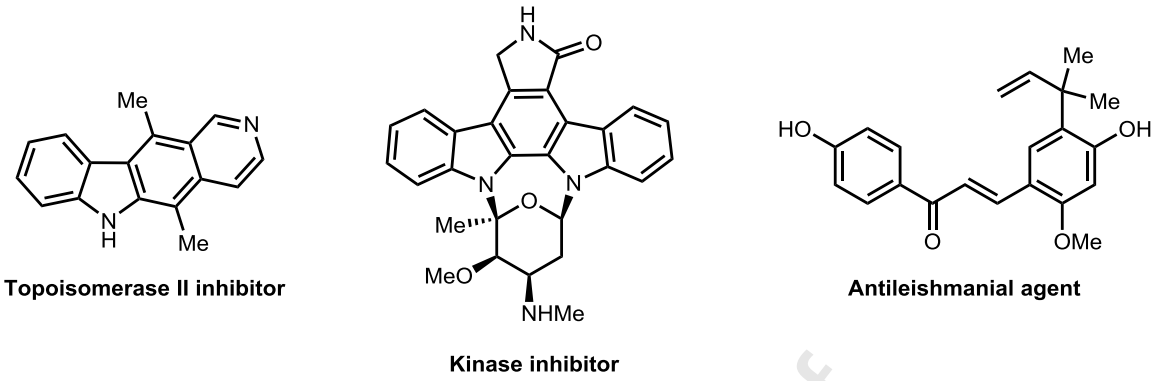


Fig. 1. Selected examples of carbazole and chalcones as useful bioactive motifs.

Urease (EC 3.5.1.5), a nickel-containing heteropolymeric amidohydrolase, is responsible for the hydrolysis of urea to ammonia and carbon dioxide [48]. It is abundantly present in fungi, bacteria, plants and vertebrates. The active site of urease contains two nickel ions coordinated to four histidine residues, carbamoylated lysine and an aspartate carboxylate [49]. This well-recognized enzyme is heavily involved in the rapid increase of ammonia level, thus providing a sustainable environment for the survival of *Helicobacter pylori* in the stomach [50]. This colonization of pathogenic microorganisms causes several urinary and gastrointestinal tract infections [51]. In agriculture, the efficacy of nitrogen fertilizers can be decreased by soil macrobiotic ureases [52] which require heavy use of urea and causes water eutrophication [53]. Several classes of structurally diverse compounds have been documented to regulate the urease activity. For instance, hydroxamic acid derivatives [54], phosphorodiamidates [55], imidazoles [56], phosphonates [57], phosphinates [58], urea and thiourea derivatives [59], triazoles, thiadiazoles [60], coumarins [61], semicarbazones [62], Schiff bases [63], oxadiazoles [64], piperidines [65], thiols [66], ebselen derivatives [67] and metal ions [68]. However, due to severe side effects and toxicity [69] associated with these inhibitors, there remained a suitable and unmet therapeutic need to unveil new, potent and safe inhibitors of urease enzyme addressing the key issues highlighted in pharmaceutical research and agriculture.

Examples described herein clearly demonstrate the medicinal chemistry value of carbazole and chalcone motifs. To combat several pathological conditions associated with ureolytic enzyme (urease), and to increase

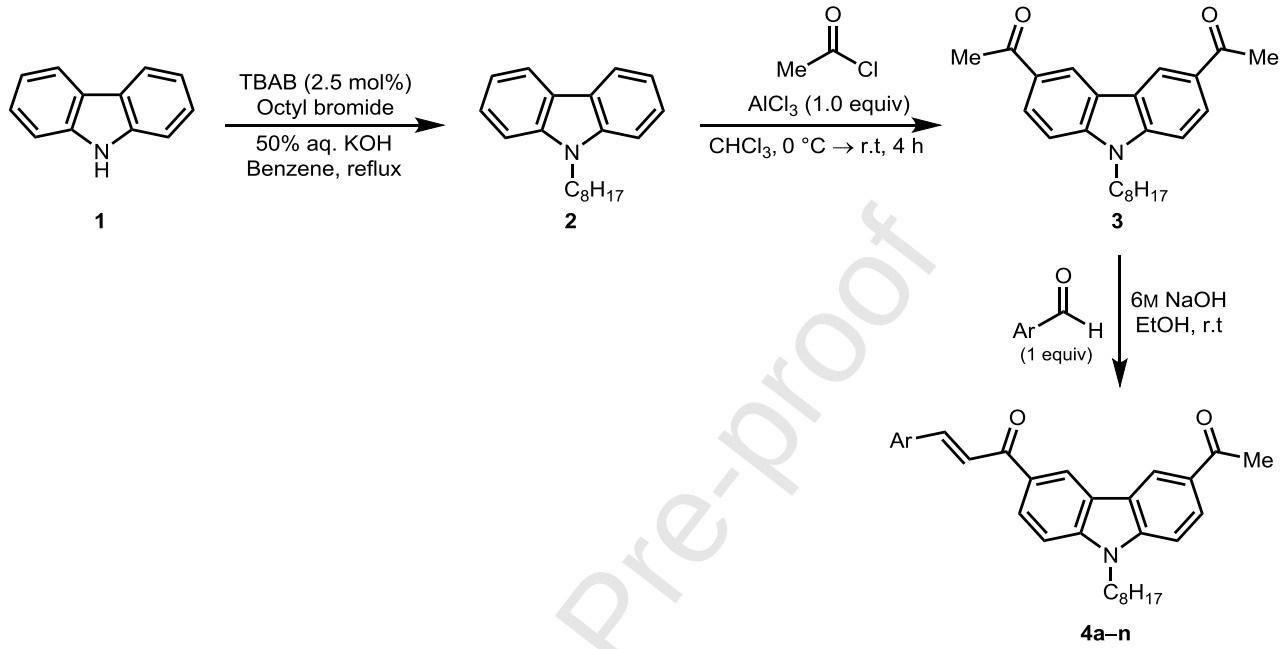
the size of structural libraries of new inhibitors with broadened diversity, we herein report the synthesis of carbazole-chalcone hybrids as a new combined template to function as novel urease inhibitors. This integrated approach combining heterocyclic and non-heterocyclic fragments would play a guiding role in the generation of new molecules with robust therapeutic potential against urease enzyme. In addition, the *in vitro* inhibitory results produced by these chemical scaffolds were rationalized using molecular docking tools.

2. Results and Discussion

2.1. Synthetic chemistry

The synthetic route adopted to access a small library of carbazole-chalcone hybrids is depicted in Scheme 1. *N*-alkylation of carbazole **1** was achieved with octyl bromide in the presence of KOH and catalytic amount (2.5 mol%) of *n*-tetrabutylammonium bromide (TBAB) under reflux conditions [70]. The formation of *N*-octylcarbazole was confirmed by its FTIR spectrum where characteristic stretching bands for C_{sp3}–H appeared at 2846 cm^{-1} alongside the disappearance of NH band around 3400 cm^{-1}. ^{1}H NMR spectral data revealed the formation of *N*-octylcarbazole by the disappearance of NH proton of carbazole moiety and by the appearance of aliphatic protons in the range of 4.41–0.86 ppm. ^{13}C NMR spectrum also confirmed the formation of *N*-octylcarbazole **2** as the signal appeared at 63.5–24.3 ppm for aliphatic carbons of octyl chain. Friedal-Crafts acylation of *N*-octyl carbazole **2** with acetyl chloride (2.0 equiv) afforded the diacylated carbazole **3** in 92% yield. FTIR spectroscopy confirmed the acetylation of *N*-octylcarbazole by the appearance of characteristic ketone carbonyl (C=O) peak at 1710 cm^{-1}. In ^{1}H NMR, methyl protons at 2.79 ppm confirmed the diacetylated product **3**. ^{13}C NMR further confirmed the acetylation, where carbonyl (C=O) carbon appeared at 197.8 ppm. Good quality crystals were grown at room temperature using ethanol and molecular structure of **3** was confirmed through X-ray crystallography [71]. Aldol condensation of **3** with appropriate aldehydes (1.0 equiv) afforded the desired carbazole-chalcones (**4a–n**) under basic conditions at room temperature [70]. The title products were isolated in good yields (59–73%). A diverse range of electron-rich, electron-deficient aryl as well as hetero-aryl aldehydes was well tolerated. The formation of desired hybrid structures (**4a–n**) was recognized by FTIR spectroscopy where characteristic bands appeared at 1723–1699 cm^{-1} were assigned to two carbonyl (C=O) functionalities of α,β-unsaturated

ketone. ^{1}H NMR spectroscopy revealed the synthesis of target compounds by the appearance of prominent signals for unsaturated unit alongside disappearance of the methyl protons. ^{13}C NMR spectroscopy further confirmed the formation of **4a–n** by the appearance of aromatic carbons at their respective chemical shift values.



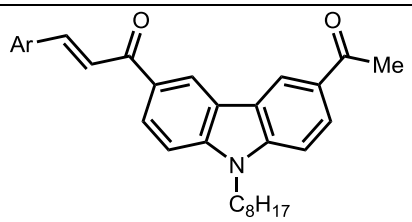
Scheme 1. Synthesis of carbazole-chalcone hybrids (**4a-n**).

2.2. Biology

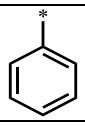
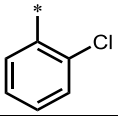
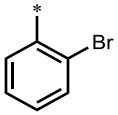
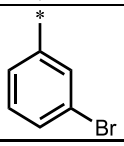
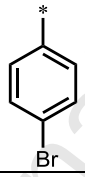
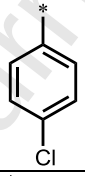
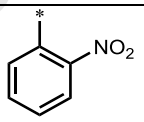
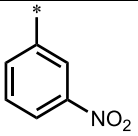
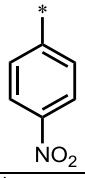
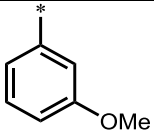
2.2.1. Urease inhibition

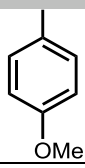
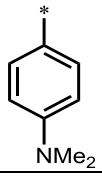
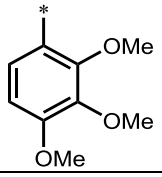
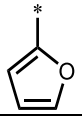
With the aim to explore a new class of compounds incorporating two distinct pharmacophores in one combined unit, carbazole-chalcones (**4a-n**) were investigated for their biological potential against urease enzyme. Thiourea was used as an internal standard (IC_{50} = 20.8 ± 0.59 μM). These medicinal chemistry efforts have produced several potent inhibitors bearing various substituents. *In vitro* biological activity results are summarized in Table 1.

Table 1: *In Vitro* urease inhibitory potential of carbazole-chalcone hybrids (**4a–n**)



4a–n

Compound	Substituent (Ar)	Urease inhibition	
		%Inhibition at 0.5 mM	IC_{50} ± SEM (μM)
4a		19.3	—
4b		24.5	—
4c		94.8	8.93 ± 0.21
4d		10.9	—
4e		24.9	—
4f		95.1	24.68 ± 0.94
4g		18.6	—
4h		94.4	14.15 ± 0.50
4i		29.3	—
4j		7.9	—

4k		98.5	6.88 ± 0.42
4l		22.6	149.02 ± 2.04
4m		74.5	149.9 ± 1.02
4n		78.7	24.78 ± 1.96
Thiourea	—	98.0	20.8 ± 0.59

2.3. Molecular docking and structure-activity relationship analyses

Compounds **4k**, **4c**, **4f**, **4n**, **4h** and **4l** exhibited anti-urease activities in the range of 6.88–149 μM. The mode of interaction of these compounds was predicted by molecular docking in the active site of urease. The binding interactions, docking scores and bond distances of active ligand with its interacting residues are tabulated in Table 2. Among all the compounds, **4k** and **4c** were the most active compounds. Compound **4h** exhibited activity with an IC_{50} value of 14.15 ± 0.50 μM, whereas **4f** and **4n** showed comparable strength to standard with IC_{50} values of ~24 μM. Compound **4l** was found to be the least active compound (~149 μM). A comparative structure-activity relationship of selected inhibitors with substitutional effect is represented in Fig. 2.

The docked view of the most active compound **4k** suggests that the compound is fitted neatly at the active site interface and mediates interaction with both nickel atoms present in the active site. The propenone oxygen facilitates bidentate interaction with the Ni1 and Ni2 at a distance of 2.35 and 2.51 Å, respectively. The side chain (NE2) of His222 further donates H-bond to this oxygen. The carbazole moiety interacts with the side chain of Cys322 and His323 and stabilized *via* hydrophobic interactions. The binding score of **4k** is higher than all the other compounds suggesting its highest activity. Similar to **4k**, compound **4c** also mediates

metal-ligand interactions with both the nickel atoms and His222. Moreover, the bromophenyl ring in **4c** makes contact with the side chains of His139, Phe274 and His137, while its carbzole moiety forms hydrophobic interactions with the side chain of His323 at the entrance of the active site. Furthermore, in compound **4h,** the carbonyl moiety interacts with the Ni2 at a distance of 2.25 Å while the substituted nitrophenyl ring interacts with the main chain nitrogen of His275. Moreover, side chains of His137 and Phe274 provide π–π interactions. Compounds **4f** and **4n** exhibited IC_{50} in the range of 24 μM. Both compounds interact with the nickel atoms while their long hydrocarbon chain remains surface exposed and do not contribute in protein-ligand binding. The furan oxygen of **4n** accepts hydrogen bond from the side chain –OH of Thr301. Finally, the docked view of the least active compound **4l** showed interaction with the Ni1 and His222, while several residues (His137, His272, His323) provide π–π interactions. The docking interactions of each compound are tabulated in Table 2. The docking score shows strong correlation with our experimental IC_{50} values, and a linear trend between experimental and docking results was observed. The binding modes of all the active compounds are presented in Fig. 3.

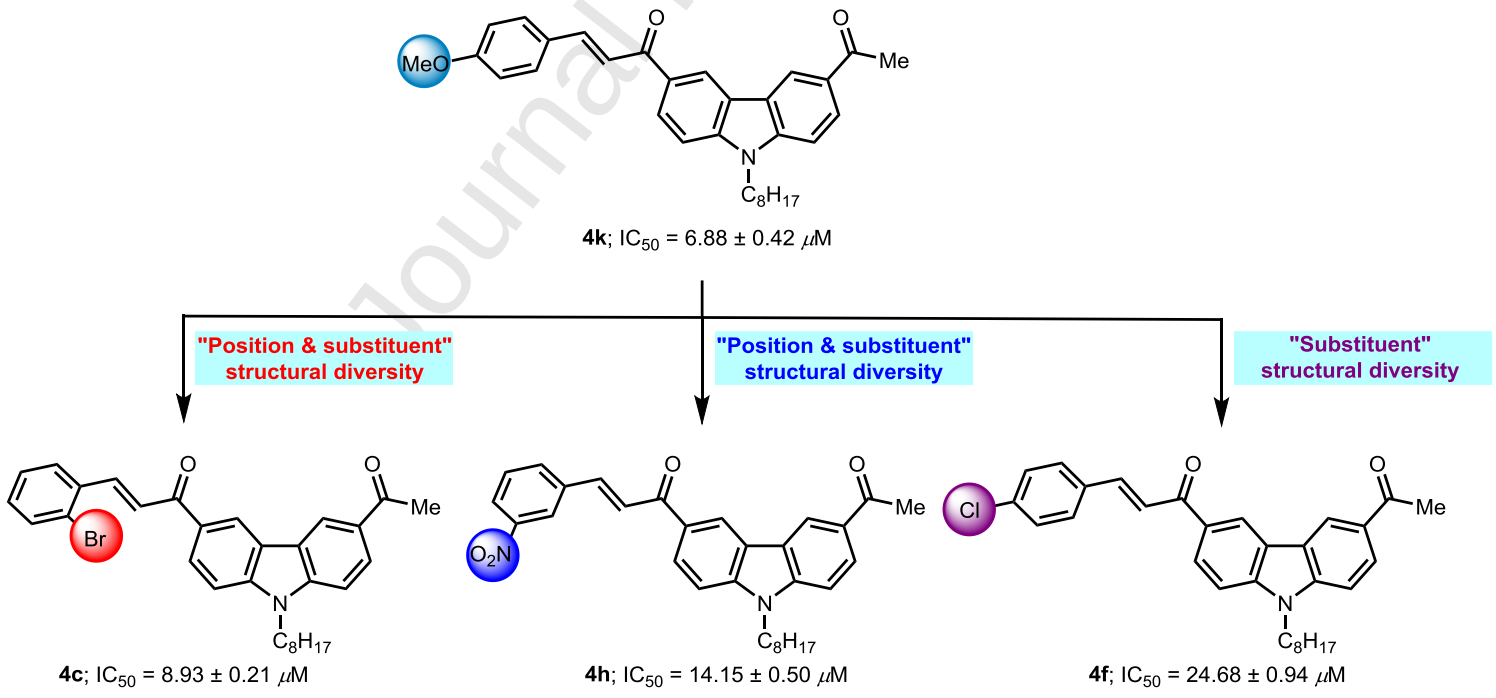


Fig. 2. Structure-activity relationship of compound **4k** with **4c**, **4f**, and **4h**.

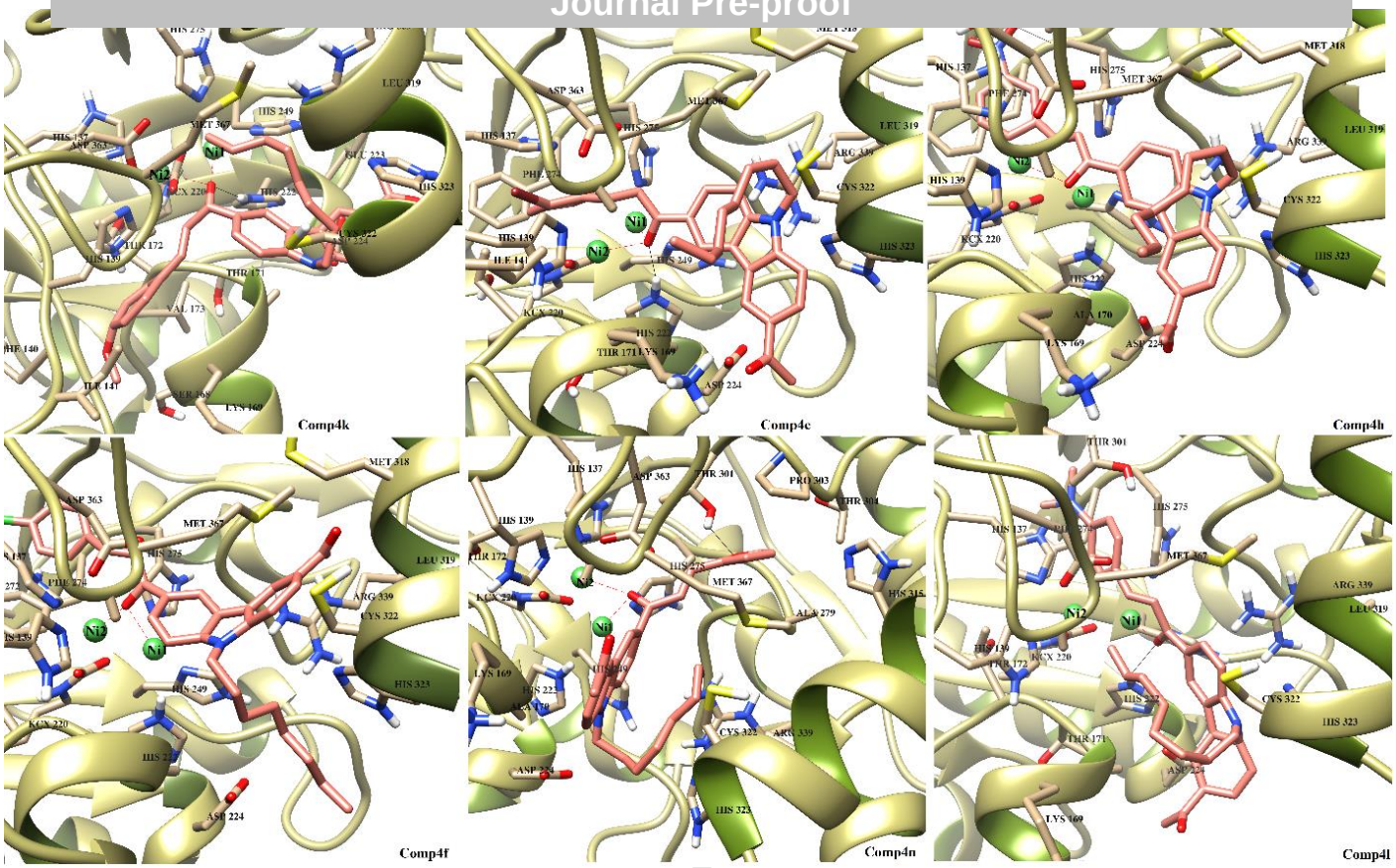


Fig. 3. Docked view of compounds **4k**, **4c**, **4f**, **4n**, **4h** and **4l** in the active site of urease. Compounds are presented in salmon sticks, active site residues are depicted in tan sticks, nickel atoms are shown in green ball model, hydrogen bonds are displayed in black lines, metal-ligand interactions are shown in red dotted lines, and the protein is depicted in gold ribbon.

Table 2. Docking scores and protein-ligand interactions of selected carbazole-chalcone hybrids.

Compound	Docking Score	Binding interactions			
		Ligand atoms	Receptor atoms	Interaction type	Distance (Å)
4k	–7.98	O28	NE2-His222	HBA	3.34
		O28	Ni1	Metal	2.35
		O28	Ni2	Metal	2.51
		6-ring	5ring-His137	π–π	3.37
4c	–7.45	O28	NE2-His222	HBA	2.67
		O28	Ni1	Metal	2.33
		O28	Ni2	Metal	2.30
		6-ring	5ring-His137	π–π	2.96
4h	–7.22	O65	N-His275	HBA	2.66
		O28	Ni2	Metal	2.25
		6-ring	6ring-Phe274	π–π	3.45
		6-ring	5ring-His137	π–π	2.72
4f	–7.00	O28	Ni1	Metal	2.25
		O28	Ni2	Metal	2.27

4h	–6.97	O28	Ni1	Metal	2.44
		O28	Ni2	Metal	2.45
		O35	OG1-Thr301	HBA	1.99
4l	–3.64	O28	NE2-His222	HBA	2.93
		O28	Ni1	Metal	2.33
		5-ring	5ring-His323	π–π	3.62
		6-ring	5ring-His137	π–π	3.46
		6-ring	5ring-His275	π–π	3.23

HBA: Hydrogen Bond Acceptor; HBD: Hydrogen Bond Donor.

3. Conclusions

A series of hybrid compounds (carbazole-chalcones) containing a heterocyclic and non-heterocyclic motif was accessed *via* a facile synthetic approach. *In vitro* biochemical assay showed that several compounds inhibited the urease enzyme with significantly greater efficacy compared to standard drug (thiourea). However, the preliminary structure-activity relationship analysis suggested the strong dependence of inhibition strength on appropriately substituted aryl/heteroaryl ring of chalcone motif which may enforce to generate an adaptable conformation in the active site of the enzyme. Importantly, compounds **4c** and **4k** turned out to be the lead candidates with superior efficacy displaying IC_{50} values of 8.93 ± 0.21 and 6.88 ± 0.42 μM, respectively. In addition, the *in vitro* inhibitory results produced by these chemical scaffolds were rationalized using molecular docking tools. Hence, carbazole-chalcone hybrids can serve as a new combined template for the development of novel urease inhibitors.

4. Experimental

4.1. General chemistry methods

Unless otherwise noted, all materials were obtained from commercial suppliers (Aldrich and Merck companies) and used without further purification. Thin layer chromatography (TLC) was performed on Merck DF-Alufoilien $60F_{254}$ 0.2 mm precoated plates. Product spots were visualized under UV light at 254. Melting points were recorded on a Stuart melting point apparatus (SMP3) and are uncorrected. Infra-red (IR) spectra were recorded on FTS 3000 MX, Bio-Rad Merlin (Excalibur model) spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance (300 MHz) spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) downfield of tetramethylsilane, using residual solvent as internal standard ($CDCl_3$ at 7.26

and DMSO-d_6 at 2.50 ppm). Abbreviations used in the description of resonances are: s (singlet), d (doublet), t (triplet), q, (quartet), m (multiplet), Ar (aromatic). Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker Avance (75 MHz) spectrometer using deuterated solvent as internal standard ($CDCl_3$ at 77.0 and DMSO-d_6 at 39.52 ppm). The elemental analysis was performed on Leco CHNS-932 Elemental Analyzer, Leco Corporation (USA).

4.2. General Procedure for the synthesis of *N*-octylcarbazole (2)

To a stirred mixture of carbazole (1.0 equiv) and octyl bromide (1.05 equiv) in benzene (25 mL) was added 50% KOH solution (60 mL) followed by tetrabutylammonium bromide (TBAB, 2.5 mol%) and mixture was heated at 80 °C for 3 h. The reaction progress was monitored by thin layer chromatography (TLC). On completion of reaction, the mixture was cooled to room temperature. The organic layer was separated and diluted with water (50 mL), extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the desired product [70]. The data was consistent to those reported in literature [72].

Yield: 84%; R_f: 0.76 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2846 (C_{sp3}–H), 1589, 1497 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85–7.79 (m, 2H), 7.27–7.21 (m, 2H,), 4.41 (t, 2H, *J* = 7.2 Hz), 1.73–1.51 (m, 12H), 0.86 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 133.9, 125.2, 121.1, 119.8, 118.0, 114.1, 63.5, 39.4, 33.8, 30.4, 29.7, 27.1, 24.3; Anal. Calcd. for $C_{20}H_{25}N$: C, 85.97; H, 9.02; N, 5.01. Found: C, 85.86; H, 8.96; N, 4.93.

4.3. General procedure for the synthesis of 3,6-diacetyl-*N*-octylcarbazole (3)

To a stirred solution of *N*-octyl carbazole **2** (1.0 equiv) in chloroform was added a solution of acetyl chloride (2.0 equiv) in chloroform followed by aluminum chloride (1.0 equiv) at 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with water, extracted with chloroform and concentrated under reduced pressure. The crude solid was recrystallized from ethanol to afford the diacetylated carbazole **3** [71].

Yield: 92%; m.p.: 87–89 °C; R_f: 0.66 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2859 (C_{sp3}–H), 1710 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.97–7.93 (m, 2H), 7.77–7.69 (m, 2H), 7.49–7.43 (m, 2H), 4.12

(t, 2H, J = 7.8 Hz), 2.79 (s, 3H), 1.78–1.63 (m, 12H), 0.79 (t, 3H, J = 7.2 Hz), ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.8, 136.4, 129.2, 125.8, 123.5, 121.0, 118.1, 65.2, 38.9, 37.6, 33.1, 31.4, 29.7, 27.5, 21.9; Anal. Calcd. for $C_{24}H_{29}NO_2$: C, 79.30; H, 8.04; N, 3.85. Found: C, 79.23; H, 7.96; N, 3.77.

4.4. General procedure for the synthesis of substituted carbazole–chalcones (4a-n)

To a stirred mixture of 3,6-diacetyl-*N*-octylcarbazole **3** (1.0 equiv) was added appropriate aldehyde (1.0 equiv) in methanol, followed by 6M NaOH. The reaction mixture was stirred at room temperature for 40 min and then cooled to 0 °C. The precipitated solid was filtered, washed with cold water and methanol, dried and recrystallized (MeOH) to afford the desired carbazole-chalcones (**4a-n**) in good isolated yields [70].

4.4.1. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-phenylprop-2-en-1-one (4a)

Yield: 73%; m.p.: 140–142 °C; R_f: 0.56 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2849 (C_{sp3}–H), 1715 (C=O), 1590, 1501 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.04 (d, 1H, J = 7.8 Hz), 7.99 (d, 1H, J = 7.8 Hz), 7.89–7.77 (m, 3H), 7.59–7.49 (m, 2H), 7.39–7.33 (m, 3H), 7.27–7.19 (m, 3H), 4.11 (t, 2H, J = 7.8 Hz), 2.50 (s, 3H), 1.79-1.55 (m, 12H), 0.89 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 198.8, 190.1, 45.9, 142.3, 136.2, 133.8, 131.9, 130.7, 129.3, 127.9, 126.6, 125.8, 123.5, 121.8, 120.5, 117.1, 115.4, 113.6, 111.9, 110.2, 59.4, 39.6, 33.4, 31.6, 30.3, 29.2, 28.0, 21.1; Anal. Calcd. for $C_{31}H_{33}NO_2$: C, 82.45; H, 7.37; N, 3.10. Found: C, 82.37; H, 7.28; N, 3.02.

4.4.2. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(2-chlorophenyl)prop-2-en-1-one (4b)

Yield 69%; m.p.: 101–103 °C; R_f: 0.52 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2839 (C_{sp3}–H), 1719 (C=O), 1600, 1507 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.09 (d, 1H, J = 7.8 Hz), 7.98 (d, 1H, J = 7.2 Hz), 7.85–7.79 (m, 3H), 7.60–7.49 (m, 2H), 7.43–7.35 (m, 2H), 7.19–7.10 (m, 3H), 4.19 (t, 2H, J = 7.2 Hz), 2.65 (s, 3H), 1.71–1.53 (m, 12H), 0.78 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 196.9, 190.5, 145.0, 141.2, 135.2, 133.8, 131.0, 130.3, 129.3, 127.9, 125.9, 125.0, 123.5, 121.9, 120.0, 119.3, 117.8, 114.7, 113.5, 112.4, 111.6, 55.9, 39.6, 34.7, 33.0, 30.9, 29.5, 27.3, 26.1, 21.3; Anal. Calcd. for $C_{31}H_{32}ClNO_2$: C, 76.60; H, 6.64; N, 2.88. Found: C, 76.51; H, 6.56; N, 2.79.

4.4.3. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(2-bromophenyl)prop-2-en-1-one (4c)

Yield: 67%; m.p.: 217–220 °C; R_f: 0.55 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2845 (C_{sp3}–H), 1711 (C=O), 1598, 1497 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.14 (d, 1H, *J* = 7.8 Hz), 7.80 (d, 1H, *J* = 7.8 Hz), 7.77–7.73 (m, 3H), 7.57–7.51 (m, 2H), 7.39–7.31 (m, 2H), 7.21–7.11 (m, 3H), 4.07 (t, 2H, *J* = 7.3 Hz), 2.45 (s, 3H), 1.72-1.60 (m, 12H), 0.87 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.6, 191.1, 145.6, 142.7, 135.7, 133.3, 131.3, 130.5, 129.8, 127.5, 126.9, 125.4, 122.8, 121.4, 120.9, 119.1, 117.4, 115.6, 113.9, 111.9, 110.4, 58.9, 38.6, 33.9, 32.6, 31.3, 29.9, 28.8, 27.0, 20.7; Anal. Calcd. for $C_{31}H_{32}BrNO_2$: C, 70.19; H, 6.08; N, 2.64. Found: C, 70.10; H, 6.01; N, 2.56.

4.4.4. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(3-bromophenyl)prop-2-en-1-one (4d)

Yield 63%; m.p.: 211–213 °C; R_f: 0.58 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2829 (C_{sp3}–H), 1720 (C=O), 1598, 1491 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.19 (d, 1H, *J* = 7.8 Hz), 7.97 (d, 1H, *J* = 7.8 Hz), 7.83–7.75 (m, 3H), 7.67–7.53 (m, 2H), 7.43–7.37 (m, 2H), 7.19–7.07 (m, 3H), 4.24 (t, 2H, *J* = 7.6 Hz), 2.35 (s, 3H), 1.47-1.32 (m, 12H), 0.93 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.0, 190.1, 145.4, 141.7, 135.3, 133.9, 130.8, 130.0, 129.3, 127.7, 126.4, 125.3, 123.7, 121.8, 119.5, 118.0, 116.6, 113.7, 111.6, 110.5, 109.9, 108.5, 58.2, 39.0, 33.4, 31.8, 28.3, 27.6, 23.6, 21.4, 15.6; Anal. Calcd. for $C_{31}H_{32}BrNO_2$: C, 70.19; H, 6.08; N, 2.64. Found: C, 70.11; H, 6.00; N, 2.54.

4.4.5. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(4-bromophenyl)prop-2-en-1-one (4e)

Yield: 68%; m.p.: 201-203 °C; R_f: 0.54 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2832 (C_{sp3}–H), 1716 (C=O), 1592, 1497 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.05 (d, 1H, *J* = 7.2 Hz), 7.75 (d, 1H, *J* = 7.8 Hz), 7.79–7.67 (m, 3H), 7.61–7.53 (m, 2H), 7.37–7.29 (m, 3H), 7.21–7.17 (m, 2H), 4.10 (t, 2H, *J* = 7.8 Hz), 2.29 (s, 3H), 1.39-1.27 (m, 12H), 0.98 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 198.0, 190.6, 146.4, 142.7, 136.3, 133.7, 131.8, 130.5, 129.8, 128.7, 126.9, 125.7, 123.8, 121.5, 120.9, 119.3, 117.0, 114.7, 112.6, 109.4, 108.1, 57.6, 39.4, 33.9, 30.8, 29.2, 27.1, 25.6, 23.4, 17.6; Anal. Calcd. for $C_{31}H_{32}BrNO_2$: C, 70.19; H, 6.08; N, 2.64. Found: C, 70.10; H, 6.01; N, 2.55.

4.4.6. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(4-chlorophenyl)prop-2-en-1-one (4f)

Yield 62%; m.p.: 120–123 °C; R_f: 0.58 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2835 (C_{sp3}–H), 1710 (C=O), 1698, 1502 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.07 (d, 1H, *J* = 7.8 Hz), 7.89 (d, 1H, *J* = 7.6

Hz), 7.79–7.71 (m, 3H), 7.63–7.54 (m, 2H), 7.37–7.29 (m, 3H), 7.17–7.11 (m, 2H), 4.23 (t, 2H, *J* = 7.3 Hz), 2.43 (s, 3H), 1.71–1.59 (m, 12H), 0.80 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 196.1, 190.3, 145.7, 141.4, 135.9, 133.2, 131.7, 130.8, 129.4, 128.5, 126.9, 125.4, 123.8, 121.7, 120.3, 119.0, 117.6, 114.7, 113.4, 110.6, 108.5, 55.2, 39.0, 35.7, 32.6, 30.8, 28.5, 26.4, 21.9, 16.2; Anal. Calcd. for $C_{31}H_{32}ClNO_2$: C, 76.60; H, 6.64; N, 2.88. Found: C, 76.53; H, 6.54; N, 2.80.

4.4.7. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(2-nitrophenyl)prop-2-en-1-one (4g)

Yield 66%; m.p.: 102–105 °C; R_f: 0.59 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2831 (C_{sp3}–H), 1699 (C=O), 1593, 1501 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.25 (d, 1H, *J* = 8.1 Hz), 8.15 (d, 1H, *J* = 8.4 Hz), 8.00–7.88 (m, 3H), 7.63–7.61 (m, 3H), 7.55–7.44 (m, 2H), 7.28–7.17 (m, 2H), 4.36 (t, 2H, *J* = 7.3 Hz), 2.75 (s, 3H), 1.42–1.32 (m, 12H), 0.92 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.7, 188.3, 144.1, 143.9, 140.9, 136.8, 134.3, 132.4, 130.9, 130.0, 129.8, 128.8, 127.4, 127.1, 124.5, 123.0, 122.8, 122.1, 109.4, 109.0, 68.1, 38.6, 31.7, 30.3, 29.3, 28.9, 27.2, 26.7, 23.7, 22.6, 14.1; Anal. Calcd. for $C_{31}H_{32}ClNO_2$: C, 74.98; H, 6.50; N, 5.64. Found: C, 74.87; H, 6.41; N, 5.57.

4.4.8. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(3-nitrophenyl)prop-2-en-1-one (4h)

Yield 58%; m.p.: 108–110 °C; R_f: 0.57 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2841 (C_{sp3}–H), 1723 (C=O), 1599, 1507 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.29 (d, 1H, *J* = 8.3 Hz), 8.04 (d, 1H, *J* = 8.4 Hz), 7.97–7.85 (m, 3H), 7.61–7.56 (m, 3H), 7.52–7.45 (m, 2H), 7.22–7.19 (m, 2H), 4.23 (t, 2H, *J* = 7.2 Hz), 2.71 (s, 3H), 1.39–1.24 (m, 12H), 0.88 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.8, 188.6, 148.6, 144.0, 143.1, 140.3, 136.3, 134.2, 132.1, 130.3, 129.4, 128.1, 127.7, 125.6, 124.7, 124.1, 123.6, 122.4, 121.7, 110.6, 109.9, 108.5, 67.5, 38.9, 31.4, 29.1, 28.2, 26.8, 23.2, 22.0, 17.5; Anal. Calcd. for $C_{31}H_{32}N_2O_4$: C, 74.98; H, 6.50; N, 5.64. Found: C, 74.89; H, 6.40; N, 5.54.

4.4.9. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(4-nitrophenyl)prop-2-en-1-one (4i)

Yield 64%; m.p.: 113–115 °C; R_f: 0.56 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2839 (C_{sp3}–H), 1719 (C=O), 1610, 1503 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.17 (d, 2H, *J* = 8.4 Hz), 8.16–8.11 (m, 2H), 7.89–7.83 (m, 3H), 7.53–7.48 (m, 3H), 7.45–7.28 (m, 2H), 4.35 (t, 2H, *J* = 7.2 Hz), 2.73 (s, 3H), 1.37–1.26 (m, 12H), 0.91 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 196.8, 187.6, 147.3, 143.5, 142.7,

139.3, 136.1, 134.9, 133.6, 131.3, 130.4, 129.6, 128.6, 126.7, 125.3, 123.7, 122.3, 121.3, 111.4, 109.6, 108.9, 67.9, 39.2, 30.7, 29.5, 28.6, 25.4, 23.9, 21.7, 17.4; Anal. Calcd. for $C_{31}H_{32}N_2O_4$: C, 74.98; H, 6.50; N, 5.64. Found: C, 74.90; H, 6.41; N, 5.56.

4.4.10. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (4j)

Yield 65%; m.p.: 212–215 °C; R_f: 0.49 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2821 (C_{sp3}–H), 1713 (C=O), 1596, 1489 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.03 (d, 1H, *J* = 8.2 Hz), 7.81–7.73 (m, 3H), 7.61–7.53 (m, 3H), 7.31–7.23 (m, 2H), 6.98–6.81 (m, 3H), 4.29 (t, 2H, *J* = 7.5 Hz), 3.35 (s, 3H), 2.56 (s, 3H), 1.41–1.33 (m, 12H), 0.83 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.8, 188.7, 157.6, 146.2, 140.0, 136.9, 132.6, 130.9, 129.4, 127.9, 126.2, 123.8, 122.4, 121.7, 119.3, 115.6, 114.2, 113.5, 112.1, 111.5, 110.9, 108.2, 61.2, 56.9, 31.4, 30.8, 29.7, 28.4, 25.3, 22.2, 17.1; Anal. Calcd. for $C_{32}H_{35}NO_3$: C, 79.80; H, 7.32; N, 2.91. Found: C, 79.71; H, 7.25; N, 2.83.

4.4.11. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (4k)

Yield 61%; m.p.: 220–223 °C; R_f: 0.51 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2838 (C_{sp3}–H), 1716 (C=O), 1599, 1500 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.98 (d, 1H, *J* = 8.5 Hz), 7.73–7.68 (m, 3H), 7.57–7.51 (m, 3H), 7.27–7.19 (m, 3H), 6.83 (d, 2H, *J* = 7.6 Hz), 4.17 (t, 2H, *J* = 7.3 Hz), 3.56 (s, 3H), 2.34 (s, 3H), 1.67–1.53 (m, 12H), 0.81 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 198.2, 187.2, 157.1, 145.7, 140.9, 136.4, 131.2, 130.0, 129.6, 128.3, 125.9, 123.5, 121.9, 121.0, 120.4, 118.5, 116.2, 113.7, 111.8, 110.5, 109.2, 63.0, 55.4, 30.6, 29.4, 28.0, 26.4, 25.7, 23.8, 21.5, 16.9; Anal. calcd. for $C_{32}H_{35}NO_3$: C, 79.80; H, 7.32; N, 2.91. Found: C, 79.70; H, 7.23; N, 2.81.

4.4.12. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(4-*N*,*N*-dimethylphenyl)prop-2-en-1-one (4l)

Yield 59%; m.p.: 150–152 °C; R_f: 0.53 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2831 (C_{sp3}–H), 1709 (C=O), 1592, 1488 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.93 (d, 1H, *J* = 8.1 Hz), 7.80–7.71 (m, 3H), 7.61–7.54 (m, 3H), 7.21–7.13 (m, 3H), 6.79 (d, 2H, *J* = 7.3 Hz), 4.31 (t, 2H, *J* = 7.6 Hz), 2.98 (s, 6H), 2.28 (s, 3H), 1.59–1.27 (m, 12H), 0.73 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 196.5, 186.7, 149.2, 145.0, 139.9, 133.6, 131.9, 129.4, 128.1, 127.8, 127.2, 125.9, 124.5, 123.8, 122.4, 121.7, 119.2, 117.6, 115.3,

112.6, 110.9, 108.2, 61.6, 45.5, 31.6, 30.8, 29.7, 28.2, 25.4, 22.5, 19.1; Anal. Calcd. for $C_{33}H_{38}N_2O_2$: C, 80.13; H, 7.74; N, 5.66. Found: C, 80.02; H, 7.67; N, 5.57.

4.4.13. 1-(6-Acetyl-9-octyl-9*H*-carbazole-3-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4m)

Yield 68%; m.p.: 236–239 °C; R_f: 0.47 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2821 (C_{sp3}–H), 1705 (C=O), 1598, 1501 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 8.08 (d, 1H, *J* = 8.4 Hz), 7.74–7.69 (m, 3H), 7.65–7.56 (m, 2H), 7.27–7.21 (m, 2H), 6.68 (m, 2H), 4.16 (t, 2H, *J* = 7.4 Hz), 3.78 (s, 9H), 2.51 (s, 3H), 1.73–1.40 (m, 12H), 0.79 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 196.7, 187.7, 154.2, 146.4, 141.3, 138.7, 132.8, 130.9, 129.7, 128.5, 127.7, 126.8, 125.3, 123.4, 122.0, 121.6, 118.5, 117.2, 116.7, 114.9, 112.8, 111.2, 109.7, 108.6, 60.2, 55.9, 31.2, 30.6, 29.9, 29.0, 27.6, 25.8, 23.5, 19.8; Anal. calcd. for $C_{34}H_{35}NO_5$; (541); C, 75.39; H, 7.26; N, 2.59. Found: C, 75.30; H, 7.17; N, 2.50.

4.4.14. 1-(6-Acetyl-9-octyl-9*H*-carbazol-3-yl)-3-(furan-2-yl)prop-2-en-1-one (4n)

Yield 57%; m.p.: 197–199 °C; R_f: 0.56 (20% EtOAc/*n*-hexane); FTIR (ATR, cm^{-1}) 2821 (C_{sp3}–H), 1721 (C=O), 1590, 1483 (C=C); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.93–7.80 (m, 2H), 7.71–7.63 (m, 3H), 7.53–7.47 (m, 2H), 7.23–7.11 (m, 2H), 6.89–6.73 (m, 2H), 4.20 (t, 2H, *J* = 7.2 Hz), 2.39 (s, 3H), 1.59–1.37 (m, 12H), 0.84 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 197.3, 188.1, 151.5, 145.7, 141.9, 139.7, 131.8, 130.5, 129.2, 128.7, 127.3, 126.1, 124.8, 123.7, 122.4, 121.2, 120.5, 117.6, 115.7, 113.5, 111.7, 59.2, 30.9, 29.6, 28.4, 25.4, 23.1, 20.7, 15.8; Anal. Calcd. for $C_{29}H_{31}NO_3$: C, 78.88; H, 7.08; N, 3.17. Found: C, 78.79; H, 7.01; N, 3.09.

4.5. Urease inhibition assay

The reaction mixtures consisting of 25 μL of *Jack bean* (*Canavalia ensiformis*) urease, 55 μL of buffer at pH 6.8, 100 mM of urea, and 5 μL of various concentrations of test compounds (from 0.5 to 0.00625 mM) were incubated at 30 °C for 15 min in 96-well plates. In kinetics experiments, various concentrations of both substrates and test compounds were used. Subsequently 45 μL phenol reagents (1% w/v phenol and 0.005% w/v sodium nitroprussside), and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% w/v NaOCl) were added to each well. Urease activity through indophenols method was measured by the production of ammonia, as described by Weatherburn [73]. After 50 min, the increasing absorbance at 630 nm was measured in a

microplate reader (xMark™ Microplate Spectrophotometer, BIO-RAD). All reactions were performed in triplicate in a final volume of 200 μL. Thiourea was used as the standard inhibitor of urease [74]. Finally, the results were processed by software SoftMax Pro (Molecular Devices, CA, USA), MS-Excel and Ez-fit programs. The percent inhibition was calculated from the formula given below:

$$\%\text{Inhibition} = 100 - (\text{OD test} / \text{OD control}) \times 100$$

4.6. Statistical analysis

The EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA) was employed to calculate the IC_{50} values.

4.7. Molecular docking protocol

Molecular docking studies were carried out on the 3D-structure of urease enzyme (PDB code 4UBP, resolution 1.55Å) using MOE docking suite (MOEv2014.09) [75]. Hydrogen atoms were added by protonoate3D utility of MOE, and partial charges were calculated based on OPLSAA force field. Nickel (Ni) parameters were set as mass = 58.6930, r = 1.4170Å, q = +2.0, and van der Waals well depth of 0.1225 kcal/mol. Protein atoms were minimized until gradient was reached to 0.1RMS kcal/mol/A^2. The structures of compounds (**4c**, **4f**, **4h**, **4k**, **4l**, **4n**) were prepared by MOE, hydrogen atoms were added and MMFF94x force field was applied to calculate partial charges. Finally, compound's structures were minimized until gradient was reached to 0.1RMS kcal/mol/A^2. For docking, Alpha PMI docking algorithm and London dG scoring function was applied. Induced fit docking method of MOE was applied. After docking thirty docked conformations were saved for each ligand.

Disclosure statement

The authors declare that they have no conflict of interest.

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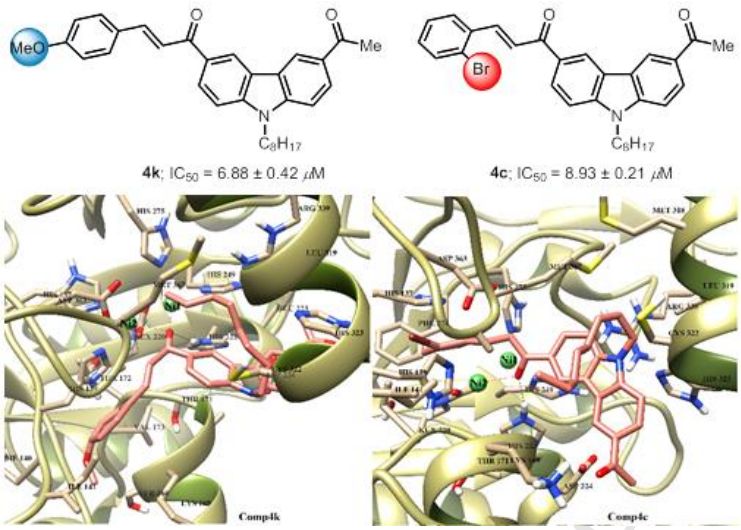
Conflict of interest

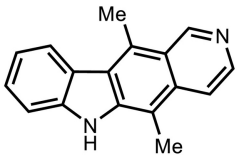
The authors declare that they have no conflict of interest.

Research Highlights

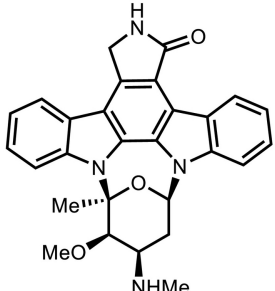
- New carbazole-chalcone hybrids were designed and synthesized.
- Good yields and broad functional group tolerance were observed.
- Compounds **4k** and **4c** were identified as the potent and lead molecules.
- Molecular docking analysis was performed to delineate several key binding interactions.

Graphical Abstract

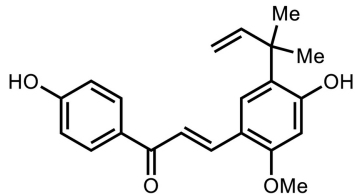




Topoisomerase II inhibitor



Kinase inhibitor



Antileishmanial agent

Figure 1

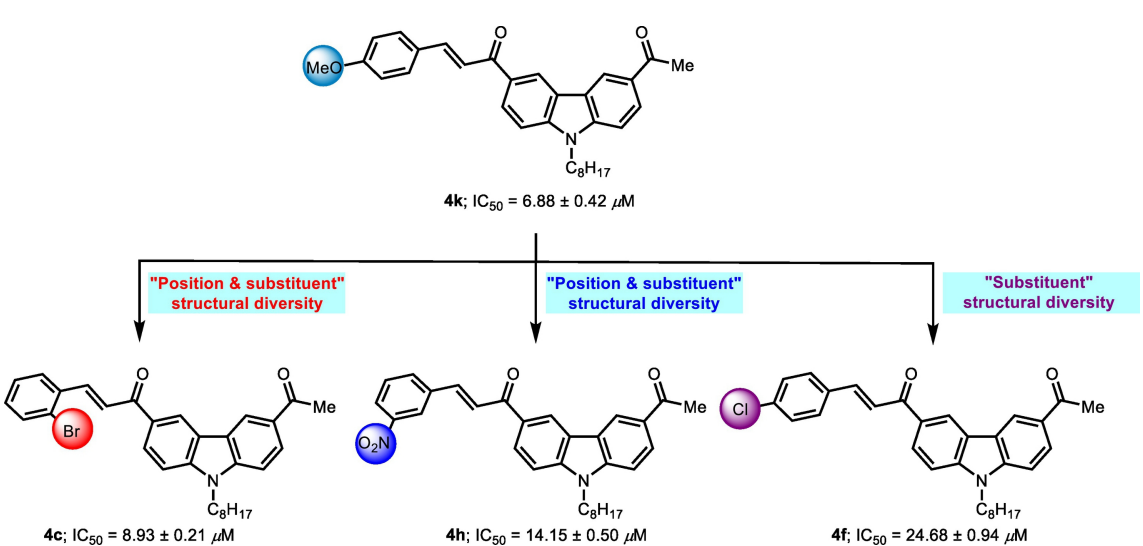

MeO
O
O
Me
N
C8H17
4k; IC50 = 6.88 ± 0.42 μM
"Position & substituent" structural diversity
"Position & substituent" structural diversity
"Substituent" structural diversity
Br
4c; IC50 = 8.93 ± 0.21 μM
O2N
4h; IC50 = 14.15 ± 0.50 μM
Cl
4f; IC50 = 24.68 ± 0.94 μM

Figure 2

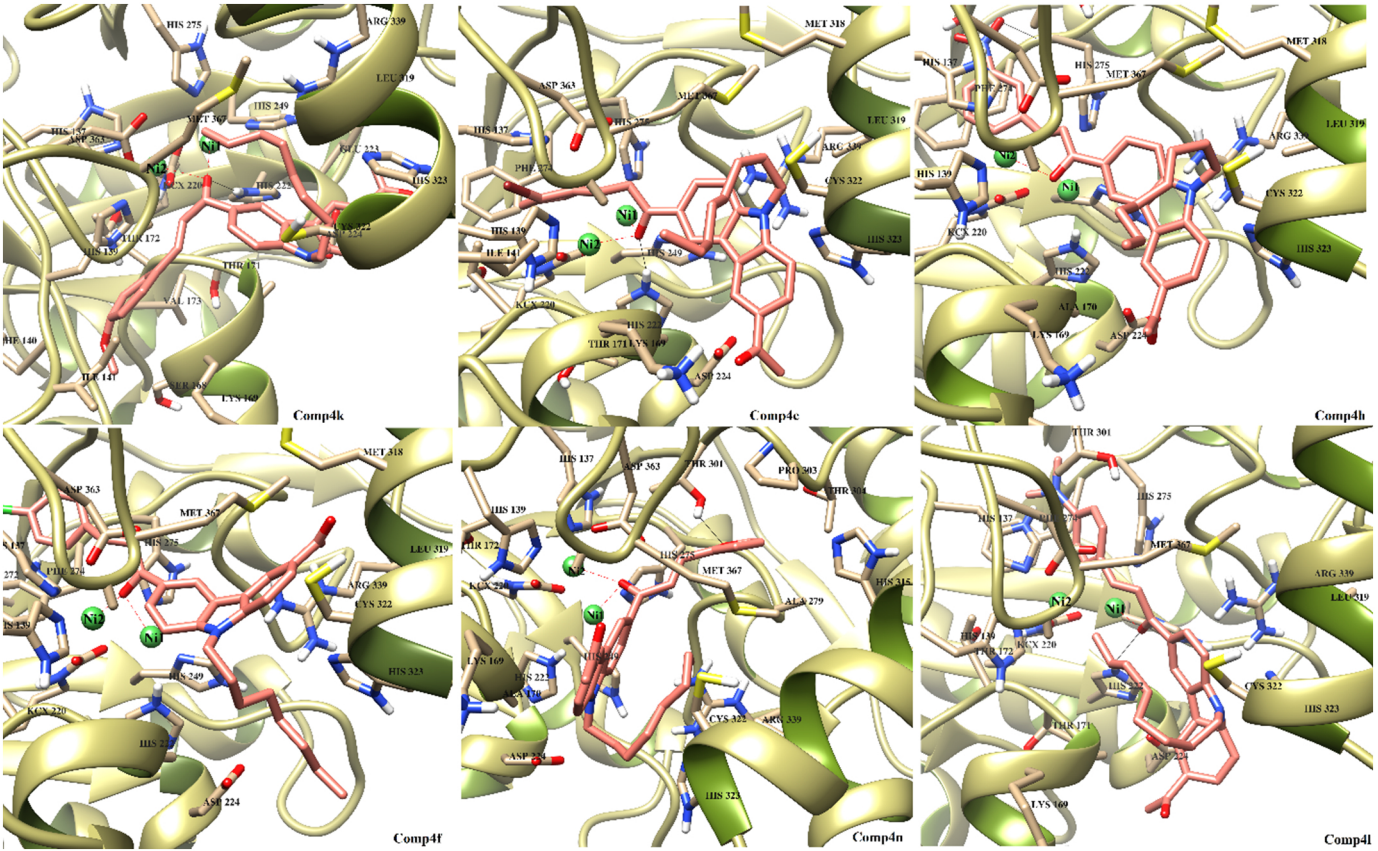


Figure 3