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Design and synthesis of 3-(3-((9*H*-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-phenylquinazolin-4(3*H*)-one derivatives to induce ACE inhibitory activity

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ABSTRACT

In an attempt to develop a new class of cardiovascular drugs, a series of novel carbazolyloxy phenylquinazoline derivatives **9a**–**g** have been synthesized and evaluated as angiotensin converting enzyme (ACE) inhibitors. Most of these compounds exhibited activity as significant ACE inhibitors and three compounds (**9b**, **9c** & **9e**) showed maximum inhibitory potency in enzyme based assays. To render support to the experimental results, a series of quinazolinone derivatives were docked into active site of ACE and identified the probable binding modes compared to Lisinopril. Also we have identified common pharmacophore hypothesis (AAADDRR) among the best docked conformers of most potent compounds in a series of compounds. The most potent **9b**, **9c**, **9e** compounds shared common active site with the Lisinopril binding site and retained the key active site residue interactions. The obtained results from pharmacological and molecular modeling studies can be utilized for further optimization of identified hits for selective inhibition of ACE.

1. Introduction

Inspite of considerable therapeutic advances, chronic heart failure diseases (CHF) remained responsible for 27% deaths worldwide, with 80% occurring in developing countries [1,2]. Several new developments in heart failure therapy have proven benefits in survival and clinical well being. ACE inhibitors are widely used for CHF diseases, which were developed in the late 1970s and early 1980s. ACE is a central component of the renin angiotensin system (RAS) [3–6], it plays a vital role in blood regulation, it controls not only blood pressure and fluid and electrolyte homeostasis but also renal and vascular function and mycocardina remodeling [7,8]. Recent development in clinical trials are ACE inhibitors, β-blockade and aldosterone receptor antagonisms to reduce mortality and morbidity in patients with chronic heart failure (CHF) [9,10]. Driven by clinical evidence which demonstrates the morbidity and mortality benefits, ACE has become an important target for drug development in treating hypertension. Discovery of Captopril [11], the first orally active ACE inhibitor, encouraged researchers, worldwide to design and develop ACE inhibitors (using the structure of carboxy peptidase) and were clinically tested [12]. After Captopril, Lisinopril, Moexipril [13], Quinapril [14] and other several ACE inhibitors have been invented (Fig. 1), but those were hampered by common side effects like skin rash, loss of taste and dry cough in patients on long term treatment. Recent studies have demonstrated that the early combination therapy of Carvedilol and an ACE inhibitor in mild heart failure and left ventricular systolic dysfunction [15,16]. The addition of Carvedilol to ACE inhibitor and other routine heart failure therapy results in a valuable improvement in the heart failure patients [17–22]. Therefore, the design of both ACE inhibitor and β-blocker activity by structure-based drug discovery is expected to produce next generation drugs that might be safer and more effective [23]. Thus, it becomes our interest to develop new therapy of a combined ACE inhibitor and a β-blocker in CHF treatment.

A survey of literature revealed that quinazolin-4(3*H*)-one and their derivatives have received much attention during recent years due to its versatile biological activities such as anticonvulsant

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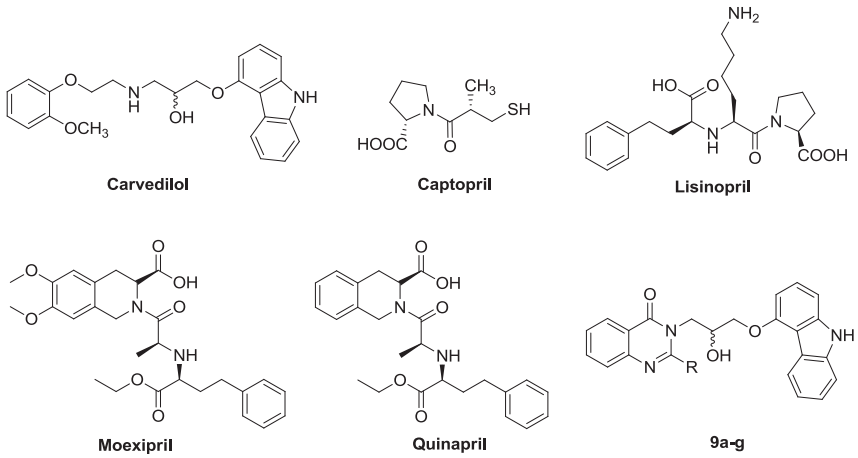


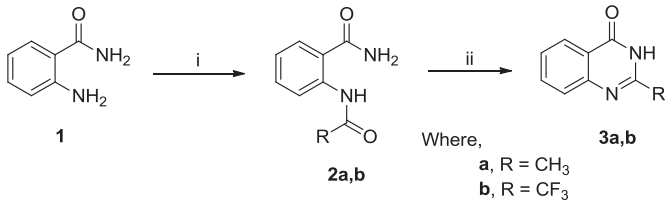
Fig. 1. Structure of Carvedilol, Captopril, Lisinopril, Moexipril, Quinapril and 3-(3-((9*H*-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-phenylquinazolin-4(3*H*)-one derivatives (**9a**–**g**).

[24–28] and CNS depressant activity [29,30]. Our on-going research programme is on the development of quinazolin-4(3*H*)-one derivatives [31–36]. Here in we devised the efficient synthesis of 3-(3-((9*H*-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-phenylquinazolin-4(3*H*)-one derivatives which has ACE inhibitory activity. The pharmacophore of quinazolin-4(3*H*)-one was designed through rational drug design and molecular hybridization approach. In addition, their ACE inhibition activity computational study was carried out to highlight the probable binding mode, key active site residue interactions and pharmacokinetic parameters [37,38]. In this study, Lisinopril-tryptophan bound crystal structure (PDB ID: 3L3N) of human ACE was considered followed by a docking study for prediction of their binding affinity. A pharmacophore modeling approach is also employed here, to help in understanding the key structural features of the ligand responsible for inhibition.

2. Results and discussion

2.1. Chemistry

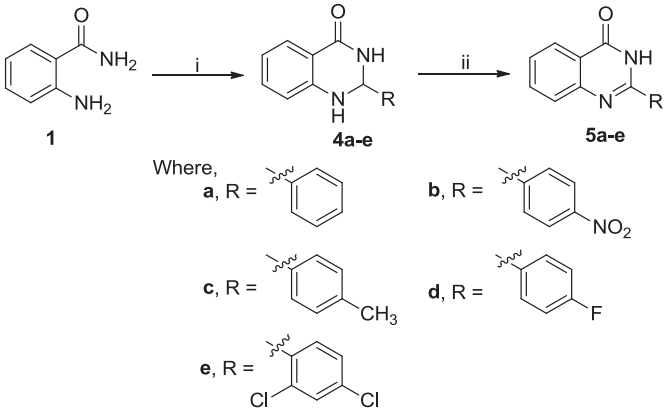
The synthesis of desired quinazolin-4(3*H*)-one derivatives could be obtained by the reaction between anthranilamide (**1**) and different anhydrides in the presence of pyridine and a catalytic amount of *N*,*N*-Dimethyl amino pyridine(DMAP) in dichloromethane(DCM) for 3h and followed by condensation with 1 N KOH in ethanol/water (1:1) system for 30 min to produce corresponding cyclic compounds (**3a**,**b**) in good yields (Scheme 1). The cyclic compounds, 2-Aryl quinazolin-4(3*H*)-one derivatives (**5a**–**e**) have been achieved in two steps starting from anthranilamide (**1**). The first synthetic step involved the condensation of anthranilamide (**1**) with aromatic aldehydes and a catalytic amount of Tetrabutyl ammonium hydrogensulphate(TBAHS) in methanol under reflux temperature for 2 h followed by oxidation with $KMnO_4$ in acetone at reflux condition for 8 h (Scheme 2) [39]. The synthesis of 4-(oxiran-2-ylmethoxy)-9*H*-carbazole (**8**) have been achieved by the reaction between 4-hydroxy carbazole (**6**) and epichlorohydrin (**7**) in presence of potassium carbonate at reflux temperature in acetone for 24 h (Scheme 3). The synthesis of quinazolinone-carbazole hybrids (**9a**–**g**) have been achieved in moderate to excellent yields by the reaction between quinazolin-4(3*H*)-one derivatives (**3a**,**b**;**5a**–**e**) and 4-(oxiran-2-ylmethoxy)-9*H*-carbazole(**8**)in the presence of potassium carbonate and a catalytic amount of Tetrabutylammoniumiodide (TBAI) at reflux temperature in acetonitrile for 36 h (Scheme 4) [40]. The structures of all the synthesized compounds were confirmed by spectral data (FTIR, ^{1}H NMR, ^{13}C NMR and ESI-MS). The target model compound **9e** was evident from the appearance of $[M+H]^+$ peak at *m*/*z* 476 in the mass spectrum (ESI), –CH–OH stretching at 1101 cm^{-1} in IR and from the characteristic CH proton as doublet of doublet at δ 4.63 with coupling constant J = 13.42 Hz and 13.58 Hz in ^{1}H NMR.



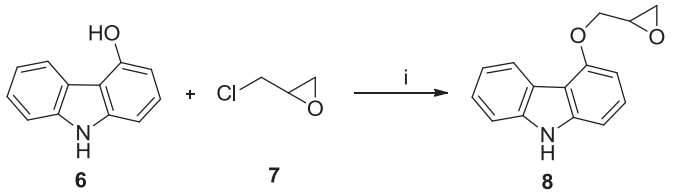
Scheme 1. Synthesis of quinazolin-4(3*H*)-one. Reagents and conditions: i) Anhydrides, Pyridine, DMAP, DCM, rt, 3 h. ii) 1 N KOH, EtOH: H_2O (1:1), reflux, 30 min.

3. Pharmacology

Angiotensin converting enzyme (ACE) inhibitory activity of newly synthesized compounds was examined *in vitro* using recently developed high-throughput colorimetric screening method [41,42]. Most of these anti-hypertensive peptides have



Scheme 2. Synthesis of 2-aryl quinazolin-4(3*H*)-one derivatives. Reagents and conditions: i) Substituted aldehydes, TBAHS, MeOH, 2 h, reflux. ii) $KMnO_4$, Acetone, 8 h, reflux.



Scheme 3. Synthesis of 4-(oxiran-2-ylmethoxy)-9*H*-carbazole. Reagents and conditions: i) K_2CO_3, Acetone, reflux, 24 h.

been characterized by the rabbit lung ACE inhibitor assay, based on the hydrolysis of the synthetic peptide hippuryl–histidyl–leucine (HHL). Angiotensin converting enzyme (ACE) hydrolyzes HHL to hippuric acid (HA) and histidyl–leucine (HL). HA released is directly proportional to the ACE activity. In this screening method, the released hippuric acid from the substrate hippuryl–histidyl–leucine (HHL) was transformed into yellow color by mixing with pyridine and benzene sulfonyl chloride. The resulted yellow color was determined colorimetrically at absorbance 410 nm. The average ACE inhibitor activity was measured in triplicate for new analogs **9a**–**g** and the standard drug Lisinopril (Fig. 2). The experiment carried out at 1.0 μM concentration of test compounds **9a**–**g** revealed 17–100% of ACE inhibition activity. Among them three compounds **9b**, **9c** and **9e** have shown better ACE inhibitory activity (>75%) compared to that of the other substituted analogs. Relating to the reference drug Lisinopril these three analogs **9b**, **9c** and **9e** were comparably active with 82.9%, 100% and 100% ACE inhibition, respectively (Fig. 2). SAR studies showed that the substitution of electron donating group on qinazolinone ring resulted in higher % of ACE inhibitory activity (see Fig. 3).

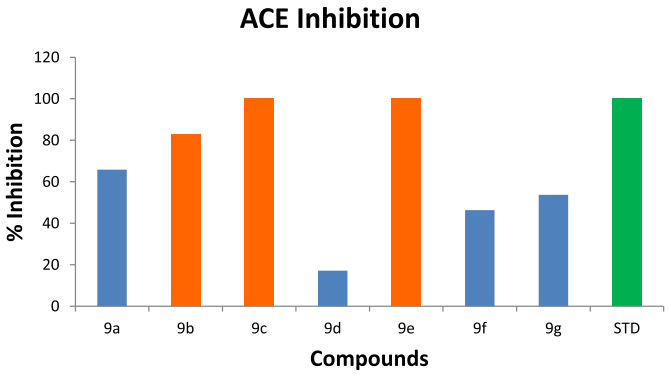


Fig. 2. *In vitro* Angiotensin Converting Enzyme (ACE) inhibition of new analogs and standard (STD) drug, Lisinopril.

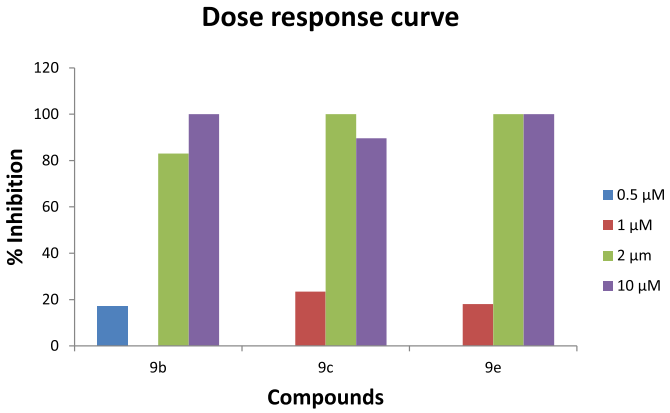


Fig. 3. Dose response curves of new derivatives **9b**, **9c** & **9e** for ACE inhibition.

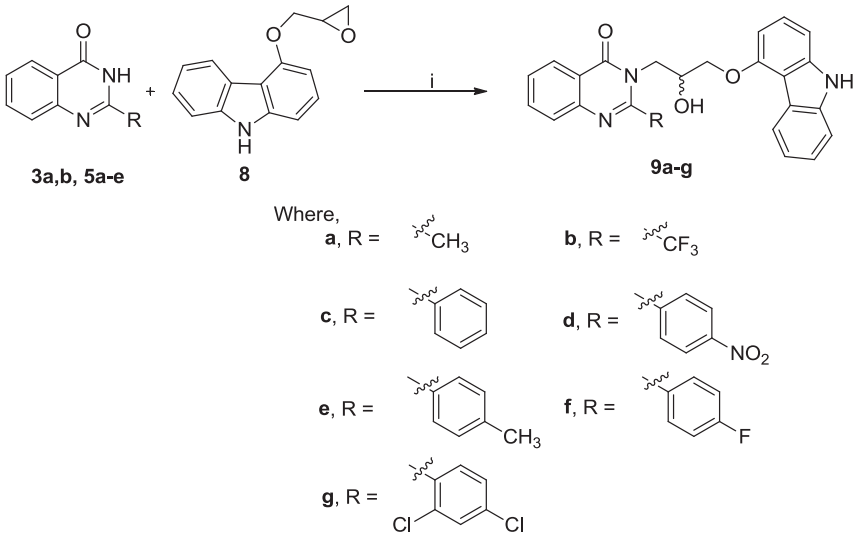
4. Molecular modeling studies

To support our hypotheses we have performed molecular modeling studies on ACE to understand the protein–ligand interactions and their binding modes as compared to Lisinopril. Even though **9e** and **9c** exhibit apparently similar level of ACE-inhibition, we chose **9e** over **9c** for molecular modeling studies. This was based on the fact that **9e** was more ACE-inhibiting than **9c** at 10 μM.

4.1. Analysis of Lisinopril and *9e* binding modes in ACE active site

4.1.1. Lisinopril drug

The 1-carboxy moiety of Lisinopril [N^2-[(1*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline] forms a network and showed hydrogen bonding and charged interaction with surrounding residues which include His383, His387, Glu411, Glu384. The His383,



Scheme 4. Synthesis of 3-(3-((9*H*-carbazol-4-yl)oxy)-2-hydroxypropyl)quinazolin-4(3*H*) one derivatives. Reagents and conditions: i) K_2CO_3, TBAI, CH_3CN, reflux, 36 h.

His387 residues showed Zn^{+2} mediated interaction with caroxylate moiety. The N2 moiety have shown hydrogen bonding interactions with Ala354 backbone residue and sidechain His353 residue. The charged amino group in lysine part of the ligand showed hydrogen bonding interactions with sidechain Asp377 and Glu162 residues. The carboxy group of proline showed hydrogen bonding interactions with sidechain Glu281, Lys511, Tyr520 residues. The proline has also showed hydrophobic interactions with Tyr520, Phe457 and Tyr523. The carbonyl moiety connected to proline showed interaction with His513 residue. The phenylprolyl moiety showed hydrophobic interactions with Val518, Phe512, Trp357 and Val351. The hydrophobic part of Lys moiety of Lisinopril was surrounded by two hydrophobic Ala354, Val380 residues (Figs. 4 and 5).

4.1.2. Compound **9e**

Similar to Lisinopril, carbonyl moiety of quinazolinone and linker hydroxyl group of compound (**9e**) showed hydrogen bonding interactions with Glu411, Glu384 and Zn^{+2} mediated interactions with His383, His387 residues. The nitrogen (N1) of quinazolinone moiety showed hydrogen bonding interactions with His513 and His353. The nitrogen of carbazole moiety showed hydrogen bonding interaction with hydroxyl group of Tyr360. The carbazole moiety has showed hydrophobic interactions with Ala63, Phe391, Trp357 and Tyr360 residues. The quinazolinone moiety was also oriented towards hydrophobic pocket and showed interactions with Tyr523, Phe457, Phe460 and Trp279 residues. Further substitution of hydrophobic moieties with electron-donating groups might be beneficial for enhancing its potency against ACE. The substituted hydrophobic moieties at 2nd position of quinazolinone ring could be favorable for enhancing its potency by interacting with neighboring hydrophobic residues i.e Phe512, Val518 and Val351. Further the substituted nitro group (**9d**) at 2nd position of quinazolinone ring was detrimental to the activity because the nitro group was oriented in close proximity to Glu143. For key active site residues around 5 Å see Supplementary Information. The probable binding mode of most potent compound (**9e**) in quinazolinone series and reference compound Lisinopril are depicted in

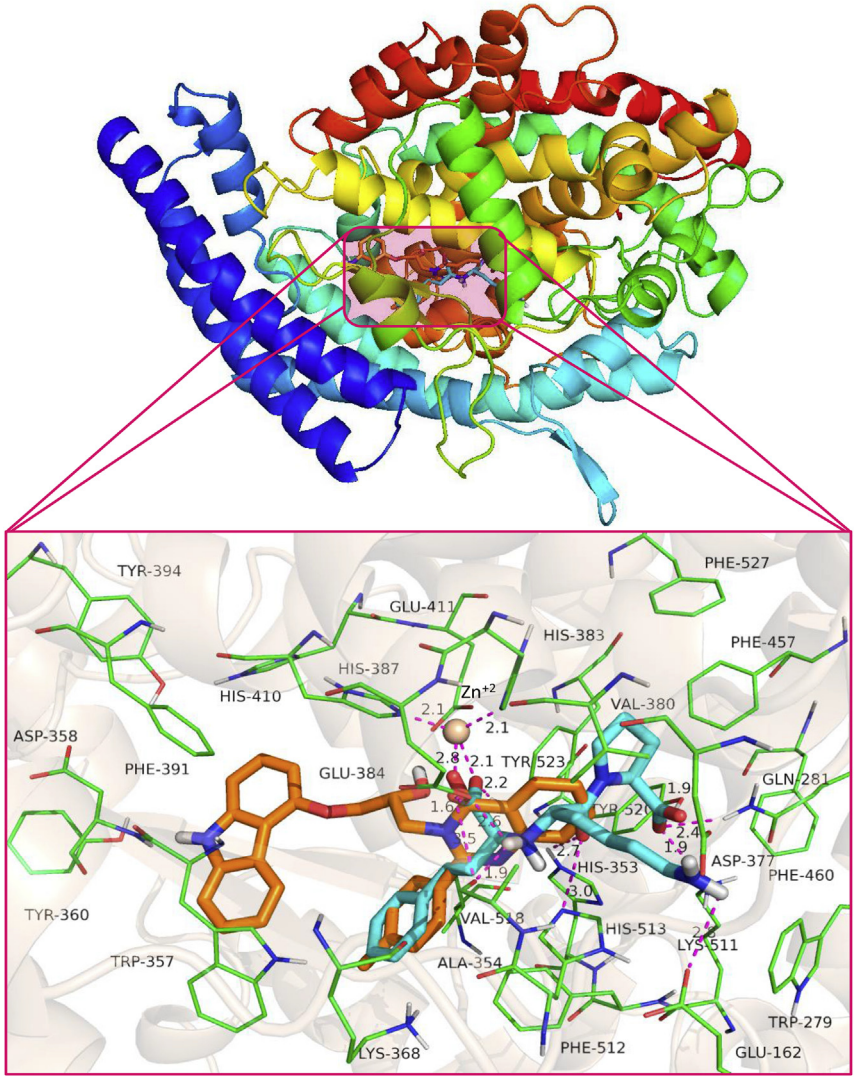


Fig. 4. Binding mode of reference Lisinopril (cyan) and **9e** in the active sites of ACE, the hydrogen bond interactions are shown in pink color dotted lines, Zn^{+2} is shown in sphere in ash color, he important residues are shown in green color and protein cartoon is represented in wheat color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

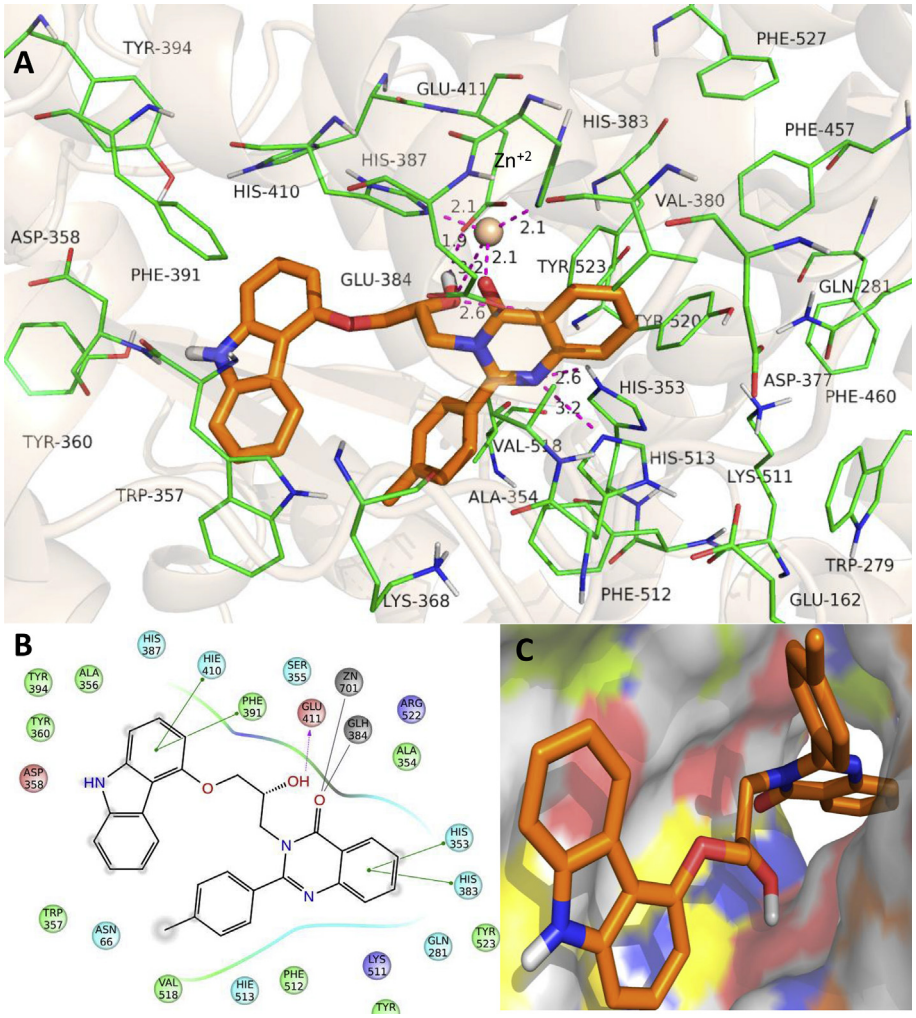


Fig. 5. A: Probable binding mode of **9e** (orange) in the active sites of ACE, the hydrogen bond interactions are shown in pink color dotted lines, Zn^{+2} is shown in sphere in ash color, the important residues are shown in green color and protein cartoon is represented in wheat color. B: 2D-ligand interaction plot and C: Surface active site pocket of ACE with **9e**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

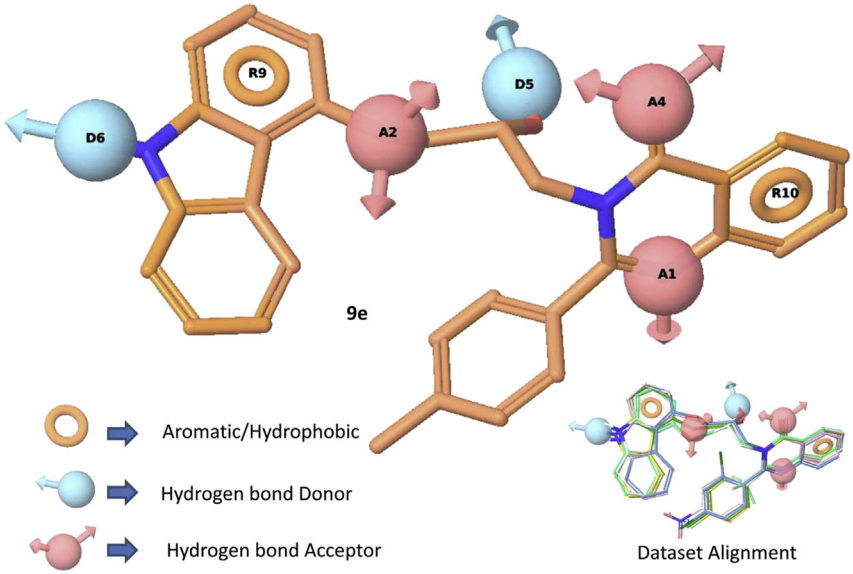


Fig. 6. A: Seven featured (AAADDRR) Pharmacophore hypothesis; A: hydrogen bond acceptor, D: hydrogen bond donor, R: aromatic ring.

Figs. 4 and 5. Also we have identified common pharmacophore hypothesis from the best docked poses of most potent compounds and is shown in Fig. 6.

5. Conclusion

In summary, we have developed and synthesized a novel class of ACE inhibitors. Most of the prepared compounds have been showed ACE inhibitory activity in enzyme based assays. Compounds **9b**, **9c** and **9e** were found to be potent ACE inhibitors. The computational techniques such as molecular docking and pharmacophore studies have been applied to support our experimental results. From our investigation we found that Lisinopril and our most potent compound **9e** have shown common hydrogen bonding interactions with Glu411, Glu384 and Zn^{+2} mediated interactions with His383, His387 residues, which are critical to access maximum ACE inhibitory potency. Apart from the common interactions compared to Lisinopril, our compounds especially quinazolinone moiety were explored in a hydrophobic binding pocket surrounded by Tyr523, Phe457, Phe460 and Trp279 residues. Substitution of lipophilic moieties along with electron-donating groups in hydrophilic pocket might be beneficial for enhancing its potency against ACE. The obtained pharmacophore and docking results may afford valuable clues to optimize the quinazolinone lead compound for the potent and selective inhibition of ACE. The results presented here may help in understanding of their β-blocker activity and further investigations.

6. Experimental section

6.1. Chemistry

All the chemicals and reagents were purchased from Sigma–Aldrich and SD Fine-Chemicals, Pvt. Ltd. India, and used as received. The reactions were monitored and R_f value were determined using analytical thin layer chromatography (TLC) with Merck Silica gel 60–120 and F_{254} pre-coated plates (0.25 mm thickness). Spot on the TLC plates were visualized using ultraviolet light (254 nm). Flash column chromatography was performed with Merck silica gel (100–200 mesh). Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DRX-300, Varian 400 and Varian-500 NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker DRX-300. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.00) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26; DMSO-d_6 δ 2.54) and multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines). Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 spectrometer; data is reported in wave numbers (cm^{-1}). Mass spectra were recorded on Agilent Technologies 1100 Series (Agilent Chemistation Software). High-resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry. All computations and molecular modeling studies have been carried out on Schrodinger software.

6.2. General procedure for the synthesis of ***3a–b***

To a stirred solution of anthranilamide (1 g, 0.007 mol) in DCM (20 ml), substituted anhydrides (0.008 mol), pyridine (0.95 mL) and DMAP (2 mol%) were added at RT. The reaction mixture was stirred at RT for 3 h. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (10 mL) and extracted with DCM (3 × 10 mL). The DCM extracts were combined, dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product obtained was purified by flash column chromatography over silica gel (20:80, ethyl acetate/hexane) to afford compounds **2a–b**.

The obtained acylated product was dissolved in EtOH: H_2O (1:1) and 1 N KOH was added at room temperature. After heating at reflux temperature for 0.5 h, the reaction mixture was cooled to RT and then generated solid was filtered, recrystallized from EtOH to give quinazolin-4(3*H*)-one derivatives **3a**,**b** in the form of white, dense needles.

6.2.1. 2-(Trifluoromethyl)quinazolin-4(3H)-one (*3b*)

Yield 81%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (t, J = 7.17 Hz, 7.55 Hz, 1H, Ar–H), 7.77–7.91 (m, 2H, Ar–H), 8.29 (d, J = 7.93 Hz, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 115.63, 119.21, 122.24, 127.84, 128.24, 134.46, 146.33, 161.44.

6.3. General procedure for the synthesis of ***5a–e***

A solution of 2-aminobenzamide (1 g, 0.007 mol), aromatic aldehyde (0.007 mol) and a catalytic amount of TBAHS (1 mol%) in MeOH was made to react at reflux temperature for 2 h. The reaction mixture was then cooled to room temperature and small amount of water (5 mL) was added to the mixture, and then generated solid was filtered to give cyclic compound **4a–e**. Intermediates **4a–e** were totally converted into **5a–e**, by the oxidation with $KMnO_4$ (0.018 mol) in acetone under reflux conditions for 8 h, The obtained solid was recrystallized from EtOH to give 2-aryl quinazolin-4(3*H*)-one derivatives **5a–e** in the form of long, white, dense needles.

6.3.1. 2-Phenylquinazolin-4(3H)-one (*5a*)

Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (t, J = 7.55 Hz, 1H, Ar–H), 7.59 (br, 4H, Ar–H), 7.84 (s, 1H, Ar–H), 8.23–8.39 (m, 3H, Ar–H), 11.80 (br, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 120.62, 125.48, 125.74, 127.11, 128.05, 130.64, 132.47, 133.73, 148.68, 151.85, 162.55.

6.3.2. 2-(p-Tolyl)quinazolin-4(3H)-one (*5c*)

Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 7.31 (d, J = 7.93 Hz, 2H, Ar–H), 7.40–7.50 (m, 1H, Ar–H), 7.76 (d, J = 3.77 Hz, 2H, Ar–H), 8.10 (d, J = 8.12 Hz, 2H, Ar–H), 8.25 (d, J = 7.74 Hz, 1H, Ar–H), 11.82–12.45 (br, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.52, 120.75, 126.33, 126.51, 127.27, 127.87, 129.72, 129.94, 134.80, 142.15, 149.59, 151.74, 163.85.

6.3.3. 2-(4-Fluorophenyl)quinazolin-4(3H)-one (*5d*)

Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (t, J = 8.49 Hz, 8.68 Hz, 2H, Ar–H), 7.47 (t, J = 7.93 Hz, 1H, Ar–H), 7.66–7.85 (m, 2H, Ar–H), 8.19–8.40 (m, 3H, Ar–H).

6.3.4. 2-(2,4-Dichlorophenyl)quinazolin-4(3H)-one (*5e*)

Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48–7.58 (m, 2H, Ar–H), 7.63–7.74 (m, 3H, Ar–H), 7.82 (t, J = 6.98 Hz, 8.12 Hz, 1H, Ar–H), 8.20 (d, J = 7.93 Hz, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 119.65, 124.08, 125.15, 125.51, 125.67, 127.43, 130.27, 130.91, 131.20, 132.49, 133.98, 146.82, 149.50.

6.4. General procedure for the synthesis of compound ***8***

To a suspension of 4-hydroxy carbazole (5 g, 0.027 mol) and K_2CO_3 (7.54 g, 0.054 mol) in acetone (80 mL), epichlorohydrin (2.50 mL, 0.032 mol) was added. The reaction mixture was refluxed for 24 h. After completion of the reaction, as indicated by TLC, the acetone in the reaction mixture was removed under reduced pressure. The obtained residue was dissolved in ethyl acetate (75 mL), organic phase was washed with water (3 × 15 mL),

separated, dried with Na_2SO_4, evaporated under reduced pressure and purified by column chromatography over silica gel (25:75, ethyl acetate/hexane) to afford **8** as solid compound.

Yield 64%; mp 167–169 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81–2.88 (dd, J = 2.45, 2.64 Hz, $1H_a$, CH_2), 2.90–2.97 (dd, J = 4.34, 4.91 Hz, $1H_b$, CH_2), 3.50–3.57 (m, $1H_c$, CH), 4.04–4.14 (dd, J = 6.23 Hz, $1H_e$, CH_2), 4.52–4.59 (dd, J = 2.26 Hz, $1H_d$, CH_2), 6.70 (d, J = 7.93 Hz, 1H, Ar–H), 7.05–7.20 (m, 2H, Ar–H), 7.25–7.39 (m, 2H, Ar–H), 7.46 (d, J = 7.93 Hz, 1H, Ar–H), 8.17 (d, J = 7.14 Hz, 1H, Ar–H), 11.27 (s, 1H, NH); ESI MS: m/z = 239 [M^+].

6.5. General procedure for the synthesis of **9a–g**

A mixture of 2-methyl quinazolin-4(3*H*)-one **3a** (150 mg, 0.937 mmol), 4-(oxiran-2-ylmethoxy)-9*H*-carbazole (**8**, 222 mg, 0.937 mmol), potassium carbonate (258 mg, 1.875 mmol) and tetrabutylammoniumiodide (5 mg, 0.014 mol) were heated in acetonitrile (15 mL) at 80 °C for 36 h under nitrogen atmosphere. The reaction mass was extracted with ethyl acetate, separated and the organic layer was dried over sodium sulfate and concentrated. The crude product was purified by column chromatography over silica gel (65:35, ethyl acetate/hexane) to give following compounds:

6.5.1. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-methylquinazolin-4(3H)-one (**9a**)

Yield 77%; mp 111–114 °C; IR (KBr) υ in cm^{-1}: 1096, 1631, 2923, 3435; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (s, 3H, CH_3), 4.39 (d, J = 5.28 Hz, $1H_a$, CH_2), 4.50 (s, $1H_b$, CH_2), 4.53 (s, $1H_a$, CH_2), 4.57 (d, J = 3.02 Hz, $1H_b$, CH_2), 4.61–4.71 (m, 1H, CH), 6.72 (d, J = 7.55 Hz, 1H, Ar–H), 7.10 (d, J = 7.55 Hz, 1H, Ar–H), 7.32–7.52 (m, 4H, Ar–H), 7.66 (d, J = 7.55 Hz, 1H, Ar–H), 7.72–7.80 (m, 1H, Ar–H), 8.13 (s, 1H, Ar–H), 8.28 (d, J = 8.30 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.70, 48.40, 66.29, 69.83, 104.31, 109.79, 110.21, 119.71, 122.66, 126.74, 129.97, 130.89, 131.12, 134.01, 135.20, 141.33, 142.41, 145.58, 148.34, 151.85, 154.58, 157.32, 160.40, 160.52; ESI MS: m/z = 400 [$M+H$]$^+$; HRMS calculated for $C_{24}H_{21}N_3O_3$: 400.1600, Found: 400.1666.

6.5.2. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-(trifluoromethyl)quinazolin-4(3H)-one (**9b**)

Yield 72%; mp 99–102 °C; IR (KBr) υ in cm^{-1}: 1099, 1213, 1607, 2927, 3401; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80–4.11 (m, 2H, CH_2), 4.30–4.39 (m, 2H, CH_2), 4.42–4.67 (m, 1H, CH), 6.68 (d, J = 8.30 Hz, 1H, Ar–H), 7.07 (d, J = 8.30 Hz, 1H, Ar–H), 7.30–7.47 (m, 3H, Ar–H), 7.51–7.68 (m, 2H, Ar–H), 7.87 (d, J = 3.77 Hz, 1H, Ar–H), 8.08–8.17 (m, 1H, Ar–H), 8.22 (d, J = 8.30 Hz, 1H, Ar–H), 8.35 (d, J = 8.30 Hz, 1H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 53.64, 68.40, 71.24, 101.24, 104.10, 110.12, 119.75, 122.35, 122.70, 124.99, 126.67, 126.86, 128.71, 129.28, 135.52, 136.64, 139.29, 140.98, 143.42, 150.59, 154.42, 154.83, 160.82, 162.09; ESI MS: m/z = 476 [$M+Na$]$^+$; HRMS calculated for $C_{24}H_{18}F_3N_3O_3$: 476.1300, Found: 476.1409.

6.5.3. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-phenylquinazolin-4(3H)-one (**9c**)

Yield 78%; mp 105–108 °C; IR (KBr) υ in cm^{-1}: 1107, 1262, 1667, 2926, 3326; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.05–4.20 (m, 5H, $2\times CH_2$, $1\times CH$), 6.52 (s, 1H, Ar–H), 7.01–7.15 (m, 2H, Ar–H), 7.21–7.45 (m, 6H, Ar–H), 7.49–7.64 (m, 3H, Ar–H), 7.74 (s, 2H, Ar–H), 8.02 (d, J = 7.55 Hz, 1H, Ar–H), 8.35 (s, 1H, Ar–H), 9.90 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.37, 64.96, 69.08, 98.80, 102.79, 108.81, 110.61, 117.46, 119.51, 120.59, 121.51, 123.09, 124.83, 125.11, 125.33, 125.84, 126.92, 127.36 (2C), 128.06, 132.87, 134.37, 137.76, 140 (2C), 145.89, 153.36, 155.55, 160.98; ESI MS: m/z = 462 [$M+H$]$^+$; HRMS calculated for $C_{29}H_{23}N_3O_3$: 462.1700, Found: 462.1806.

6.5.4. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-(4-nitrophenyl)quinazolin-4(3H)-one (**9d**)

Yield 71%; mp 101–104 °C; IR (KBr) υ in cm^{-1}: 1104, 1349, 1682, 2924, 3420; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.88–3.94 (dd, J = 11.44 Hz and 11.90 Hz, $1H_a$, CH_2), 3.97–4.03 (dd, J = 11.44 Hz, $1H_b$, CH_2), 4.12–4.17 (dd, J = 11.90 Hz, 2H, CH_2), 4.36 (dd, 1H, CH), 6.66–6.70 (dd, J = 7.78 Hz, 1H, Ar–H), 7.06–7.09 (dd, J = 8.08 Hz, 1H, Ar–H), 7.11–7.14 (dd, J = 8.54 Hz and 8.69 Hz, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.31 (d, J = 3.81 Hz, 1H, Ar–H), 7.33 (d, J = 3.96 Hz, 1H, Ar–H), 7.34 (d, J = 3.81 Hz, 1H, Ar–H), 7.36 (s, 1H, Ar–H), 7.38–7.42 (d, J = 8.85 Hz, 2H, Ar–H), 7.53 (d, J = 8.69 Hz, 1H, Ar–H), 7.95 (t, J = 8.69 Hz and 9.00 Hz, 1H, Ar–H), 8.14–8.19 (br, 1H, Ar–H), 8.21 (d, J = 7.62 Hz, 1H, Ar–H), 8.28 (t, J = 8.69 Hz, 1H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 48.10, 62.32, 69.94, 96.29, 100.04, 106.26, 108.39, 117.73, 118.01, 120.08, 121.10, 123.76, 124.79 (2C), 126.02, 128.07, 132.92, 134.56, 138.20, 140.01, 153.04, 155.57, 161.02; ESI MS: m/z = 507 [$M+H$]$^+$; HRMS calculated for $C_{29}H_{22}N_4O_5$: 507.1600, Found: 507.1705.

6.5.5. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-(p-tolyl) quinazolin-4(3H)-one (**9e**)

Yield 80%; mp 97–100 °C; IR (KBr) υ in cm^{-1}: 1101, 1346, 1607, 2923, 3404; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.13 (s, 3H, CH_3), 4.07–4.23 (dd, J = 14.80 Hz and 15.25 Hz, 2H, CH_2), 4.42–4.52 (dd, J = 13.58 Hz and 17.09 Hz, 2H, CH_2), 4.63 (dd, J = 13.42 Hz and 13.58 Hz, 1H, CH), 6.55 (d, J = 7.93 Hz, 1H, Ar–H), 7.01–7.10 (dd, J = 20.75 Hz and 21.05 Hz, 2H, Ar–H), 7.14–7.18 (m, 1H, Ar–H), 7.28 (d, J = 8.08 Hz, 1H, Ar–H), 7.35–7.42 (dd, J = 10.07 Hz and 15.10 Hz, 3H, Ar–H), 7.53 (t, J = 6.71 Hz, 1H, Ar–H), 7.73–7.80 (m, 2H, Ar–H), 7.92 (d, J = 7.78 Hz, 1H, Ar–H), 8.16 (s, 1H, Ar–H), 8.36 (d, J = 7.93 Hz, 1H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.09, 49.66, 69.28, 69.69, 100.90, 103.97, 109.89, 112.56, 119.61, 120.42, 122.23, 122.88, 124.90 (2C), 126.46, 126.74, 127.12, 127.46 (2C), 127.95, 129.32, 132.04, 134.70, 138.62, 140.09, 140.84, 147.22, 154.60, 156.50, 164.12; ESI MS: m/z = 476 [$M+H$]$^+$; HRMS calculated for $C_{30}H_{25}N_3O_3$: 476.1900, Found: 476.1966.

6.5.6. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-(4-fluorophenyl)quinazolin-4(3H)-one (**9f**)

Yield 72%; mp 116–119 °C; IR (KBr) υ in cm^{-1}: 1025, 1102, 1668, 2254, 3439; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.21 (d, J = 4.42 Hz, $1H_a$, CH_2), 4.30–4.33 (s, $1H_b$, CH_2), 4.41–4.44 (dd, J = 2.59 Hz, $1H_a$, CH_2), 4.45–4.47 (m, $1H_b$, CH_2), 4.48–4.58 (m, 1H, CH), 6.57 (d, J = 7.93 Hz, 1H, Ar–H), 6.67 (d, J = 7.93 Hz, 1H, Ar–H), 6.93 (t, J = 8.54 Hz, 1H, Ar–H), 7.07 (t, J = 8.08 Hz and 10.37 Hz, 1H, Ar–H), 7.17 (t, J = 7.78 Hz and 7.93 Hz, 1H, Ar–H), 7.40 (t, J = 6.56 Hz, 1H, Ar–H), 7.50 (dd, J = 8.39 Hz and 8.54 Hz, 1H, Ar–H), 7.55 (t, J = 7.32 Hz and 7.78 Hz, 1H, Ar–H), 7.74 (d, J = 7.78 Hz, 1H, Ar–H), 7.78–7.83 (m, 1H, Ar–H), 7.90 (d, J = 7.78 Hz, 1H, Ar–H), 8.11–8.17 (m, 1H, Ar–H), 8.22 (d, J = 7.78 Hz, 1H, Ar–H), 8.30 (d, J = 7.78 Hz, 1H, Ar–H), 8.37 (d, J = 7.32 Hz, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.37, 66.56, 69.71, 99.79, 103.69, 109.65, 111.79, 114.86, 115.15, 118.45, 120.36, 121.58, 122.34, 124.08, 125.66, 126.12, 126.42, 126.84, 130.33, 130.44, 131.25, 133.87, 138.60, 140.84, 146.67, 154.21, 155.50, 162.34, 164.20; ESI MS: m/z = 480 [$M+H$]$^+$; HRMS calculated for $C_{29}H_{22}FN_3O_3$: 480.1600, Found: 480.1716.

6.5.7. 3-(3-((9H-carbazol-4-yl)oxy)-2-hydroxypropyl)-2-(2,4-dichlorophenyl) quinazolin-4(3H)-one (**9g**)

Yield 84%; mp 112–115 °C; IR (KBr) υ in cm^{-1}: 1025, 1102, 1680, 2924, 3418; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99–4.13 (m, $1H_a$, CH_2), 4.18 (s, $1H_b$, CH_2), 4.32 (s, $1H_a$, CH_2), 4.47–4.64 (m, $1H_b$, CH_2), 4.75 (dd, 1H, CH), 6.45 (d, J = 7.55 Hz, 1H, Ar–H), 6.55 (d, J = 7.74 Hz, 1H, Ar–H), 6.71 (d, J = 7.55 Hz, 1H, Ar–H), 7.06 (d, J = 7.55 Hz, 1H, Ar–H), 7.13–7.37 (m, 2H, Ar–H), 7.38–7.45 (m, 1H, Ar–H),

7.46–7.50 (m, 1H, Ar–H), 7.55 (t, $J = 8.12$ Hz, 1H, Ar–H), 7.68–7.83 (m, 2H, Ar–H), 7.92 (d, $J = 6.42$ Hz, 1H, Ar–H), 8.18 (d, $J = 7.17$ Hz, 1H, Ar–H), 8.36 (d, $J = 7.93$ Hz, 1H, Ar–H), 10.59–10.80 (m, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.21, 66.06, 69.54, 99.58, 103.49, 109.50, 111.44, 118.24, 120.46, 121.33, 122.12, 123.84, 125.45, 125.86, 126.66, 128.04, 130.54, 132.01, 132.33, 132.51, 133.67, 135.25, 135.46, 138.41, 140.62, 146.50, 152.89, 154.02, 161.22; ESI MS: $m/z = 530$ $[M + H]^+$; HRMS calculated for $C_{29}H_{21}Cl_2N_3O_3$: 530.1000, Found: 530.1034.

6.6. Methodology for molecular modeling studies

All computations and molecular modeling studies have been carried out on Schrodinger software [Maestro version 9.3, Schrödinger, LLC, New York, NY 2013.].

6.6.1. Ligand preparation

A series of seven quinazolinone compounds were synthesized and tested for biological assays against ACE. These quinazolinone compounds were drawn in ISIS draw and converted into 3D-molecules with all possible tautomers. The converted 3D-molecules were minimized with OPLS-2005 force field using water as solvent.The minimization of molecules was carried out using Polak-Ribiere Conjugate Gradient (PRCG) method with maximum of 5000 iterations. The minimized compounds were used for molecular docking study.

6.6.2. Protein preparation

The co-crystal structure of ACE (Uniport ID: P12821) with Lisinopril-tryptophan analog (PDB ID: 3L3N) has been selected for the docking studies. The ACE structure was prepared by adjusting bond orders, tautomers and adding hydrogen atoms using protein preparation wizard of Schrödinger software graphical user interface Maestro v9.3. Further the proteins were minimized by OPLS_2005 force field with converge heavy atoms to RMSD 0.3 Å relative to original protein structure.

6.6.3. Docking studies

The docking study has been carried out using Schrödinger docking program Glide. The prepared protein complex (3L3N) was used for grid generation using the default value of protein atom scaling (1.0) within a cubic box centered on the co-crystal ligand. No constraints were imposed and standard precision (SP) docking of ligands was carried out with default value of ligand atom scaling (0.8). The post docking minimization has been carried out and maximum of 5 poses per ligand was saved. The protein-ligand interactions, hydrophobic residue contributions of quinazolinone series of compounds were analyzed and compared with reference ligand Lisinopril in the active site of ACE. The obtained docked modes of quinazolinone series of compounds were subjected to pharmacophore modeling. The identified seven featured common pharmacophore hypothesis (AAADDRR) can be used to screen the large dataset of compounds.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.04.009.

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