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Original article

Design and green synthesis of 2-(diarylalkyl)aminobenzothiazole derivatives and their dual activities as angiotensin converting enzyme inhibitors and calcium channel blockers

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ABSTRACT

A series of novel 2-(diarylalkyl)aminobenzothiazoles were designed based on commercial synthetic calcium channel blockers (CCBs) and angiotensin converting enzyme (ACE) inhibitors. Which are further modified based on the natural products which are angiotensin converting enzyme (ACE) inhibitors. Completely green protocol was developed for their synthesis. As they are initially designed based on CCBs, they were screened for their ACE inhibition property believing that almost all the compounds will be CCBs. Out of 42 compounds two lead molecules were identified as ACE inhibitors, which were further screened for CCB. As expected both were identified as CCBs with different selectivity over ACE inhibition. Their selectivity over ACE and CCB can be used to treat resistant hypertension.

1. Introduction

Cardiovascular disease remains number one cause of death worldwide [1]. Although the causes of cardiovascular disease are diverse, hypertension remains the most common and crucial one. Elevated blood pressure is the most common disorder in developed as well as developing countries. Because of increasing prevalence of hypertension, efforts were made to find new drug molecules which may reduce blood pressure by inhibiting several important mediators of hypertension. Multiple etiologies and environmental factors contribute to the development of hypertension [2,3]. Despite extensive ongoing research in academia and industry, some forms of hypertension remain untreatable. Several drugs, available in the market as antihypertensives, are targeting different pathways or modulators of hypertension. But treating resistant hypertension is still in infancy. Combination of three or more classes of drugs like ACE inhibitors (or) angiotensin receptor blocker, CCBs and diuretics might be the right approach to reduce hypertension-related cardiovascular deaths [4]. Among all those classes of drugs, angiotensin converting enzyme (ACE) inhibitors got a wide acceptance and become first line of therapy. Several ACE inhibitors like Lisinopril, Enalapril (Fig. 1) etc. are in clinical use for the treatment of hypertension [5,6]. All these compounds are arylalkylamines in which aryl and amine groups are separated by a three membered carbon chain. However, severe associated side effects (i.e. dry cough, hyperkalemia, rashes, loss of taste and first dose hypotension) limit their use justifying the development of new safe ACE inhibitors [7–9]. On the other hand there are several natural products such as chalcones (Butein, Fig. 1) [10], Styrenes [11], flavanoids (Astragalin, Isoquercitrin etc.) [12], Xanthones [13] with multiple hydroxyl groups on aromatic rings. It is also evident that blocking the free hydroxyl groups on Xanthones with acetyl group decreases the ACE inhibition property of the compounds indicating that free hydroxyl groups playing a key role in inhibition of ACE [13].

Abbreviations: ACE, Angiotensin Converting Enzyme; BSC, Benzenesulfonyl Chloride; tACE, Testicular Angiotensin Converting Enzyme; CCB, Calcium Channel Blocker; MVD, Molegro Virtual Docker; KHB, Krebs–Hensleit Buffer; PDB, Protein Data Bank; RCSB, Research Collaboratory for Structural Bioinformatics; HHL, Hippuryl-Histidyl-Leucine; HA, Hippuric Acid; PMA, Phosphomolybdic Acid.

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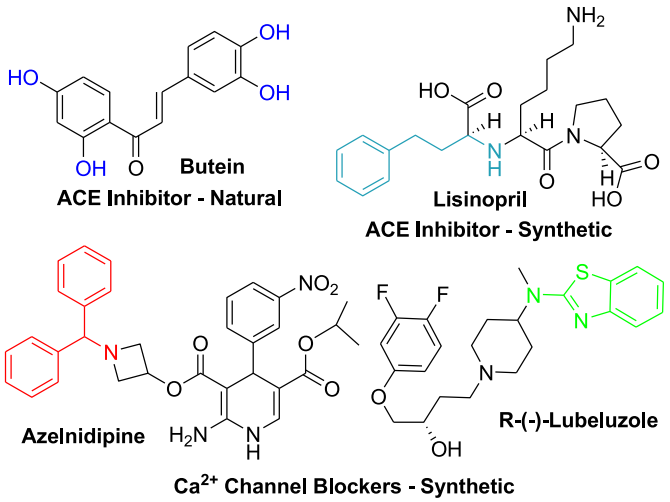


Fig. 1. Structures of ACE Inhibitors and Ca^{2+} Channel blockers.

Second most common antihypertensive drugs are calcium channel blockers (CCB) (Figs. 1 and 2). Most of these drug molecules are having the basic skeleton of arylalkylamine, with varying the length of alkyl chain from one to three and with different substituents on aryl, amine or on both the groups. There are very few reports on diarylalkylamines as antihypertensive agents such as Azelnidipine (Fig. 1) and Flunarizine (Fig. 2) which act as CCB.

Similar to arylalkylamines, several 2-aminobenzothiazoles-derivatives (Figs. 1 and 2) were also explored for their antihypertensive property. 2-aminobenzothiazoles act as antihypertensive by inhibiting acid induced Ca^{2+} uptake [14], altering intracellular Ca^{2+} concentration [15] and inhibiting veratridine-induced atrium contraction [16]. These derivatives are also effective inhibitor of histamine-induced angiogenesis [17] and veratridine-induced increase in oxygen consumption and uptake of Ca^{2+} ions [18], and showed protection in the veratridine-induced calcium (Ca^{2+}) overload model [19]. Commercially available drug Riluzole, a derivative of 2-aminobenzothiazole is known to act as glutamate uptake stimulator [20], membrane channel activity stimulator [21], intracellular Ca^{2+} level reducer [22], high voltage-activated calcium channel inhibitor [23].

It was observed that a diarylalkylamine i.e. Flunarizine and a 2-aminobenzothiazole i.e. R 56865 (Fig. 2) suppress veratridine-induced increase in oxygen consumption and uptake of Ca^{2+} ions [18]. Based on all above data, we decided to replace the amino group in diarylalkylamine with 2-aminobenzothiazole and thus forming 2-(diarylalkyl)aminobenzothiazole derivatives to screen CCB properties. It is note worthy that all the CCBs are having phenyl group as aromatic group and they do not carry any hydroxyl group, whereas most of the natural products such as Xanthones, Flavones etc. which are ACE inhibitors, carry multiple hydroxyl groups on aromatic rings. Based upon earlier report that decrease in free hydroxyl groups on them decreases ACE inhibition [13]. We are interested to substitute 2-(diarylalkyl)aminobenzothiazole derivatives with at least one hydroxyl group on aryl groups. In over all we designed hybrid molecules in which two aryl groups and one 2-aminobenzothiazole group are attached to the same carbon with the properties based on CCB property and with atleast one hydroxyl group on aryl groups based on natural products which are ACE inhibitors. With the above hybrid structure we are expecting that the new analogues may also act as ACE inhibitors along with CCB, which is their inherent property.

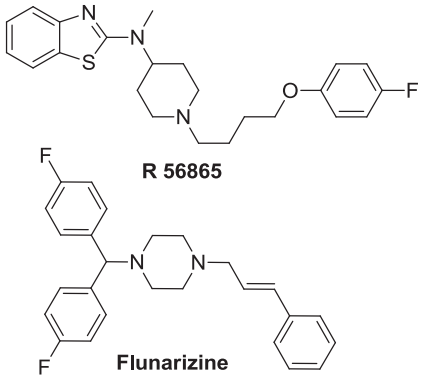


Fig. 2. Structures of R 56865 and Flunarizine.

2. Chemistry

In search for the existence of compounds with the above skeleton we found few methodologies in which the hydroxyl group is placed on the napthyl ring [24–26]. However all these methodologies suffer from serious draw backs such as high temperatures, long reaction times, use of solvent and catalyst. As a result the reaction mixture needed to be purified by traditional workup and column chromatography techniques. The low solubility of the product in several organic solvents makes the purification difficult and requires huge amounts of organic solvents for purification. Moreover, yield of the purified product after column chromatography is very low. Hence there is a need to develop a method for the synthesis of these compounds with better yield, good purity and least solvent consumption. Several attempts (Tables 1 and 2) were made to develop a methodology which gives higher yields with more purity. Finally we found the reaction proceeds smoothly on a preheated oilbath at a temperature of about 110–120 °C using no solvent and no catalyst with a relatively short span of time.

Initially we attempted a three component reaction using Benzaldehyde(1), β-Napthol(2) and 2-Aminobenzothiazole **(3)** as substrates to stabilize the reaction. The methodology so developed is further applied to the synthesis of several of its derivatives (**4aa**–**4bp**). The reaction proceeds smoothly with a variety of substituents on both aldehyde and 2-aminobenzothiazole moieties and even with aryl substituted aliphatic aldehydes (compound **4aa**, entry 1, Table 3). Schematically the reaction can be represented as in Scheme 1.

From the above experiments it was observed that the rate of reaction is fast under solvent free conditions. But yield is very low without a catalyst. This is because during the traditional heating process β-Napthol is reacting with aldehyde forming xanthenes leaving small amount of β-Napthol available when it reaches 100 °C. This was further confirmed by carrying the reaction at lower temperature which forms xanthenes exclusively. To overcome the

Table 1
Preliminary studies of three component coupling reaction.

Entry	Solvent (mL)	Temp °C	Time (min)	Yield (%)
1	PEG-400 (3)	100	100	30
2	Toluene (3)	100	100	32
3	Toluene (3)	100	150	30
4	Toluene (1)	100	100	35
5	*m*-Xylene (1)	100	100	35
6	–	100	100	20
7	–	100	150	25
8	–	50	100	0
9	–	75	150	23

Table 2
Reactions on a preheated oilbath without solvent.

Entry	Temp (°C)	Time (min)	Yield (%)
1	50	100	0
2	80	100	60
3	100	100	80
4	110	100	95
5	110	60	94
6	110	40	94
7	110	20	47
8	130	20	70

problem we kept the reaction over a preheated oil bath without any solvent and catalyst, good yields were obtained as shown in Table 2.

As observed above 110 °C was found to be the ideal temperature to carry out the reaction within a short span of time i.e. 40 min. Because of the low solubility of the product in many organic solvents the reaction mixture was cooled to room temperature and the so formed solid is powdered and washed with ethanol or methanol (3 × 8 mL) to obtain the pure product 1-((benzo[d]thiazol-2-ylamino)(phenyl)methyl) naphthalen-2-ol in 94% yield. It's analogues can be washed with 3 × 8 mL in case of halogenated compounds except fluorinated, 3 × 4 mL in case of fluoro and methoxy derivatives. It is noteworthy to handle fluoro and methoxy derivatives carefully which otherwise may easily washed off in methanol/ethanol. The scope and generality of the reaction is shown in Table 3.

Reactions with *ortho*-substituted aldehydes were relatively slow when compared to their *meta*- and *para*- counter parts. So such reactions were kept at a temperature of 120 °C and heated for few minutes excess. As expected the product **4aa** formed using 2-Phenylpropanal produced four isomers forming a pair of enantiomers as major leaving the other pair of enantiomers as minor products. The reaction mixture was washed repeatedly with methanol to remove the minor enantiomers. However it was observed that it is not always possible to purify these isomers by this method. Only major enantiomeric pair was screened for its ACE inhibitory activity.

3. Pharmacology

3.1. In vitro inhibition of ACE

The ACE inhibition activity for new analogues **4aa**–**4bp** and the standard drug, Lisinopril were presented in Fig. 3. The experiment was carried out at 1.0 μM concentration of test and standard compounds. Our data revealed that all compounds tested in the present study have 7–100% of ACE inhibition activity. Further analysis ACE inhibition activity indicated that two derivatives **4as** and **4bn** exhibited 61.75% and 80.08% ACE inhibition, respectively and are comparable to standard drug Lisinopril. Further, IC_{50} value for test compounds and reference drug was calculated from dose–response curves obtained by plotting the percentage inhibition verses the concentration (Fig. 4). IC_{50} values of **4as** and **4bn** are 0.837 μM and 0.607 μM, respectively.

3.2. Effect of Nifedipine, 4as and 4bn on dose–response curve induced by $CaCl_2$

Preincubation of rat aortic strips with Nifedipine or **4as** or **4bn** resulted in non-parallel shift to the right of calcium dose response curves. In addition, preincubation of aortic ring with Nifedipine caused a significant reduction of maximal contraction to $CaCl_2$. (Figs. 5 and 6). Vasorelaxants effects of newly synthesized derivatives **4aa**–**4bp** showed concentration dependent depressant effect on calcium-induced contractions. Our experimental data indicated that preincubation of both **4as** and **4bn,** antagonized the calcium-induced aortic contractions in concentration dependent manner. Among, both the compounds tested, **4as** showed better inhibition property of Ca^{2+} induced contraction in isolated aortic ring. As smooth muscle of blood vessel contracts in response to the activation of voltage-dependent and receptor-operated Ca^{2+} channels, our data suggest that both molecules cause vaso-relaxation through inhibition of voltage-operated Ca^{2+} channels in the isolated rat aortic rings. IC_{50} values of **4as** and **4bn** are 3 mM and 50 mM respectively.

4. Results and discussion

4.1. Chemistry

We developed an efficient atom economic three component coupling reaction under completely green protocols. The reactions are fast, easy to handle and the products formed can be easily purified by simply washing the reaction mixture with methanol or ethanol which are considered as green solvents with low environmental factor.

4.2. Pharmacology

The compounds synthesized above were primarily screened for their ACE inhibition activity as they were designed based on CCBs and assuming all of they will be CCBs. Out of 42 compounds screened for their ACE inhibition activity two lead molecules **4as** and **4bn** were identified as strong ACE inhibitors and were further proved to be CCBs with varying activity against ACE and CCB. The extent of ACE inhibition is comparably best when compared with the natural product Butein (IC_{50} = 730 mM) based on which the molecules were designed. The structures and IC_{50} values of the compounds **4as** and **4bn** which are dual inhibitors were shown in Fig. 7.

4.3. Molecular docking

To gain insight into the interaction of the ACE enzyme with two potent analogues **4as** and **4bn**, they were docked on to surface of tACE (PDB code: 1O86) using Molegro Virtual Docker (MVD) software. The ligands were generated using Marvin sketch and saved in mol2 format. The typical poses obtained were presented in Fig. 8. Results suggest that the docking position of **4aa**–**4bp** analogues showed a similar binding mode to that of A-ring of Lisinopril. The interactions were mainly seen with the His 353, Ala 354, Tyr 523, Tyr 520 and Glu 152 which are comparable to the standard drug, Lisinopril binding mode. We found that docking scores were matched with *in vitro* activity data.

5. Conclusion

In conclusion, we designed hybrid molecules which are effective against both ACE inhibition and CCB activity. Demonstrated an efficient three-component coupling for their synthesis. The synthesis and purification processes were completely green. The reaction procedure did not utilize any solvent or catalyst and did not use traditional workup and chromatography techniques for purification which generally requires huge amounts of solvents. Thus, we prepared a wide range of 2-(diarylalkyl)aminobenzothiazole derivatives in a single-step process through a multi-component reaction and were initially screened for their angiotensin-converting-enzyme (ACE) inhibition property. Two compounds

Table 3
Different derivatives of Aldehydes (R^1) and 2-Aminobenzothiazoles (R^2) reacting with β-Napthol to produce 2-(diarylalkyl)aminobenzothiazoles.

Entry	Compound	R^1	R^2	Time (min)	Temp (°C)	Yield (%)
1	**4aa**	1-Phenylethyl	H	40	110	86
2	**4ab**	2,4-Dichlorophenyl	4-Chloro	55	120	92
3	**4ac**	3-Chlorophenyl	4-Chloro	45	110	89
4	**4ad**	4-Fluorophenyl	4-Chloro	45	110	88
5	**4ae**	Phenyl	6-Chloro	45	110	90
6	**4af**	4-Methylphenyl	6-Chloro	45	110	88
7	**4ag**	4-Bromophenyl	6-Chloro	50	110	91
8	**4ah**	4-(Dimethylamino)phenyl	H	40	110	93
9	**4ai**	4-Cyanophenyl	H	40	110	92
10	**4aj**	2-Bromo-5-fluorophenyl	H	50	120	92
11	**4ak**	Phenyl	H	40	110	94
12	**4al**	2,4-Dichlorophenyl	H	50	120	92
13	**4am**	3-Chlorophenyl	H	40	110	93
14	**4an**	4-Methylphenyl	H	45	110	91
15	**4ao**	4-Bromophenyl	H	50	110	94
16	**4ap**	4-Fluorophenyl	H	40	110	95
17	**4aq**	3-Phenoxyphenyl	H	45	110	92
18	**4ar**	2-Napthyl	H	45	110	90
19	**4as**	4-Methoxyphenyl	H	45	110	91
20	**4at**	3,4,5-Trimethoxyphenyl	H	45	110	88
21	**4au**	3-Bromophenyl	H	40	110	94
22	**4av**	3,4-Dichlorophenyl	H	40	110	94
23	**4aw**	3-Fluorophenyl	H	40	110	93
24	**4ax**	4-Isopropylphenyl	H	45	110	90
25	**4ay**	3,4-Dichlorophenyl	4-Chloro	45	110	94
26	**4az**	3,4-Dichlorophenyl	6-Chloro	43	110	94
27	**4ba**	3,4-Dichlorophenyl	4-Methyl	40	110	94
28	**4bb**	2,4-Dimethoxyphenyl	H	50	110	90
29	**4bc**	2-Chlorophenyl	H	50	120	92
30	**4bd**	4-Trifluoromethyphenyl	H	40	110	88
31	**4be**	3,4-Dimethoxyphenyl	H	45	110	90
32	**4bf**	3,4-Difluorophenyl	H	40	110	87
33	**4bg**	4-Chlorophenyl	H	40	110	91
34	**4bh**	2,3-Dichlorophenyl	H	50	120	89
35	**4bi**	Phenyl	4-Chloro	40	110	90
36	**4bj**	4-Methylphenyl	4-Chloro	45	110	91
37	**4bk**	4-Bromophenyl	4-Chloro	45	110	90
38	**4bl**	3-Phenoxyphenyl	4-Chloro	45	110	88
39	**4bm**	2-Napthyl	4-Chloro	45	110	89
40	**4bn**	4-Methoxyphenyl	4-Chloro	50	110	90
41	**4bo**	3,4,5-TrimethoxyPhenyl	4-Chloro	50	110	88
42	**4bp**	4-(Dimethylamino)phenyl	4-Chloro	45	115	90

4as and **4bn** which were found active against ACE inhibition, were further screened for their calcium channel blocker (CCB) property by using rat aortic contraction method. Both the compounds were found effective against calcium channel blocking property. From the present study, we developed two new lead molecules **4as** and **4bn** with dual inhibitory property, which may be useful in treating resistant hypertension. Treating resistant hypertension uses combination of drugs which usually contains an ACE inhibitor (or) angiotensin receptor blocker, CCBs and diuretics The compounds **4as** and **4bn** being CCB along with ACE inhibition could be utilized as novel molecules to treat resistant hypertension.

6. Experimental protocols

6.1. Chemistry

Commercially available reagent grade chemicals and solvents were used in all the reaction and purification processes. All the reactions were monitored by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light, charring with PMA charring solution or exposure to I_2 vapours. The ^1H (300 and 500 MHz) and ^{13}C NMR (75, 100 and 125 MHz) were recorded on Avance-300, Inova-400, Avance-500 and Inova-500 in DMDO-d_6 and $CDCl_3$. Chemical shift values are reported in ppm relative to TMS (Tetramethylsilane) or DMSO as internal reference unless stated. Coupling constant value *J* is measured in hertz. s (singlet), d (doublet), t (triplet), brs (broad singlet), dd (double doublet), m (multiplet). Mass spectra were recorded on an Agilent LC/MSD trap SL 1100 series spectrometer with a 70 eV (ESI probe) and high-resolution mass spectra were obtained by using ESI-QTOF mass spectrometry. Infrared spectra recorded on a Thermo nicolet Nexus 670 FT-IR spectrometer.

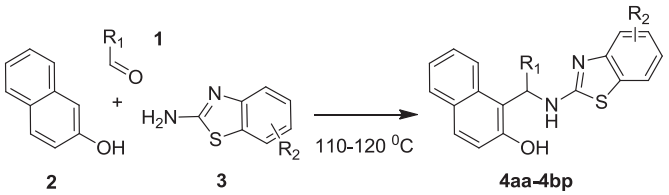
6.1.1. Typical procedure for the synthesis of 2-(diarylalkyl) aminobenzothiazole derivatives (*4aa*–*4bp*)

To a 10 mL RB flask aldehyde (1.0 mmol), 2-aminobenzothiazole (1.0 mmol) and β-naphthol (1.0 mmol) were added, mixed thoroughly and heated in preheated oil bath at 110–120 °C for appropriate time (Table 3). After completion of reaction as indicated by TLC the reaction mixture was allowed to cool. The so formed solid is powdered and washed with methanol or ethanol (3 × 8 mL in case of halogenated compounds except fluorinated, 3 × 4 mL in case of fluoro and methoxy derivatives) to obtain the corresponding pure 2-(diarylalkyl)aminobenzothiazole derivative. It is noteworthy to handle fluoro and methoxy derivatives carefully which otherwise may easily washed off in methanol/ethanol.

*6.1.1.1. 1-(1-(benzo[d]thiazol-2-ylamino)-2-phenylpropyl)naphthalen-2-ol (**4aa**).* The reaction forms two enantiomeric pairs as expected. One of them being major, only that was obtained after several successful washings with methanol while the minor enantiomeric pair washed off with methanol. White solid; mp = 197–199 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.12–9.95 (brs, 1H), 8.41–8.08 (brs, 1H), 7.59 (d, 2H, *J* = 7.4 Hz), 7.51 (d, 1H, *J* = 8.7 Hz), 7.40–7.28 (m, 2H), 7.21–6.82 (m, 11H), 6.07–5.98 (brs, 1H), 4.00–3.88 (brs, 1H), 1.51 (d, 1H, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.6, 153.2, 152.2, 143.9, 130.2, 128.6, 128.1, 127.9, 127.5, 127.2, 125.8, 125.6, 125.4, 121.9, 120.7, 120.6, 118.2, 117.7, 57.6, 42.7, 19.8; IR (KBr): ν_{max} 3450, 3371, 3062, 2964, 1602, 1551, 1448, 1331, 1266, 741, 701 cm^{-1}; ESI-MS: *m*/*z*: 411(M + H); HRMS calcd. for $C_{26}H_{23}ON_2S$: 411.15256, found: 411.15381.

*6.1.1.2. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(2,4-dichlorophenyl) methyl)naphthalen-2-ol (**4ab**).* Pink solid; mp = 212–214 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.04 (s, 1H), 9.22 (d, 1H, *J* = 7.2 Hz), 8.17 (d, 1H, *J* = 8.7 Hz), 7.79 (t, 2H, *J* = 7.2 Hz), 7.67–7.58 (m, 2 h), 7.53 (d, 1H, 2.1 Hz), 7.48–7.38 (m, 2H), 7.32–7.11 (m, 4H), 6.98 (t, 1H, 7.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.0, 153.9, 148.8, 138.2, 133.3, 132.6, 132.1, 132.0, 131.2, 130.1, 128.7, 128.6, 128.3, 126.6, 125.6, 122.9, 122.5, 121.7, 121.7, 119.9, 118.4, 115.8, 52.5; IR (KBr): ν_{max} 3336, 2932, 1585, 1537, 1434, 1326, 1252, 882, 812, 735 cm^{-1}; ESI-MS: *m*/*z*: 485 (M + H); HRMS calcd. for $C_{24}H_{16}ON_2Cl_3S$: 485.00434, found: 485.00457.

*6.1.1.3. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(3-chlorophenyl) methyl)naphthalen-2-ol (**4ac**).* White solid; mp = 221–223 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.33–10.13 (brs, 1H), 8.17 (d, 1H,



Scheme 1. Schematic representation of condensation of an Aldehyde, β-Napthol and a 2-Aminobenzothiazole derivative producing 2-(diarylalkyl)aminobenzothiazole.

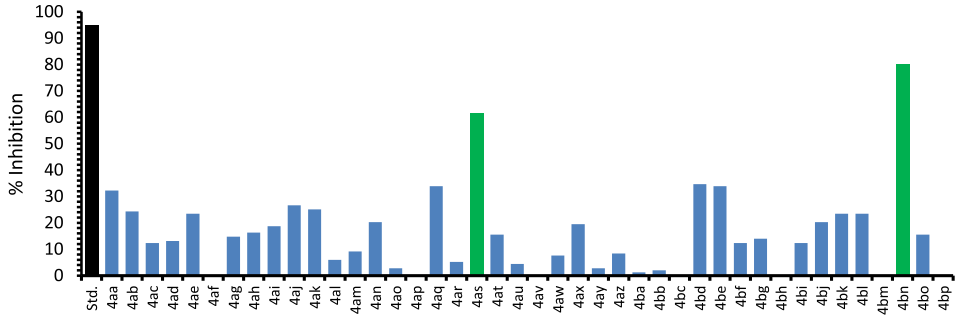


Fig. 3. *In vitro* Angiotensin Converting Enzyme (ACE) inhibition of new Diarylalkylamine analogues **4aa**–**4bp** and standard drug, Lisinopril.

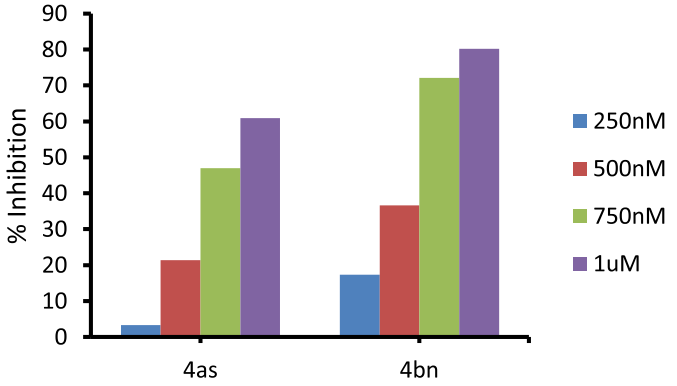


Fig. 4. Dose Response Curves of new Diarylalkylamine derivatives **4as** and **4bn** for ACE inhibition.

J = 6.4 Hz), 8.02 (d, 1H, J = 6.8 Hz), 7.86–7.78 (m, 2H), 7.64 (d, 1H, J = 9.1 Hz), 7.46–7.16 (m, 9H), 6.99 (t, 1H, J = 7.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 153.4, 148.6, 144.7, 132.9, 132.1, 130.1, 129.9, 128.6, 128.5, 126.6, 126.4, 125.7, 125.6, 124.8, 123.3, 122.6, 121.7, 119.9, 118.4, 118.0, 52.9; IR (KBr): ν_{max} 3376, 3064, 1590, 1546, 1413, 1307, 1264, 817, 770 cm^{-1}; ESI-MS: m/z: 451 (M + H); HRMS calcd. for $C_{24}H_{17}ON_2Cl_2S$: 451.04332, found: 451.04224.

*6.1.1.4. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(4-fluorophenyl) methyl)naphthalen-2-ol (**4ad**).* White solid; mp = 205–207 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25–10.11 (brs, 1H), 9.16 (d, 1H, J = 7.6 Hz), 7.97 (d, 1H, 7.6 Hz), 7.84–7.76 (m, 2H), 7.63 (d, 1H, J = 7.7 Hz), 7.43–7.21 (m, 7H), 7.11 (t, 2H, J = 8.7 Hz), 6.99 (t, 1H, J = 7.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 162.4, 159.2,

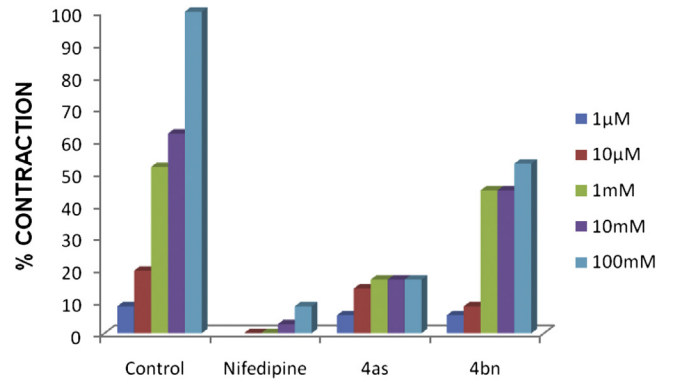


Fig. 5. Dose-response curve of calcium-induced aortic ring contraction in presence and absence of standard (Nifedipine) and test compounds (**4as** and **4bn**).

153.3, 148.6, 137.8, 132.0, 129.7, 128.5, 128.0, 127.9, 126.3, 125.5, 123.4, 122.4, 121.6, 119.7, 118.3, 118.1, 114.9, 114.6, 52.9; IR (KBr): ν_{max} 3337, 2938, 1591, 1542, 1507,1436, 1271, 1157, 812, 764 cm^{-1}; ESI-MS: m/z: 435 (M + H); HRMS calcd. for $C_{24}H_{17}ON_2ClFS$: 435.07287, found: 435.07248.

*6.1.1.5. 1-((6-chlorobenzo[d]thiazol-2-ylamino)(phenyl)methyl) naphthalen-2-ol (**4ae**).* White solid; mp = 202–204 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.16 (s, 1H), 8.92 (d, 1H, J = 7.6 Hz), 7.90–7.75 (m, 4H), 7.39–7.12 (m, 11H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 153.1, 151.0, 142.2, 132.4, 132.0, 129.5, 128.5, 128.5, 128.0, 126.2, 125.9, 125.5, 124.6, 123.6, 122.3, 120.4, 118.9, 118.4, 118.3, 53.1; IR (KBr): ν_{max} 3375, 3059, 3021, 1598, 1546, 1515, 1447, 1260, 806, 738 cm^{-1}; ESI-MS: m/z: 417 (M + H); HRMS calcd. for $C_{24}H_{18}ON_2ClS$: 417.08229, found: 417.08234.

*6.1.1.6. 1-((6-chlorobenzo[d]thiazol-2-ylamino)(p-tolyl)methyl) naphthalen-2-ol (**4af**).* White solid; mp = 191–193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 10.18–10.10 (brs, 1H), 8.91 (d, 1H, J = 7.6 Hz), 7.96–7.71 (m, 4H), 7.39–7.02 (m, 10H), 2.22 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 153.1, 151.0, 139.1, 137.1, 135.2, 132.4, 132.1, 129.4, 128.6, 128.5, 126.1, 125.9, 125.5, 124.6, 123.7, 122.3, 120.4, 118.9, 118.5, 118.3, 53.1, 20.5; IR (KBr): ν_{max} 3364, 3054, 2915, 1599, 1547, 1513, 1447, 1260, 806, 765 cm^{-1}; ESI-MS: m/z: 431 (M + H); HRMS calcd. for $C_{25}H_{20}ON_2ClS$: 431.09794, found: 431.09840.

*6.1.1.7. 1-((6-chlorobenzo[d]thiazol-2-ylamino)(4-bromophenyl) methyl)naphthalen-2-ol (**4ag**).* White solid; mp = 217–219 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25–10.16 (brs, 1H), 8.92 (d, 1H, J = 7.3 Hz), 7.87–7.76 (m, 4H), 7.46 (d, 2H, J = 8.5 Hz), 7.41–7.33 (m, 2H), 7.30–7.14 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 153.2, 150.9, 141.9, 132.5, 132.0, 130.9, 129.8, 128.6, 128.3, 126.4, 125.5, 124.9, 123.4, 122.5, 120.5, 119.2, 119.0, 118.3, 118.0, 52.8; IR (KBr): ν_{max} 3560, 3366, 3054, 1598, 1545, 1447, 1258, 1070, 804, 767,

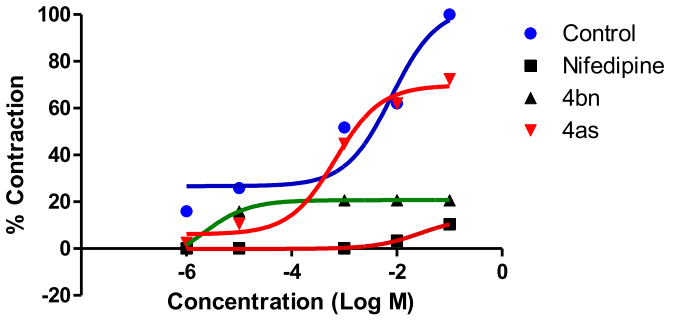


Fig. 6. Inhibitory effect of **4as** and **4bn** on aortic ring contraction induced by extracellular Ca^{2+} influxes at different cumulative concentration (10^{-1} M, 10^{-2} M, 10^{-3} M, 10^{-5} M and 10^{-6} M).

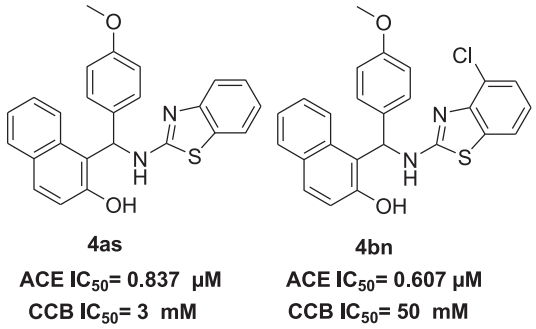


Fig. 7. Structures and IC_{50} values of compounds **4as** and **4bn** which are showing dual activity as ACE inhibitors and CCBs.

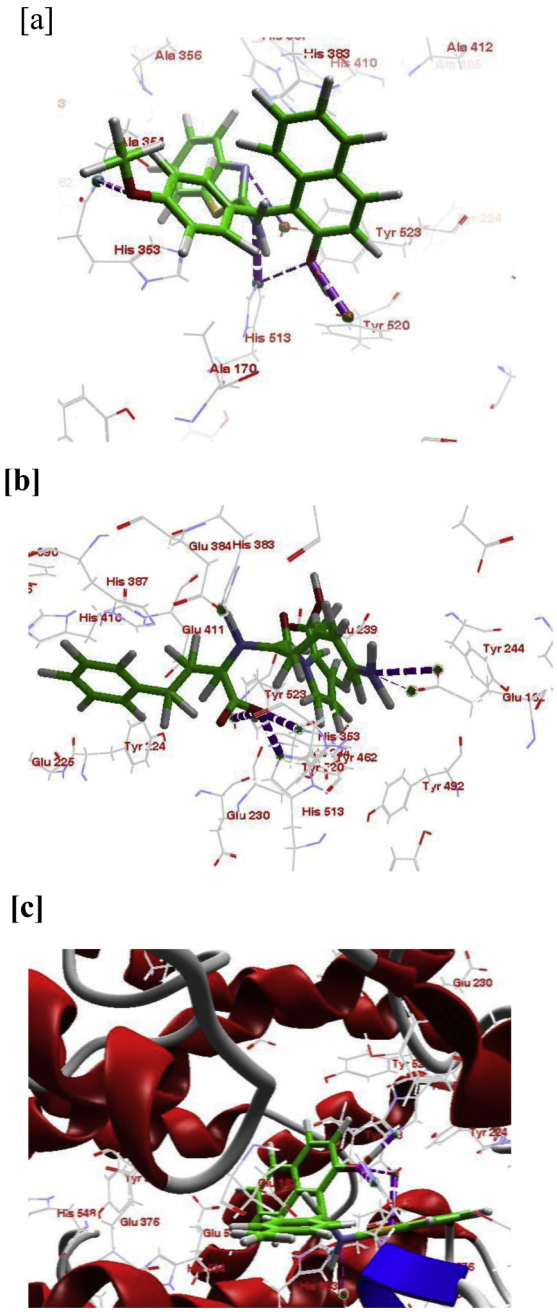


Fig. 8. a) Best predicted binding mode of **4as** at tACE binding site. b) Best predicted binding mode of lisinopril at tACE binding site. c) Best predicted binding mode of **4bn** at tACE binding site.

566 cm^{-1}; ESI-MS: *m/z*: 495 (M + H); HRMS calcd. for $C_{24}H_{17}N_2OSClBr$: 494.9933, found: 494.9928.

*6.1.1.8. 1-((benzo[d]thiazol-2-ylamino)(4-(dimethylamino)phenyl) naphthalen-2-ol (**4ah**).* White solid; mp = 156–158 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.22–10.04 (brs, 1H), 8.71 (d, 1H, *J* = 7.4 Hz), 7.93 (d, 1H, *J* = 7.5 Hz), 7.77 (t, 2H, *J* = 9.1 Hz), 7.64 (d, 1H, *J* = 7.4 Hz), 7.39–7.29 (m, 2H), 7.28–7.11 (m, 4H), 7.07 (d, 2H, *J* = 8.5 Hz), 6.99 (t, 1H, *J* = 7.9 Hz), 6.63 (d, 2H, *J* = 8.9 Hz), 2.80 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.2, 153.0, 152.1, 149.0, 132.1, 130.5, 129.5, 129.1, 128.6, 128.4, 126.8, 125.9, 125.3, 122.2, 120.7, 118.9, 118.4, 117.9, 112.2, 53.1, 40.1; IR (KBr): ν_{max} 3315, 2849, 1602, 1538, 1446, 1318, 1264, 1165, 810, 745 cm^{-1}; ESI-MS: *m/z*: 426 (M + H); HRMS Calcd. for $C_{26}H_{24}N_3OS$: 426.1640, found: 426.1639.

*6.1.1.9. 4-((benzo[d]thiazol-2-ylamino)(2-hydroxynaphthalen-1-yl) methyl)benzonitrile (**4ai**).* White solid; mp = 213–215 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.31–10.20 (brs, 1H), 8.85 (d, 1H, *J* = 7.0 Hz), 7.89–7.78 (m, 3H), 7.76–7.65 (m, 3H), 7.46–7.33 (m, 5H), 7.31–7.17 (m, 3H), 7.03 (t, 1H, *J* = 7.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.2, 151.9, 148.8, 132.0, 130.8, 130.0, 128.6, 128.5, 127.0, 126.5, 125.4, 123.2, 122.5, 121.2, 120.9, 118.8, 118.3, 117.8, 108.9, 53.0; IR (KBr): ν_{max} 3379, 3056, 2226, 1573, 1541, 1437, 1329, 1270, 1026, 751 cm^{-1}; ESI-MS: *m/z*: 408 (M + H); HRMS Calcd. for $C_{25}H_{18}N_3OS$: 408.1170, found:408.1163.

*6.1.1.10. 1-((benzo[d]thiazol-2-ylamino)(2-bromo-5-fluorophenyl) methyl)naphthalen-2-ol (**4aj**).* white solid; mp = 198–200 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 9.92 (brs, 1H), 8.90 (d, 1H, *J* = 7.4 Hz), 8.05 (d, 1H, *J* = 8.7 Hz), 7.80 (t, 2H, *J* = 9.3 Hz), 7.66 (d, 1H, *J* = 7.7 Hz), 7.61–7.54 (m, 1H), 7.39–7.34 (m, 3H), 7.28 (t, 1H, *J* = 7.4 Hz), 7.20 (t, 1H, *J* = 7.6 Hz), 7.14–6.97 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.0, 162.6, 159.4, 153.7, 152.0, 144.2, 144.1, 134.1, 134.0, 132.8, 130.6, 129.9, 128.5, 128.1, 126.5, 125.3, 122.4, 122.3, 121.0, 120.8, 118.5, 118.2, 116.9, 116.7, 116.6, 115.6, 115.3, 54.7; IR (KBr): ν_{max} 3383, 1598, 1543, 1512, 1453, 1431, 1265, 812, 752 cm^{-1}; ESI-MS: *m/z*: 479 (M + H); HRMS calcd. for $C_{24}H_{17}BrFN_2OS$: 479.0204, found: 479.0190.

*6.1.1.11. 1-((benzo[d]thiazol-2-ylamino)(phenyl)methyl) naphthalen-2-ol (**4ak**).* White solid: mp = 200–202 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.17 (brs, 1H), 8.78 (d, 1H, *J* = 7.6 Hz), 7.94–7.74 (m, 3H), 7.66 (d, 1H, *J* = 7.6 Hz), 7.40–7.13 (m, 10H), 7.0 (t, 1H, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.3, 153.2, 152.1, 142.5, 132.1, 130.8, 129.6, 128.6, 128.6, 128.1, 126.2, 126.0, 125.4, 123.8, 122.4, 121.0, 120.9, 118.7, 118.4, 118.1, 53.1; IR (KBr): ν_{max} 3376, 3060, 1598, 1547, 1514, 1444, 1331, 1263, 744 cm^{-1}; ESI-MS: *m/z*: 383 (M + H); HRMS calcd. for $C_{24}H_{19}N_2OS$: 383.1218, found: 383.1206.

*6.1.1.12. 1-((benzo[d]thiazol-2-ylamino)(2,4-dichlorophenyl)methyl) naphthalen-2-ol (**4al**).* White solid: mp = 190–192 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.02–9.91 (brs, 1H), 8.88 (d, 1H, *J* = 7.0 Hz), 8.02 (d, 1H, *J* = 8.5 Hz), 7.79 (t, 2H, *J* = 9.3 Hz), 7.70–7.63 (m, 2H), 7.49 (d, 1H, *J* = 2.1 Hz), 7.46–7.35 (m, 3H), 7.27 (t, 1H, *J* = 7.6 Hz), 7.23–7.12 (m, 3H), 7.01 (t, 1H, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.2, 153.8, 152.1, 139.0, 133.0, 132.7, 131.8, 131.1, 130.7, 130.0, 128.7, 128.3, 126.6, 126.5, 125.5, 122.6, 122.4, 121.1, 120.9, 118.5, 118.3, 115.9, 52.3; IR (KBr): ν_{max} 3378, 3068, 1592, 1542, 1438, 1371, 1331, 1268, 751 cm^{-1}; ESI-MS: *m/z*: 451 (M + H); HRMS calcd. for $C_{24}H_{17}N_2OSCl_2$: 451.0438, found: 451.0451.

*6.1.1.13. 1-((benzo[d]thiazol-2-ylamino)(3-chlorophenyl)methyl) naphthalen-2-ol (**4am**).* White solid: mp = 193–194 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.19 (brs, 1H), 8.4 (brs, 1H), 7.96 (brs, 1H), 7.77–7.64 (m, 2H), 7.52 (d, 1H, *J* = 7.7 Hz), 7.45–7.30 (m, 3H),

7.30–7.09 (m, 7H), 6.99 (t, 1H, $J = 7.5$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.1, 151.9, 145.3, 132.8, 131.9, 130.9, 130.7, 129.9, 129.8, 128.6, 128.5, 126.5, 126.1, 125.6, 125.4, 124.8, 122.5, 121.1, 120.9, 118.3, 118.2, 118.0, 52.6; IR (KBr): ν_{max} 3360, 3062, 1596, 1573, 1548, 1515, 1444, 1332, 1268, 749 cm^{-1}; ESI-MS: *m/z*: 417 (M + H); HRMS calcd. for $C_{24}H_{18}N_2OSCl$: 417.0828, found: 417.0809.

6.1.1.14. 1-((benzo[d]thiazol-2-ylamino)(p-tolyl)methyl) naphthalen-2-ol ***(4an)***. White solid: mp = 180–182 °C; (300 MHz, DMSO-d_6): δ 10.18–10.11 (brs, 1H), 8.78 (d, 1H, $J = 7.6$ Hz), 7.91–7.73 (m, 3H), 7.66 (d, 1H, $J = 7.4$ Hz), 7.39–6.96 (m, 11H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.2, 153.1, 152.1, 139.4, 135.1, 132.1, 130.7, 129.4, 128.6, 128.5, 126.1, 125.9, 125.4, 123.9, 122.4, 120.9, 120.8, 118.7, 118.3, 118.0, 52.9, 20.5; IR (KBr): ν_{max} 3307, 1535, 1441, 1317, 1261, 744 cm^{-1}; ESI-MS: *m/z*: 397 (M + H); HRMS calcd. for $C_{25}H_{21}N_2OS$: 397.1374, found: 397.1373.

6.1.1.15. 1-((benzo[d]thiazol-2-ylamino)(4-bromophenyl)methyl) naphthalen-2-ol ***(4ao)***. White solid; mp = 202–204 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.24–10.16 (brs, 1H), 8.82 (d, 1H, $J = 7.0$ Hz), 7.89–7.75 (m, 3H), 7.67 (d, 1H, $J = 7.6$ Hz), 7.49–7.36 (m, 4H), 7.30–7.12 (m, 6H), 7.02 (t, 1H, $J = 7.6$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.1, 151.9, 142.1, 131.9, 130.9, 130.8, 129.7, 128.5, 128.2, 126.3, 125.3, 123.5, 122.4, 121.0, 120.8, 119.1, 118.3, 118.1, 52.6; IR (KBr): ν_{max} 3373, 1627, 1541, 1442, 1332, 1267, 1206, 1070, 749, 571 cm^{-1}; ESI-MS: *m/z*: 461 (M + H); HRMS calcd. for $C_{24}H_{18}N_2OSBr$: 461.0323, found: 461.0320.

6.1.1.16. 1-((benzo[d]thiazol-2-ylamino)(4-fluorophenyl)methyl) naphthalen-2-ol ***(4ap)***. White solid; mp = 178–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.22–10.13 (brs, 1H), 8.81 (d, 1H, $J = 7.4$ Hz), 7.93–7.76 (m, 3H), 7.67 (d, 1H, $J = 7.7$ Hz), 7.42–7.32 (m, 2H), 7.31–7.15 (m, 6H), 7.93–6.95 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 162.2, 159.0, 153.1, 151.9, 138.4, 131.9, 130.7, 129.5, 128.5, 127.9, 127.8, 126.2, 125.3, 123.5, 122.3, 120.9, 120.8, 118.3, 118.0, 114.8, 114.5, 52.6; IR (KBr): ν_{max} 3308, 1537, 1436, 1319, 1286, 1060, 810, 746 cm^{-1}; ESI-MS: *m/z*: 401 (M + H).

6.1.1.17. 1-((benzo[d]thiazol-2-ylamino)(3-phenoxyphenyl)methyl) naphthalen-2-ol ***(4aq)***. White solid; mp = 171–173 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.16 (s, 1H), 8.78 (d, 1H, $J = 7.4$ Hz), 7.89–7.73 (m, 3H), 7.66 (d, 1H, $J = 7.0$ Hz), 7.43–7.16 (m, 9H), 7.06–6.93 (m, 4H), 6.87 (d, 2H, $J = 7.6$ Hz), 6.79 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 2.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 156.5, 156.0, 153.1, 152.0, 145.0, 132.0, 130.7, 129.7, 128.6, 128.5, 126.2, 125.4, 123.0, 122.4, 121.3, 121.0, 120.8, 118.3, 118.1, 117.9, 116.6, 116.3, 52.7; IR (KBr): ν_{max} 3314, 3052, 1541, 1511, 1451, 1267, 814, 749 cm^{-1}; ESI-MS: *m/z*: 475 (M + H); HRMS calcd. for $C_{30}H_{23}N_2O_2S$: 475.1480, found: 475.1471.

6.1.1.18. 1-((benzo[d]thiazol-2-ylamino)(naphthalen-2-yl)methyl) naphthalen-2-ol ***(4ar)***. White solid; mp = 190–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.21–10.16 (brs, 1H), 8.91 (d, 1H, $J = 7.4$ Hz), 7.95 (d, 1H, $J = 7.9$ Hz), 7.89–7.74 (m, 6H), 7.68 (d, 1H, $J = 7.9$ Hz), 7.53–7.40 (m, 3H), 7.38–7.15 (m, 6H), 7.02 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.2, 153.1, 152.0, 140.1, 132.6, 132.1, 131.7, 130.7, 129.6, 128.5, 128.4, 127.5, 127.3, 126.1, 126.0, 125.4, 125.3, 124.9, 123.8, 123.6, 122.3, 120.9, 120.8, 118.5, 118.3, 118.0, 53.3; IR (KBr): ν_{max} 3313, 3052, 1541, 1442, 1264, 814, 749 cm^{-1}; ESI-MS: *m/z*:433 (M + H); HRMS Calcd. for $C_{28}H_{21}N_2OS$: 433.1374, found: 433.1387.

6.1.1.19. 1-((benzo[d]thiazol-2-ylamino)(4-methoxyphenyl)methyl) naphthalen-2-ol ***(4as)***. White solid; mp = 190–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19–10.11 (brs, 1H), 8.79 (d, 1H, $J = 7.4$ Hz), 7.94–7.84 (brs, 1H), 7.79 (t, 2H, $J = 8.7$ Hz), 7.66 (d, 1H, $J = 7.6$ Hz), 7.42–7.12 (m, 9H), 7.0 (t, 1H, $J = 7.9$ Hz), 6.83 (d, 1H, $J = 8.7$ Hz), 3.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.2, 157.7, 153.0, 152.1, 134.1, 132.1, 130.6, 129.4, 128.6, 128.5, 127.2, 126.1, 125.3, 123.8, 122.3, 120.9, 120.8, 118.7, 118.4, 118.0, 113.5, 54.9, 52.8; IR (KBr): ν_{max} 3313, 3052, 1541, 1442, 1264, 814, 749 cm^{-1}; ESI-MS: *m/z*: 413 (M + H); HRMS calcd. for $C_{25}H_{21}N_2O_2S$: 413.1323, found: 413.1319.

6.1.1.20. 1-((benzo[d]thiazol-2-ylamino)(3,4,5-trimethoxyphenyl) methyl) naphthalen-2-ol ***(4at)***. White solid; mp = 170–172 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42–10.30 (brs, 1H), 8.63–8.37 (brs, 1H), 7.98–7.87 (brs, 1H), 7.75–7.63 (m, 2H), 7.52–7.31 (m, 3H), 7.28–7.15 (m, 3H), 7.11–6.94 (m, 2H), 6.58 (s, 2H), 3.70 (s, 3H), 3.66 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.3, 153.3, 152.4, 137.0, 136.1, 132.0, 129.9, 129.1, 128.6, 128.1, 125.8, 125.1, 123.0, 122.2, 120.8, 120.1, 119.1, 118.8, 117.9, 103.6, 95.6, 59.8, 55.4, 54.3; IR (KBr): ν_{max} 3317, 2923, 2833, 1590, 1538, 1441, 1315, 1248, 1171, 1030, 813, 745 cm^{-1}; ESI-MS: *m/z*: 473 (M + H).

6.1.1.21. 1-((benzo[d]thiazol-2-ylamino)(3-bromophenyl)naphthalen-2-ol ***(4au)***. White solid; mp = 201–203 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.27–10.20 (brs, 1H), 8.84 (d, 1H, $J = 7.4$ Hz), 7.92–7.77 (m, 3H), 7.68 (d, 1H, $J = 7.6$ Hz), 7.45–7.15 (m, 11H), 7.02 (t, 1H, $J = 7.6$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.0, 153.1, 151.9, 145.5, 131.9, 130.7, 130.2, 129.8, 129.0, 128.5, 126.4, 125.4, 125.1, 123.3, 122.4, 121.4, 121.0, 120.8, 118.3, 118.1, 118.0, 52.6; IR (KBr): ν_{max} 3377, 3058, 1543, 1511, 1435, 1327, 1268, 747 cm^{-1}; MS-ESI: *m/z*: 461 (M + H); HRMS calcd. for $C_{24}H_{18}BrN_2OS$: 461.0323, found: 461.0312.

6.1.1.22. 1-((benzo[d]thiazol-2-ylamino)(3,4-dichlorophenyl)naphthalen-2-ol ***(4av)***. White solid; mp = 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.28–10.24 (brs, 1H), 8.87 (d, 1H, $J = 7.2$ Hz), 7.93–7.79 (m, 3H), 7.69 (d, 1H, $J = 7.6$ Hz), 7.53 (d, 1H, $J = 8.3$ Hz), 7.47–7.37 (m, 3H), 7.32–7.15 (m, 5H), 7.03 (t, 1H $J = 7.7$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.0, 153.2, 151.8, 144.0, 131.9, 130.8, 130.6, 130.2, 130.0, 128.6, 128.4, 127.7, 126.6, 126.5, 125.4, 123.1, 122.5, 121.1, 120.9, 118.3, 118.2, 117.6, 52.3; IR (KBr): ν_{max} 3379, 1577, 1540, 11461, 1328, 1268, 1205, 1028, 816, 747 cm^{-1}; ESI-MS: *m/z*: 451 (M + H); HRMS calcd. for $C_{24}H_{17}Cl_2N_2OS$: 451.0438, found: 451.0439.

6.1.1.23. 1-((benzo[d]thiazol-2-ylamino)(3-fluorophenyl)naphthalen-2-ol ***(4aw)***. White Solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.26–10.19 (brs, 1H), 8.84 (d, 1H, $J = 7.4$ Hz), 7.90–7.78 (m, 3H), 7.68 (d, 1H, $J = 7.7$ Hz), 7.43–7.17 (m, 7H), 7.07–6.96 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 163.7, 160.5, 153.1, 151.9, 145.8, 145.8, 131.9, 130.7, 130.0, 129.9, 129.7, 128.5, 126.3, 125.4, 123.4, 122.4, 122.1, 121.0, 120.8, 118.3, 118.1, 113.0, 112.8, 112.5, 52.7; IR (KBr): ν_{max} 3387, 1587, 1536, 1482, 1439, 1329, 1244, 1053, 749 cm^{-1}; ESI-MS: *m/z*: 401 (M + H); HRMS calcd. for $C_{24}H_{18}FN_2OS$: 401.1123, found: 401.1118.

6.1.1.24. 1-((benzo[d]thiazol-2-ylamino)(4-isopropylphenyl)naphthalen-2-ol ***(4ax)***. White solid; mp = 191–193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17–10.09 (brs, 1H), 8.78 (d, 1 h, $J = 7.6$ Hz), 7.98–7.87 (brs, 1H), 7.79 (t, 2H, $J = 7.8$ Hz), 7.66 (d, 1H, $J = 7.6$ Hz), 7.4–7.32 (m, 2H), 7.30–7.10 (m, 8H), 7.00 (t, 1H, $J = 8.1$ Hz), 2.89–2.74 (m, 1H), 1.14 (dd, 6H, $J_1 = 7.0$ Hz, $J_2 = 1.5$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.2, 153.0, 152.1, 146.1, 139.6, 132.1, 130.6, 129.3, 128.5, 128.4, 126.1, 126.0, 125.9, 125.3, 123.7, 122.3, 120.8, 120.8, 118.7, 118.4, 118.0, 53.0, 32.9, 23.8, 23.7; IR (KBr): ν_{max} 3314, 3055, 2957, 1540, 1450, 1268, 1060, 746 cm^{-1}; ESI-MS: *m/*

z: 425 (M + H); HRMS calcd. for $C_{27}H_{25}N_2OS$: 425.1687, found: 425.1704.

*6.1.1.25. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(3,4-dichlorophenyl) methyl)naphthalen-2-ol (**4ay**).* White solid; mp = 208–210 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.24 (s, 1H), 9.20 (d, 1H, *J* = 7.7 Hz), 8.07–7.97 (brs, 1H), 7.86–7.79 (m, 2H), 7.66 (d, 1H, *J* = 7.9 Hz), 7.55 (d, 1H, *J* = 8.3 Hz), 7.48–7.40 (m, 2H), 7.36–7.14 (m, 5H), 7.0 (t, 1H, 7.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.6, 153.4, 148.5, 143.4, 132.1, 132.0, 130.9, 130.3, 130.0, 128.8, 128.6, 128.4, 127.8, 126.7, 126.5, 125.6, 123.1, 122.6, 121.7, 119.8, 118.3, 117.6, 52.5; IR (KBr): ν_{max} 3352, 2935, 1590, 1544, 1254, 817, 738 cm^{-1}; ESI-MS: *m*/*z*: 485 (M + H); HRMS calcd. for $C_{24}H_{16}N_2OSCl_3$: 485.0048, found: 485.0032.

*6.1.1.26. 1-((6-chlorobenzo[d]thiazol-2-ylamino)(3,4-dichlorophenyl) methyl)naphthalen-2-ol (**4az**).* White solid; mp = 215–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.28–10.23 (brs, 1H), 8.98 (d, 1H, *J* = 7.4 Hz), 7.9307.77 (m, 4H), 7.53 (d, 1H, *J* = 8.5 Hz), 7.47–7.14 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.6, 153.2, 150.8, 143.8, 132.6, 131.9, 130.7, 130.3, 130.0, 128.7, 128.6, 128.4, 127.7, 126.6, 126.5, 125.5, 124.9, 123.0, 122.6, 120.6, 119.1, 118.3, 117.5, 52.4; IR (KBr): ν_{max} 3360, 2865, 1598, 1545, 1446, 1254, 813, 740 cm^{-1}; ESI-MS: *m*/*z*: 485 (M + H); HRMS calcd. for $C_{24}H_{16}N_2OSCl_3$: 485.0048, found: 485.0032.

*6.1.1.27. 1-((4-methylbenzo[d]thiazol-2-ylamino)(3,4-dichlorophenyl)methyl)naphthalen-2-ol (**4ba**).* White solid; mp = 222–224 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.26–10.16 (brs, 1H), 8.98 (d, 1H, *J* = 7.9 Hz), 8.17–8.02 (brs, 1H), 7.86–7.77 (m, 2H), 7.58–7.40 (m, 4H), 7.03 (d, 1H, *J* = 7.2 HZ), 6.91 (t, 1H, *J* = 7.7 Hz), 2.41 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.1, 153.3, 150.6, 143.7, 132.1, 130.6, 130.2, 130.1, 129.8, 128.7, 128.5, 128.3, 127.9, 127.3, 126.6, 126.5, 126.1, 123.1, 122.5, 121.0, 118.3, 118.3, 118.1, 52.4, 17.9; IR (KBr): ν_{max} 3340, 2923, 1585, 1545, 1516, 1252, 816, 739 cm^{-1}; ESI-MS: *m*/*z*: 465 (M + H); HRMS calcd. for $C_{25}H_{19}Cl_2N_2OS$: 465.0595, found: 465.0575.

*6.1.1.28. 1-((benzo[d]thiazol-2-ylamino)(2,4-dimethoxyphenyl) methyl)naphthalen-2-ol (**4bb**).* White solid; mp = 174–176 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.22–10.08 (brs, 1H), 8.81 (d, 1H, *J* = 7.2 Hz), 7.95 (d, 1H, *J* = 8.5 Hz), 7.78 (t, 2H, *J* = 8.9 Hz), 7.65 (d, 1H, *J* = 7.7 Hz), 7.41–7.31 (m, 2H), 7.29–7.15 (m, 4H), 7.04–6.91 (m, 4H), 6.84 (d, 1H, *J* = 8.5 Hz), 6.73 (d, 1H, 8.7 Hz), 3.68 (s, 3H), 3.60 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.0, 152.0, 148.4, 147.4, 134.5, 132.1, 130.5, 129.3, 128.5, 128.4, 126.0, 125.3, 123.8, 122.3, 120.8, 118.6, 118.4, 117.9, 111.6, 110.6, 55.4, 53.1; IR (KBr): ν_{max} 3368, 2930, 2834, 1573, 1542, 1509, 1271, 752 cm^{-1}; ESI-MS: *m*/*z*: 443 (M + H); HRMS calcd. for $C_{26}H_{23}O_3N_2S$: 443.14239, found: 443.14463.

*6.1.1.29. 1-((benzo[d]thiazol-2-ylamino)(2-chlorophenyl)methyl) naphthalen-2-ol (**4bc**).* White solid; mp = 197–199 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.99–9.93 (brs, 1H), 8.86 (d, 1H, *J* = 7.4 Hz), 8.05 (d, 1H, *J* = 8.7 Hz), 7.78 (t, 2H, *J* = 9.3 Hz), 7.64 (d, 2H, *J* = 7.7 Hz), 7.47–7.12 (m, 10H), 6.99 (t, 1H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.3, 153.7, 152.2, 139.4, 132.7, 132.4, 130.6, 129.7, 129.4, 128.6, 128.3, 126.4, 126.3, 125.4, 122.9, 122.3, 120.9, 120.8, 118.5, 118.2, 116.5, 52.7; IR (KBr): ν_{max} 3456, 3381, 3066, 1571, 1528, 1438, 1319, 1270, 1040, 750 cm^{-1}; ESI-MS: *m*/*z*: 417 (M + H); HRMS calcd. for $C_{24}H_{18}ON_2ClS$: 417.08229, found: 417.08426.

*6.1.1.30. 1-((benzo[d]thiazol-2-ylamino)(4-(trifluoromethyl)phenyl) methyl)naphthalen-2-ol (**4bd**).* White solid; mp = 203–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.20–10.13 (brs, 1H), 8.90 (d, 1H, *J* = 7.6 Hz), 7.91–7.72 (m, 4H), 7.44–7.16 (m, 7H), 7.15–7.02 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.2, 151.9, 147.7, 132.0, 130.8, 129.9, 128.6, 128.5, 127.4, 127.0, 126.7, 126.6, 126.5, 126.1, 125.4, 124.99, 124.96, 123.3, 122.5, 121.1, 120.9, 118.3, 118.2, 118.0, 52.9; IR (KBr): ν_{max} 3380, 3059, 2867, 2599, 1574, 1541,1326, 1267, 1158, 1125, 1068, 749 cm^{-1}; ESI-MS: *m*/*z*: 451 (M + H); HRMS calcd. for $C_{25}H_{18}ON_2F_3S$: 451.10865, found: 451.10976.

*6.1.1.31. 1-((benzo[d]thiazol-2-ylamino)(3,4-dimethoxyphenyl) methyl)naphthalen-2-ol (**4be**).* White solid; mp = 176–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.21–10.11 (brs, 1H), 8.78 (d, 1H, *J* = 7.6 Hz), 7.95 (d, 1H, *J* = 7.4 Hz), 7.77 (t, 2H, *J* = 8.9 Hz), 7.64 (d, 1H, *J* = 7.4 Hz), 7.41–7.31 (m, 2H), 7.28–7.14 (m, 4H), 7.03–6.92 (m,2H), 6.83 (d, 1H, *J* = 8.5 Hz), 6.73 (d, 1H, *J* = 7.6 Hz), 3.68 (s, 3H), 3.60 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.3, 153.1, 152.1, 148.5, 147.5, 134.5, 132.2, 130.6, 129.4, 128.6, 128.5, 126.1, 125.5, 123.8, 122.4, 121.0, 120.9, 118.7, 118.5, 118.0, 111.7, 110.6, 55.5, 53.3; IR (KBr): ν_{max} 3368, 2930, 2834, 1573, 1543, 1510, 1330, 1271, 752 cm^{-1}; ESI-MS: *m*/*z*: 443 (M + H); HRMS calcd. for $C_{26}H_{23}O_3N_2S$: 443.14239, found: 443.14472.

*6.1.1.32. 1-((benzo[d]thiazol-2-ylamino)(3,4-difluorophenyl)methyl) naphthalen-2-ol (**4bf**).* White solid; mp = 177–179 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.38–10.13 (brs, 1H), 8.84 (d, 1H, *J* = 7.0 Hz), 7.94–7.78 (m, 3H), 6.8 (d, 1H, *J* = 7.6 Hz), 7.46–7.16 (m, 8H), 7.02 (t, 2H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.0, 153.1, 151.8, 140.4, 131.9, 130.7, 129.8, 128.5, 128.5, 126.4, 125.3, 123.2, 122.6, 122.4, 121.0, 120.8, 118.3, 118.2, 117.8, 117.1, 116.9, 114.9, 114.7, 52.4; IR (KBr): ν_{max} 3390, 3052, 1598, 1541, 1511, 1431, 1274, 754, 467 cm^{-1}; ESI-MS: *m*/*z*: 419 (M + H); HRMS calcd. for $C_{24}H_{17}ON_2F_2S$: 419.10242, found: 419.10338.

*6.1.1.33. 1-((benzo[d]thiazol-2-ylamino)(4-chlorophenyl)methyl) naphthalen-2-ol (**4bg**).* White solid; mp = 214–216 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.22–10.18 (brs, 1H), 8.82 (d, 1H, *J* = 7.4 Hz), 7.87–7.66 (m, 3H), 7.68 (d, 1H, *J* = 7.4 Hz), 7.42–7.16 (m, 10H), 7.02 (t, 3H, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.1, 153.1, 152.0, 141.6, 132.0, 130.8, 130.6, 129.7, 128.5, 128.0, 127.9, 126.3, 125.4, 123.5, 122.4, 121.0, 120.8, 118.3, 118.2, 52.6; IR (KBr): ν_{max} 3375, 3056, 1574, 1541, 1514, 1436, 1330, 1269, 749 cm^{-1}; ESI-MS: *m*/*z*: 417 (M + H); HRMS calcd. for $C_{24}H_{18}ON_2ClS$: 417.08229, found: 417.08458.

*6.1.1.34. 1-((benzo[d]thiazol-2-ylamino)(2,3-dichlorophenyl)methyl) naphthalen-2-ol (**4bh**).* White solid; mp = 200–202 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.03–9.92 (brs, 1H), 8.93 (d, 1H, *J* = 6.6 Hz), 8.01 (d, 1H, *J* = 8.5 Hz), 7.80 (t, 2H, 8.9 Hz), 7.66 (d, 2H, *J* = 7.7 Hz), 7.53 (d, 1H, *J* = 7.7 Hz), 7.46–7.11 (m, 7H), 7.01 (t, 1H, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.1, 153.8, 152.1, 142.6, 132.7, 131.7, 130.7, 130.0, 128.6, 128.6, 128.3, 127.2, 126.5, 125.4, 122.6, 122.4, 121.0, 120.8, 118.5, 118.3, 115.8, 53.3; IR (KBr): ν_{max} 3350, 3014, 1597, 1538,1512, 1448, 1270, 1154, 810, 748, 586, 482 cm^{-1}; ESI-MS: *m*/*z*: 451 (M + H); HRMS calcd. for $C_{24}H_{17}Cl_2N_2OS$: 451.04332, found: 451.04577.

*6.1.1.35. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(phenyl)methyl) naphthalen-2-ol (**4bi**).* White solid; mp = 214–216 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19–10.11 (brs, 1H), 9.18 (d, 1H, *J* = 7.4 Hz), 8.02–7.89 (brs, 1H), 7.84–7.76 (m, 2H), 7.64 (d, 1H, *J* = 7.7 Hz), 7.42–7.15 (m, 10H), 6.98 (t, 1H, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.9, 153.3, 148.7, 141.8, 132.1, 132.0, 129.6, 128.5, 128.4, 128.1, 126.3, 126.2, 126.0, 125.5, 123.7, 122.4, 121.5, 119.7, 118.3, 53.3; IR (KBr): ν_{max} 3350, 3314, 3059, 2937, 1587, 1537, 1416, 1325, 1253, 815, 747, 697 cm^{-1}; ESI-MS: *m*/*z*: 417 (M + H); HRMS calcd. for $C_{24}H_{18}N_2ClOS$: 417.08229, found: 417.08168.

*6.1.1.36. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(p-tolyl)methyl) naphthalen-2-ol (**4bj**).* White solid; mp = 219–221 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.18–10.07 (brs, 1H), 9.23–9.11 (brs, 1H), 8.04–7.88 (brs, 1H), 7.83–7.74 (m, 2H), 7.63 (d, 1H, *J* = 7.0 Hz), 7.41–7.31 (m, H), 7.30–7.20 (m, 4H), 7.17–7.05 (m, 4H), 6.98 (t, 1H, *J* = 7.9 Hz), 2.24 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.1, 153.4, 148.8, 138.7, 135.5, 132.2, 132.0, 129.6, 128.8, 128.6, 128.5, 126.3, 126.0, 125.7, 123.8, 122.5, 121.6, 121.6, 119.8, 118.5, 118.4, 53.4, 20.6; IR (KBr): ν_{max} 3310, 2927, 1586, 1533, 1417, 1320, 1271, 1051, 883, 812, 679 cm^{-1}; ESI-MS: *m/z*: 431 (M + H); HRMS calcd. for $C_{25}H_{20}ClN_2OS$: 431.09794, found: 431.09731.

*6.1.1.37. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(4-bromophenyl) methyl)naphthalen-2-ol (**4bk**).* White solid; mp = 223–225 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.22–10.16 (brs, 1H), 9.18 (d, 1H, *J* = 7.6 Hz), 8.02–7.88 (brs, 1H), 7.84–7.77 (m, 2H), 7.65 (d, 1H, *J* = 7.7 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 7.39 (t, 1H, *J* = 7.7 Hz), 7.32–7.15 (m, 6H), 6.99 (t, 1H, *J* = 7.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 153.3, 148.7, 141.5, 132.0, 130.9, 129.8, 128.5, 128.3, 126.4, 125.5, 123.5, 122.5, 121.6, 119.7, 119.3, 118.3, 117.9, 52.9; IR (KBr): ν_{max} 3343, 3297, 1711, 1586, 1528, 1416, 881, 814, 744, 498 cm^{-1}; ESI-MS: *m/z*: (M + H); HRMS calcd. for $C_{24}H_{17}BrClN_2OS$: 494.9933, found: 494.9948.

*6.1.1.38. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(3-phenoxyphenyl) methyl)naphthalen-2-ol (**4bl**).* White solid; mp = 208–210 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.28–10.14 (brs, 1H), 9.23–9.12 (brs, 1H), 8.02–7.88 (brs, 1H), 7.84–7.78 (m, 2H),7.64 (d, 1H, *J* = 7.2 Hz), 7.46–7.28 (m, 9H), 7.07–6.95 (m, 4H), 6.90 (d, 2H, *J* = 7.7 Hz), 6.82 (d, 1H, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 156.6, 156.1, 153.4, 148.7, 144.4, 132.1, 129.7, 128.5, 126.3, 125.5, 123.7, 123.0, 122.4, 121.7, 121.6, 121.4, 119.7, 118.3, 118.1, 117.9, 116.8, 116.5, 53.1; IR (KBr): ν_{max} 3332, 2938, 1588, 1545, 1485, 1249, 1165, 884, 744, 491 cm^{-1}; ESI-MS: *m/z*: 509 (M + H); HRMS calcd. for $C_{30}H_{22}ClN_2O_2S$: 509.10850, found: 509.10754.

*6.1.1.39. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(naphthalen-2-yl) methyl)naphthalen-2-ol (**4bm**).* White solid; mp = 236–238 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25–10.14 (brs, 1H), 9.36–9.27 (brs, 1H), 8.13–7.96 (brs, 1H), 7.88–7.76 (m, 6H), 7.66 (d, 1H, *J* = 7.4 Hz), 7.55–7.21 (m, 8H), 6.99 (t, 1H, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.0, 153.5, 148.8, 139.6, 132.7, 132.3, 132.1, 131.8, 129.7, 128.6, 128.5, 127.7, 127.6, 127.3, 126.3, 125.6, 125.5, 125.0, 124.0, 123.7, 122.4, 121.7, 121.6, 119.8, 118.5, 118.4, 53.7; IR (KBr): ν_{max} 3320, 3052, 2930, 1585, 1533, 1416, 1268, 816, 660, 478 cm^{-1}; ESI-MS: *m/z*: 467 (M + H); HRMS calcd. for $C_{28}H_{19}N_2ONaSCl$: 489.0804, found: 489.0822.

*6.1.1.40. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(4-methoxyphenyl) methyl)naphthalen-2-ol (**4bn**).* White solid; mp = 211–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.22–10.08 (brs, 1H), 9.24–9.10 (brs, 1H), 8.05–7.93 (brs, 1H), 7.79 (m, 2H), 7.63 (d, 1H, *J* = 7.742 Hz), 7.42–7.13 (m, 7H), 6.98 (t, 1H, *J* = 7.9 Hz), 6.85 (d, 2H, *J* = 8.7 Hz), 3.69 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.9, 157.8, 153.3, 148.8, 133.4, 132.1, 131.9, 129.5, 128.6, 128.4, 127.3, 126.1, 125.5, 123.8, 122.4, 121.5, 121.5, 119.7, 118.4, 113.5, 54.9, 53.2; IR (KBr): ν_{max} 3315, 3066, 2925, 2830, 1583, 1510, 1433, 1319, 1250, 1173, 1032, 821, 668, 588 cm^{-1}; ESI-MS: *m/z*: 447 (M + H); HRMS calcd. for $C_{25}H_{20}O_2N_2ClS$: 447.09285, found: 447.09345.

*6.1.1.41. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(3,4,5-trimethoxyphenyl)methyl) naphthalen-2-ol (**4bo**).* White solid; mp = 215–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.27–10.10 (brs, 1H), 9.32–9.17 (brs, 1H), 8.18–8.04 (brs, 1H), 7.79 (t, 2H, *J* = 8.3 Hz), 7.63 (d, 1H, *J* = 7.9 Hz), 7.42 (t, 1H, *J* = 7.5 Hz), 7.31–7.13 (m, 4H), 6.97 (t, 1H, *J* = 7.7 Hz), 6.66 (s, 2H), 3.62 (s, 6H), 3.61 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.9, 153.4, 152.6, 148.7, 137.2, 136.3, 132.2, 131.9, 129.6, 128.5, 126.3, 125.6, 123.6, 122.4, 121.5, 121.5, 119.7, 118.4, 118.3, 104.1, 59.9, 55.8, 54.0; IR (KBr): ν_{max} 3366, 2932, 1593, 1542, 1509, 1414, 1328, 1131, 818 cm^{-1}; ESI-MS: *m/z*: 507 (M + H); HRMS calcd. for $C_{27}H_{24}O_4N_2ClS$: 507.11398, found: 507.11452.

*6.1.1.42. 1-((4-chlorobenzo[d]thiazol-2-ylamino)(4-(dimethylamino) phenyl)methyl) naphthalen-2-ol (**4bp**).* Pale yellow solid; mp = 205–207 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.20–10.03 (brs, 1H), 9.23–9.07 (brs, 1H), 8.07–7.93 (brs, 1H), 7.77 (t, 2H, *J* = 8.9 Hz), 7.62 (d, 1H, *J* = 7.9 Hz), 7.35 (t, 1H, *J* = 7.9 Hz), 7.25 (t, 3H, *J* = 7.6 Hz), 7.18–7.03 (m, 3H), 6.96 (t, 1H, *J* = 7.7 Hz), 6.64 (d, 2H, *J* = 8.9 Hz), 2.82 (S, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.0, 153.3, 149.1, 148.9, 132.2, 131.8, 129.3, 128.7, 128.6, 128.4, 126.9, 126.0, 125.5, 124.0, 122.3, 121.4, 121.4, 119.7, 118.6, 118.4, 112.2, 53.5, 40.1; IR (KBr): ν_{max} 3353, 3321, 2851, 1587, 1533, 1433, 1269, 811 cm^{-1}; HRMS calcd. for $C_{26}H_{22}N_3ONaSCl$: 482.1069, found: 482.1089.

6.2. In vitro angiotensin converting enzyme inhibition assay

ACE inhibition assay was performed using the method described by Jimsheena et al. [27,28]. Rabbit lung acetone powder (Sigma Aldrich, USA) was used as a source of ACE enzyme. 1 g of rabbit lung acetone powder was suspended in 10 mL of 0.05 M sodium borate buffer pH 8.2 containing 0.3 M NaCl and 0.5% Triton X-100 at 4 °C for 24 h followed by centrifugation at 15,000 rpm for 60 min at 4 °C. Supernatant was used as a source of ACE enzyme for this assay. ACE inhibition activity was assayed by monitoring the release of hippuric acid (HA) from the hydrolysis of the substrate hippuryl-histidyl-leucine (HHL) (Sigma–Aldrich, USA). 7 μl of ACE enzyme solution was preincubated with test and standard drug solution at 1 μM concentration for 10 min at 37 °C. Final volume was adjusted using 0.05 M sodium borate buffer (pH 8.2) containing 0.3 M NaCl to get equal concentration. The enzyme reaction was then initiated by adding 50 μl of 5 mM substrate (HHL) followed by incubation at 37 °C for 30 min. The reaction was then arrested by the addition of 0.1 mL of 1 M HCl. Hippuric acid produced in the reaction was then reacted with0.2 mL of pyridine (SD Fine chemical, India) and 0.1 mL of Benzenesulfonyl chloride(BSC) (SD Fine chemical, India)to develop yellow colour. The solution was mixed by inversion for 1 min and cooled on ice. The yellow colour developed was measured at 410 nm. The decreased concentration of HA in the test reaction compared with the control. ACE inhibition was expressed as percentage inhibition and calculated from the equation: Inhibition% = 100 − [*T*/*C*] × 100, where *T* = absorbance of test reaction and *C* = absorbance of control reaction. The therapeutic drug Lisinopril was used as reference ACE inhibitor. The inhibitory concentration 50% (IC_{50}) was calculated by nonlinear regression. The dose–response curve was obtained by plotting the percentage inhibition versus the concentrations.

6.3. Ex-vivo model for measuring aortic contraction

All the experiments were approved by the Institutional Animal Ethics Committee (IAEC, IICT, Hyderabad). Experiments were performed on male Sprague–Dawley rats that were 8–10 weeks old and weighed 180–200 g. Aortic contraction was determined by measuring isometric tension from a rat aortic ring [29]. The rats were sacrificed by cervical dislocation and under xylazine and ketamine anaesthesia. The thoracic aorta (from the arch of aorta to the diaphragm) was quickly excised and placed in 37 °C oxygenated (95% O_2 − 5% CO_2) calcium free Krebs − Hensleit buffer (KHB). Aorta

was cut into 5-mm segments and was cleared of adhering fat and adventitial tissues. Due care was taken to keep the endothelium intact. The composition of calcium free KHB (mM) was NaCl : 118, KCl : 4.7, $MgSO_4$: 1.2, KH_2PO_4 : 1.2, $NaHCO_3$: 25 and Glucose : 5.5 (pH 7.4). Each ring was suspended by stainless steel hooks in a water-jacketed organ bath, filled with 25 mL of oxygenated KHB maintained at 37 °C. Response from isometric tension was measured using an isometric force transducer (AD Instruments, Australia). A resting tension of 1 g was applied to aortic rings, which were then allowed to equilibrate for about 30 min. Once the tissue was stabilized, contraction was evoked by adding phenylephrine 1 mM to the organ bath during which the buffer was changed every 15 min. After the initial contraction by epinephrine, concentration–response curve was measured with the cumulative concentration of calcium (10^{-3} to 10^{-1} M). Similarly, the same cumulative concentration–response curve with calcium was prepared in presence of 100 mM test drugs (**4as** and **4bn**) and standard drug (Nifedipine).

6.4. Molecular docking

Molegro Virtual Docker (MVD, Ver: 2012.5.0.0) [30,31] is a program for determining the most conformations of a ligand that bind to an enzyme site. The identification of ligand binding modes is done iteratively by evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the enzyme. The highest scoring solutions are returned for further analysis. MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking. In the present study, docking studies were performed with two active ligands **4as** and **4bn** using the software. Crystal structure of tACE (PDB ID: 1O86) along with the co-factor (Zinc) was downloaded from RCSB Protein Data Bank. Structure of tACE (residues 37–625) [32–34] adopts an overall ellipsoid shape with central groove that extends for about 30 Å into molecules and divides the protein into two sub domains. Zinc is an important catalytic component of ACE, bound at the active site. The boundaries of the groove are provided by helices α and β strands. Structure of tACE–lisinopril complex used to locate the active site of the molecules. tACE contains 27 helices. Structure of tACE revealed the location of two buried chloride ions separated by 20.3 Å. Structures of both active compounds i.e. **4as** and **4bn** were prepared by using Chem Draw and saved to mol file format. These structures were further converted into mol2 file format by using Marvin Sketch. MVD was used to perform computational studies, cavity prediction, assigning bond orders, structure refinement, defining the active binding sites of the tACE and structure preparation. Residues from ACE enzyme involved in hydrogen bonding interaction with ligands are as follows: His 353, Ala 354, Tyr 523, Tyr 520 and Glu 152. As most of the ligands are surrounded by the above residues, it can be concluded that these residues are highly conserved and plays an important functional roles. Interaction of the ligands with these residues might be responsible for inhibition of ACE enzyme. Generally, low free energy is associated with high affinity binding with tACE.

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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.2014.06.035.

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