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ORIGINAL ARTICLE

Repurposing of pharmaceutical drugs by high-throughput approach for antihypertensive activity as inhibitors of angiotensin-converting enzyme (ACE) using HPLC-ESI-MS/MS method

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Abstract Angiotensin-converting enzyme (ACE) plays an important role in regulating blood pressure in the body by converting angiotensin-I into angiotensin-II. It is the basic component of Renin angiotensin aldosterone system (RAAS), imbalance of RAAS may leads to many cardiovascular and renal diseases. There are many marketed available drugs for the inhibition of ACE, but prolonged use of some drugs may cause the progressive side effects. Repurposing of existing drugs can be a way to find new inhibitors of ACE. In this study, a high-throughput and sensitive method

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Repurposing of drugs

of HPLC-ESI-QqQ-MS with good reproducibility (%RSD < 9.98) and linearity (R^2 = 0.999) was used to investigate the 77 commercial drugs for their inhibitory potential as antihypertensive drugs. Among these drugs, 41 drugs were found active and 36 of them showed moderate to good inhibition with lowest IC_{50} = 272 μM. This study showed that different pharmaceutical drugs can also be used as ACE inhibitor after necessary clinical trials or validation.

1. Introduction

The process of identifying new therapeutics indications of previously knows drugs is called as repurposing of drugs it is also known as re-tasking, reprofiling or repositioning of drug. This area of drug discovery has increasing interest in past few years due to its many advantages. It allows short times and reduced budgets for development of drugs as compared to drug development of new molecule (Pushpakom et al., 2018). The major advantage of drug repositioning is very low risk of failure, repurposed drug has already passed the toxicity tests, and it has well established safety profile in preclinical models (Rasheed et al., 2018).

Several drugs have been successful repurposed and had their impact in treatment by using this approach. Disulfiram was used to control chronic alcoholism now has been approved for cancer treatment, especially for glioblastoma, because of its ability to suppress properties of cancer stem cells (CSCs) (Rasheed et al., 2018). Topiramate which has originally indication for epilepsy was found potentially beneficial for weight loss and approved in 2012 as repurposed for treatment of obesity (Pushpakom et al., 2018; Tran et al., 2017).

Angiotensin-converting enzyme (ACE) is an important cardiovascular enzyme. It catalyzes the conversion of angiotensin I (AI) to the potent vasoconstrictor angiotensin II (AII) (Lapointe and Rouleau, 2002; Elased et al., 2006; Balasuriya and Rupasinghe, 2011; Menard and Patchett, 2001). It plays an important role in the renin angiotensin aldosterone system which regulates the blood pressure in the body (Wu et al., 2002; Peach, 1977; Bernstein et al., 2013). Inhibition of ACE leads to a decrease in the concentration of angiotensin II which causes the reduction in blood pressure (Elased et al., 2006; Lu et al., 2011; Mason et al., 2012; Sayer and Bhat, 2014).

Increased activity of ACE is associated with the high risk of cardiovascular and renal diseases and hypertension. In a statistic by American Heart Association (AHA) it was showed that, in USA, the rate of heart failure (HF) has been increased to 0.8 million new cases in last 5 years, and it is expected to rise by 46% by 2030 (Members et al., 2017). Hypertension is the world's most ubiquitous progressive disorder, about 25% of total adult population of the world is affected by the hypertension, and it may increase up to 29% by 2025 (Balasuriya and Rupasinghe, 2011). In many communities of the world approximately one adult out of three have hypertension, which is leading risk factor for disability and death (Carey, 2015).

To overcome the activity of ACE there are many marketed available drugs for inhibition, among them Captopril and Lisinopril are two potent synthetic inhibitors which were developed and used widely as drugs for the treatment of hypertension (Lapointe and Rouleau, 2002; Wu et al., 2002; Chen et al., 2013; Hsieh et al., 1998). But the prolonged use of some drugs may cause the progressive side effects. It is necessary to identify new inhibitors of clinically important enzymes with minimum side effects.

Many drugs have been identified as new cardiovascular treatment drugs among the examples, Aspirin the most common analgesic had been identified and approved in 2015 for repurposed in treatment of Colorectal cancer as well as cardiovascular diseases (Pushpakom et al., 2018). Methotrexate, a cancer treatment drug which was also used for treatment of rheumatoid arthritis and chronic inflammatory conditions, had also considered for repurposing to reduce Cardiovascular risks (e.g. hypertension, arterial stiffness and endothelial dysfunction) (Mangoni et al., 2018; Ishida et al., 2016).

Purpose of this study was to screen several pharmaceutical drugs to identify their potential as ACE inhibitor by employing a high-throughput and sensitive method of HPLC-ESI-MS/MS. Combination of HPLC with ESI-MS/MS allows sensitive and unambiguous analysis of peptides in complex matrices as well as give many advantages over other spectrophotometric analysis methods, like speed reproducibility, minimum amount of substrate and enzyme etc. This previously developed method had very low limit of detection (LOD) and limit of quantification (LOQ), very low quantification range of 20–200 nM. of Angiotensin with linearity of R^2 = 0.999, as well as it utilize very low amount of both enzyme and substrate per enzymatic reaction. This method also had good inter and intra-day reproducibility with RSD % as low as 4.14 (Musharraf et al., 2017).

This study proved to be very effective in employing a modern phenomena of drug repurposing to identify indication of standard drugs as ACE inhibitors. Most of the screened drugs showed very good inhibition activity and used method of HPLC-ESI-MS/MS also found to be effective for screening of large libraries of drugs.

2. Material and methods

2.1. Reagents, chemicals and samples

Angiotensin converting enzyme, its substrate angiotensin I and product angiotensin II were purchased from Sigma Aldrich (USA). Two standard inhibitors Captopril and Lisinopril were purchased from Tokyo Chemical Industries Co, Ltd. (Japan), both were >98% pure. Tris buffer (research grade) was purchased from Serva (Germany). The concentration of this buffer was prepared 20 mM with 3 mM dithiothreitol (DTT) for the pH 7.5 at 37 °C. Acetonitrile and DMSO were purchased from Fisher Scientific (Leicestershire, UK). All other solvents used, were of spectroscopic grade, Milli-Q water was used fresh from Milli-Q water assembly (Bedford, USA) which had 16.5 MΩ resistance. For inhibition study of standard

drugs, all drugs were collected from the compound bank of International Center for Chemical and Biological Sciences, University of Karachi, Karachi.

2.2. Angiotensin-converting enzyme assay

Enzymatic assays for inhibition study were performed in well plate in different batches, each batch contained one well as blank or control and other contain reaction mixtures with different drugs or compounds. Control or blank well contain only enzyme and substrate, but reaction mixture contains enzyme, substrate and inhibitors of different concentrations. All the wells contained same volume of each enzyme, substrate and inhibitor.

Each reaction well contained 1 μL of angiotensin converting enzyme (0.5 μM), 10 μL of angiotensin I (4 μM) and 2.5 μL of compound or drug of different concentration in different well. In control reaction 2.5 μL of water or 20% DMSO to compensate the volume of inhibitor. Both ACE and substrate were prepared in TRIS buffer of 20 mM with 3 mM DDT of pH 7.5 and standard drug were prepared in milli-Q water or 20% DMSO according to their solubility. All reactions were performed in incubator at 37 °C for a standard time of 30 min.

2.3. Preparation of drugs

Most of the compounds and drugs were soluble in water therefore their solutions were prepared in milli-Q water. Some drugs were only soluble in DMSO, their solubility was checked in different percentages of DMSO in water and 20% DMSO in water (1 part DMSO: 4 parts water) was found suitable. Those drug's solutions and their dilutions were prepared in the same 20% DMSO solvent. Standard inhibitors, Captopril and Lisinopril were prepared in milli-Q water as well as in 20% DMSO, separately. All concentrations were prepared in micro molar (μM) or milli molar (mM) and diluted with the same solvent in which bulk was prepared.

2.4. Sample preparation for LC-ESI-QqQ-MS analysis

Aliquot of 2.5 μL was taken after standard incubation time i.e. 30 min and mixed with 2.5 μL of 0.5 μM of bradykinin (Internal standard) and 7.5 μL of 0.1% formic acid solution. Formic acid solution (0.1%) was used to quench the reaction and also to make the 1/5th dilution of the reaction mixture. From this mixture duplicate runs were carried out, in each run, 5 μL of the sample was injected into the ESI-QqQ-MS system of Agilent equipped with HPLC system and ESI Jet Stream Source. Agilent Mass Hunter Data Acquisition 5.0 and Data Analysis 6.01 were used for data acquisition and interpretation, respectively.

2.5. LC-ESI-QqQ-MS analysis

All samples were analyzed using Agilent 1260 liquid chromatograph (Agilent Technologies, Wilmington, DE), coupled with Agilent 6400 triple quadruple mass spectrometer (Agilent Technologies, Wilmington, DE, USA). Peptides separation was achieved by using reverse phase HPLC with Jupiter C-18 column of Phenomenex (Torrance, California) having dimensions of 50 mm length, 1 mm i.d. and 5 μm particle size. The mobile phase was comprised of water with 1% formic acid as solvent A, and acetonitrile with 1% formic acid as solvent B. Gradient of 5–70% of B was run for a total time of 7 min. The gradient was started with 5% B, which was changed progressively to 70% B in 3 min, and then rapidly decreased again to 5% B in half minute and run for the next 3.5 min at same composition. The flow rate was set at 0.4 mL/min.

Final optimized conditions of ESI JetStream source were used as, gas temperature 300 °C, gas flow 12 L/min, nebulizers pressure 30 psi, sheath gas heater 240 °C, sheath gas flow 10 L/min, capillary voltage 3000 V and nozzle voltage (V Charging) 1000 V. Multiple reaction monitoring (MRM) mode was used to collect mass spectral data of precursor and product ion transitions. The optimized values of MRM transition, collision energy (CE) and the fragmentor voltages (FV) of peptides were as follows: Bradykinin, 530.8 → 175.2, FV = 100 V, CE = 30 eV and Angiotensin, 433 → 110.1, FV = 100 V, CE = 20 eV.

3. Results and discussion

Repurposing of standard drugs is a valid method in which marketed available standard drugs are treated and checked for the treatment of other diseases, if a drug can provide multi-function it can be used more widely. In this study, 77 drugs were selected to screened for their inhibition against ACE. Selected drugs were already known and used for other purposes like antibiotic, inhibitors of other enzymes, anti-epileptic, anti-inflammatory, etc. All drugs were selected randomly, from the drug bank and prepared according to their solubility as given in the Table 1. Solution of drugs were prepared in deionized water or 20% DMSO, each drug was prepared of 50 mM bulk concentration; further dilutions were prepared from the same solvent in which bulk was prepared. Among 77 drugs, 40 were prepared in water and 37 were prepared in DMSO. Three of these drugs Lisinopril Dihydrate, Enalapril Maleate and Ramipril, were standard inhibitors of ACE. Details of all drugs along with their properties and uses are also given in Table 1.

3.1. Enzymatic reaction in different solvents

Angiotensin Converting Enzyme reactions were performed with optimized condition of reported method (Musharraf et al., 2017). In this study as the 20% DMSO was used to prepare the solution of few drugs, therefore the effect of DMSO on enzyme reaction had to be checked. For this purpose, ACE reaction was performed while adding DMSO instead of water as a compensation of inhibitor volume. In normal reactions, 2.5 μL of water was added in 1 μL of 0.5 μM Enzyme (ACE) and 10 μL of 4 μM substrate (angiotensin I). while in DMSO reaction, 2.5 μL of 20% DMSO was added in the reaction mixture of enzyme and substrate. Aliquots of both reactions were taken at same time intervals i.e. 5, 10, 20, 30, 40 and 50 min and reaction progress was plotted as percentage conversion (%C) *vs.* time as shown in Fig. 1. In enzyme reaction of DMSO, overall intensity of both substrate and product peaks was decreased as compared to enzyme reaction of water

Table 1 Drugs screened again ACE for inhibition potential.

S. No.	Code	Name	Chemical class	Purpose(s)[‡]	Solvent used	IC_{50} (μM)
1	DB-000	Acyclovir	Purine	Antiviral	Water	Inactive
2	DB-001	Amlodipine besylate*	Pyridine	Antidepressant, Calcium channel blocker, CVD	Water	288
3	DB-002	Amoxicillin trihydrate*	Penicillin	Antibiotic	Water	364
4	DB-003	Ampicillin trihydrate*	Penicillin	Antibiotic	Water	319
5	DB-005	Atorvastatin calcium trihydrate	Statin	Statin, lower cholesterol levels in the blood, CVD	DMSO	Inactive
6	DB-007	Azithromycin dihydrate	Macrolide	Antibiotic, bacterial infections	DMSO	3957
7	DB-009	Ciprofloxacin HCl monohydrate*	Quinoline	Antibiotic, bacterial infections	Water	272
8	DB-010	Clarithromycin HCl	Macrolide	Antibiotic, bacterial infections, anti-ulcer	DMSO	Inactive
9	DB-012	Diltiazem hydrochloride*	Benzothiazepine	Calcium channel blocker, CVD, prevent chest pain (angina)	Water	490
10	DB-016	Indomethacin	Indoleacetic acid	Nonsteroidal anti-inflammatory drug (NSAID)	DMSO	Inactive
11	DB-017	Itopride hydrochloride*	Benzamide	AChE Inhibitor	Water	296
12	DB-020	Lidocaine Hydrochloride monohydrate	Acetamide	Local anesthetic	Water	645
13	DB-022	Lisinopril Dihydrate[#]	Peptide	ACE inhibitor, CVD	DMSO	2.66
14	DB-027	Ofloxacin	Quinoline	Antibiotic, bacterial infections	DMSO	2691
15	DB-031	Prednisolone Acetate	Pregnane Steroids	Anti-inflammatory, corticosteroids, eye condition	DMSO	Inactive
16	DB-033	Ropinirole HCl	Indole	Parkinson's disease treatment, anti-psychotic	DMSO	Inactive
17	DB-035	Sodium valproate	Fatty acid	Bipolar disorder, anti-psychotic	DMSO	1147
18	DB-040	Gamma-Aminobutyric Acid	Alkanoic acid	Neurotransmitter, anti-anxiety	DMSO	3766
19	DB-043	Bromazepam	Benzodiazepine	Anti-anxiety, anti-psychotic	DMSO	7231
20	DB-045	Celecoxib	Benzenesulfonamide	Nonsteroidal anti-inflammatory drug (NSAID)	DMSO	5711
21	DB-046	Chloroquine Phosphate*	Quinoline	Anti-malarial	Water	342
22	DB-052	Diclofenac Sodium	Phenylacetic acid	Nonsteroidal anti-inflammatory drug (NSAID)	Water	Inactive
23	DB-053	Diphenhydramine Hydrochloride*	Benzothiazepine	Antihistamine, anti-allergy	Water	338
24	DB-054	Doxycycline Hyclate*	Tetracycline	Antibiotic	Water	329
25	DB-056	Enalapril Maleate[#]	Pyrrolidine	ACE inhibitor, CVD	Water	10.03
26	DB-069	Mefenamic Acid	Benzoic acid	Nonsteroidal anti-inflammatory drug (NSAID)	Water	10,959
27	DB-070	Mesterolone	Androstan	Androgen and anabolic steroid (AAS)	DMSO	Inactive
28	DB-073	Nabumetone	Naphthalenes	Nonsteroidal anti-inflammatory drug (NSAID)	DMSO	3279
29	DB-076	Oxaprozin	Oxazole	Nonsteroidal anti-inflammatory drug (NSAID)	DMSO	Inactive
30	DB-077	D-Penicillamine	Amino acid	Rheumatoid arthritis, Wilson's disease	Water	Inactive
31	DB-079	Ramipril[#]	Pyrrole-carboxylic acid	ACE inhibitor, CVD	DMSO	8.12

Table 1 (*continued*)

S. No.	Code	Name	Chemical class	Purpose(s)[‡]	Solvent used	IC_{50} (μM)
32	DB-082	Tranexamic acid	Cyclohexanecarboxylic acid	Anti-fibrinolytics, Trauma bleeding prevent	Water	Inactive
33	DB-083	Valsartan	Biphenyl	Angiotensin receptor blockers (ARBs), CVD	DMSO	Inactive
34	DB-084	Epinephrine Bitartrate/ Adrenaline Bitartrate	Benzene	Neurotransmitter, relief of hypersensitivity	Water	Inactive
35	DB-086	Cefadroxil monohydrate	Cephalosporin	Antibiotic, bacterial infections	Water	Inactive
36	DB-089	Ceftriaxone Sodium 3.5 H2O	Cephalosporin	Antibiotic, bacterial infections	Water	Inactive
37	DB-091	Dextromethorphan Hydrobromide Monohydrate	Morphinan	Cough suppressant	Water	5258
38	DB-093	Gliclazide	Thiazole	Antihyperglycemic, anti-diabetic	DMSO	~50000
39	DB-094	Hydrocortisone Sodium Succinate	Pregnan	Used to treat arthritis, severe allergies, blood diseases, breathing problems	Water	~20000
40	DB-096	Mirtazapine	benzazepine	Antidepressant	DMSO	Inactive
41	DB-098	Norethisterone	Pregnane	Used for treatment of amenorrhea, endometriosis	DMSO	3839
42	DB-099	Clavulanic acid	Beta-lactam	Antibacterial	Water	Inactive
43	DB-100	Clioquinol	Quinolin	Antifungal, antibacterial	DMSO	Inactive
44	DB-101	Cloxacillin Sodium Hydrate	Penicillin	Antibiotic	Water	Inactive
45	DB-104	Lysine Hydrochloride	Amino acid	Antiviral	Water	2905
46	DB-105	Montelukast Sodium	Quinolin	Antiasthma agents	DMSO	Inactive
47	DB-106	Quinine Dihydrochloride	Cinchonan	Antiprotozoal, antimyotonic	Water	8104
48	DB-107	Salbutamol Sulfate	Ethanolamine	Antiasthma agents	Water	Inactive
49	DB-108	Sulfadoxine	Sulfonamide	Antiprotozoal, Antiasthma	Water	Inactive
50	DB-110	Bupropion Hydrochloride	Phenone	Antidepressant	Water	~55000
51	DB-113	Diclofenac Potassium	Phenylacetate	Nonsteroidal anti-inflammatory drug (NSAID)	DMSO	~46000
52	DB-114	Domperidone	Benzimidazole	Antiemetic	DMSO	1491
53	DB-117	Gabapentin	γ-Aminobutyric acid	Anti-psychotic	Water	Inactive
54	DB-120	Levetiracetam	Pyrrolidine	Anticonvulsant, anti-psychotic	DMSO	Inactive
55	DB-123	Sertraline Hydrochloride*	Naphthalene	anxiety, antidepressant, panic attacks	DMSO	581
56	DB-125	Beclomethasone Dipropionate	Pregnan	Corticosteroids, anti-inflammatory	DMSO	Inactive
57	DB-127	Cefazolin Sodium	Cephalosporin	Antibiotic, bacterial infections	Water	Inactive
58	DB-128	Crotamiton	Toluidine	Scabicides, antipruritic	DMSO	4383
59	DB-129	Folic Acid	Pteridine	Vitamin B	Water	Inactive
60	DB-132	Cefotaxime Sodium	Cephalosporin	Antibiotic, bacterial infections	Water	Inactive
61	DB-133	Cholecalciferol	Secosterol	Vitamin D3	DMSO	4691
62	DB-137	Metronidazole	Imidazole	Antibacterial, anti-parasitic	DMSO	2238

(*continued on next page*)

Table 1 (*continued*)

S. No.	Code	Name	Chemical class	Purpose(s)[‡]	Solvent used	IC_{50} (μM)
63	DB-141	Venlafaxine HCl	Cyclohexanol	Antidepressant	Water	1722
64	DB-142	Aminophylline	Purine	Phosphodiesterase inhibitor, adenosine receptor blocker, anti-asthma	Water	Inactive
65	DB-143	Fluoxetine HCl	Phenylpropylamines	Antidepressant	DMSO	962
66	DB-145	Pantoprazole Sodium	Benzimidazoles	Proton pump inhibitor (PPI), antiulcer	Water	~73000
67	DB-146	Pyridoxine HCl	Pyridine	Vitamin B6	Water	15,980
68	DB-147	Rabeprazole Sodium	Benzimidazoles	Proton pump inhibitor (PPI), antiulcer	DMSO	Inactive
69	DB-149	Terbutaline sulfate	Resorcinol	Bronchodilator	Water	Inactive
70	DB-150	(±)-Alpha-Tocopherol acetate	Chroman	Vitamin E, antioxidant	DMSO	9800
71	DB-152	Enoxacin sesquihydrate	Fluoroquinolone	Antibiotic, bacterial infections	Water	5782
72	DB-156	Suxamethonium HCl	Quaternary ammonium compound	Anesthetic	Water	Inactive
73	DB-157	Thiamine HCl	Thiopyrimidine	Vitamin B1	Water	Inactive
74	DB-158	Topiramate	Dioxolopyrans	Anti-epileptic, prevents migraine headaches	DMSO	3296
75	DB-191	Permethrin	Cyclopropanecarboxylate	Insect killer drugs, treat scabies	DMSO	1814
76	DB-214	Caffeine	Xanthenes	Central nervous system stimulants	DMSO	846
77	DB-221	Papaverine (HCl)	Alkaloid	Vasodilator, Phosphodiesterase inhibitor, direct actions on calcium channels	DMSO	2699

[*] Ten most active drugs in all screened samples.
[‡] Note: All the purposes of drugs are taken from the following websites, WebMD.com, DrugBank.Ca and Drugs.com.
[#] Standard inhibitors of Angiotensin-Converting Enzyme.

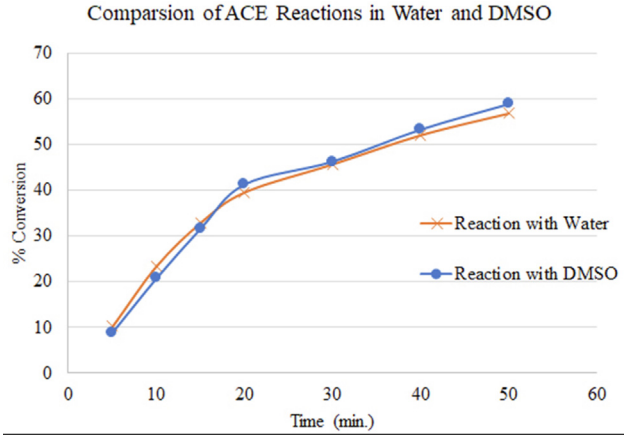

Fig. 1 Comparison of ACE reaction in different solvents (Water and 20% DMSO).

but the ratios remain the same, and no significant difference in percentage conversion was observed at specific time in both reactions. Therefore, reaction of drugs prepared in water can be compared with the reactions of drugs prepared in 20% DMSO.

3.2. HPLC-ESI-MS/MS method and validation

This HPLC-ESI-MS/MS method used in this study was developed to maximize the response with minimum substrate, enzyme and other enzymatic reagents and validated for its linearity range and enzymatic reaction by interday and intraday analysis.

The calibration curve was drawn by using 5 different calibrators of 20, 60, 100, 140 and 200 nM of Angiotensin I. The calibration showed a linear response over the range with R^2 = 0.999 and LOD and LOQ as 1.44 nM (1.866 ng/mL) and 4.37 nM (5.664 ng/mL), respectively. Interday and intraday reproducibility of the linear curve, for two qualifiers showed that it has RSD% in the range of 1.76 – 4.37, which is very low as comparation to the other methods. Interday and intra analysis of the enzymatic method and inhibition reaction also showed very low RSD% in range of 4.14 – 9.98. In this study further validation of the assay was performed by inhibition reaction of Captopril and Lisinopril.

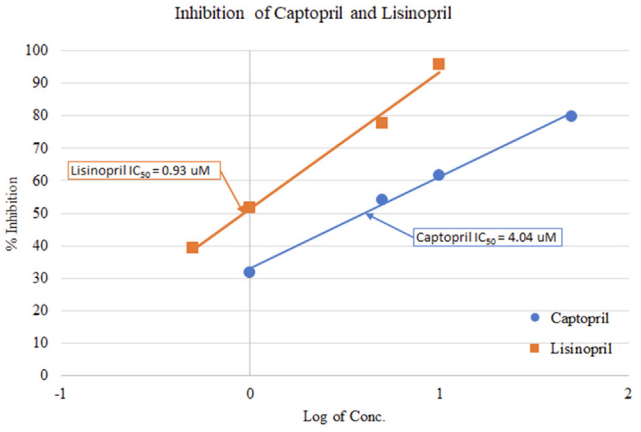

Fig. 2 Comparation of inhibition of captopril and lisinopril and their IC_{50} values.

3.3. Determination of IC_{50} of inhibitors

Once the enzyme inhibition assays were performed, all drugs and two standard inhibitors were investigated for their inhibitory potential against ACE by using the reported methodology (Musharraf et al., 2017). For concentration dependent inhibition studies, the inhibition was calculated as % inhibitory activity (% IA). It is also defined as the measurement of the degree of inhibition and it is used to determine the IC_{50} value. For IC_{50} calculation inhibition data was plotted as % IA *vs.* log of concentration of inhibitor, concentration of inhibitor correspond to 50% inhibition activity was the IC_{50} value of that inhibitor. For few drugs which have very low activity, extrapolation of curve was used and their values are reported as estimated values. The inhibitory activity is the % conversion measured in control reactions (without inhibitor) divided by the % conversion measured in a reaction with inhibitor, as represented in Equation (1) (Chen et al., 2013; Lahogue et al., 2010; Greis et al., 2006):

$$\%\ \text{Inhibition activity} = \left(1 - \frac{\%\ \text{conversion with inhibitor}}{\%\ \text{conversion without inhibition}}\right) \times 100 \quad (1)$$

Two standard inhibitors of ACE, Lisinopril and Captopril, were checked for the authentication of the assay. For Captopril concentrations of 1, 5, 10 and 50 μM and for Lisinopril concentrations of 0.5, 1, 5 and 10 μM were used. The IC_{50} values of Captopril and Lisinopril were found to be 4.04 μM and 0.93 μM, respectively, as shown in Fig. 2. These values were closely matched with the previously reported values and used as reference for the screening of other inhibitors (pharmaceutical drugs).

Total 77 pharmaceutical drugs were analyzed for their inhibitory potential and among these drugs, 41 drugs showed inhibitory activity against ACE. IC_{50} values of 13 drugs (DB-001, DB-002, DB-003, DB-009, DB-012, DB-017, DB-020, DB-046, DB-053, DB-054, DB-123, DB-143 and DB-214) were less than 1 mM, and showed good to moderate inhibition, with as low as IC_{50} = 272 μM (DB-9). 23 Drugs showed low inhibition, their IC_{50} values were in the range of 1.49 to 15.98 mM. While DB-93, DB-94, DB-110, DB-113 and DB-145 showed very low inhibition, their IC_{50} value was ranges from 20 to 73 mM, in comparison to the standard inhibitor these values are very high. Other 33 drugs were inactive, even a high concentration of those drugs did not show any inhibition at all. Remining three drugs Lisinopril Dihydrate (DB-22), Enalapril Maleate (DB-56) and Ramipril (DB-79) were the standard inhibitors of ACE and they had IC_{50} value 2.663 μM, 10.026 μM and 8.125 μM, respectively. These three inhibitors were analyzed blind folded along with other pharmaceutical drugs. Close values of these inhibitors represent the authenticity of the assay and screening method. Lisinopril (DB-22) showed insignificant high values compare to standard pure Lisinopril, because of its low purity. IC_{50} values of all the active drugs are given in the Table 1.

3.4. Repurposing of potential drugs

Among all the analyzed drugs, the most active drugs against ACE are marked with asterisk (*) in the Table 1. These drugs may serve as ACE inhibitor or their derivatives may be formed to enhance the activity. Among the most active drugs four of them, Amoxicillin trihydrate (DB-002), Ampicillin trihydrate (DB-003), Ciprofloxacin HCl monohydrate (DB-009) and Doxycycline Hyclate (DB-054), showed the IC_{50} values of 364 μM, 319 μM, 272 μM and 329 μM, respectively, were belong to the Antibiotic class of drugs. In this study total 13 drugs of Antibiotic class were screened, among them seven showed activity along with above mentioned four drugs. It seems like that Antibiotics can be used as ACE inhibitors; therefore, more Antibiotics may be screened, and potential hits may be found among them.

Amlodipine besylate (DB-001) and Diltiazem hydrochloride (DB-012) are also using for cardiovascular diseases (CVD) but their mode of action is different, they act as calcium channel blockers (CCBs). Both drugs were also found active as ACE inhibitors with IC_{50} values of 288 μM and 490 μM, respectively, it may enhance the area of treatment for these drugs. Two more CVD drugs were also screened from different mode of actions and both were found inactive. Among these CVD drugs only CCBs were found active therefore more CCBs drugs may also be screened to find more potent hits against ACE.

Itopride hydrochloride (DB-017) which is an Acetylcholinesterase (AChE) inhibitor, was also found as potential inhibitor of ACE with IC_{50} = 296 μM. Another Phosphodiesterase inhibitor was also found active, more inhibitors may also be screened to find their dual inhibition properties. Diphenhydramine Hydrochloride (DB-053) an antihistamine and anti-allergy drug, Chloroquine Phosphate (DB-046) an anti-malarial drug and Sertraline Hydrochloride (DB-123) an anxiety relief and antidepressant drugs were also among the top active drugs in screening results. These drugs had IC_{50} values of 338 μM, 342 μM and 581 μM, respectively. These drugs may serve their purpose as ACE inhibitor along with their primary approved treatment, after proper development studies for repurposing on them.

4. Conclusion

Evaluation of seventy-seven commercial drugs was carried out in this study, and more than half of them were found active against ACE. Among them, thirteen drugs showed moderate to good inhibition with as low as IC_{50} = 272 μM, twenty-three drugs showed low inhibition in range of 1.49−15.98 mM, while five drugs showed very low inhibition. Active drugs were belonging to various classes of compounds including antibiotics, inhibitors of other enzymes, anti-malarial, CVD treatment etc. Among all drugs, three ACE standard inhibitors were also screened blind folded, which give very similar results to the known standard inhibitors, which further proved the authenticity of study and reproducibility of the assay. This study proved that the used method of HPLC-ESI-MS/MS was a valid readout for enzyme inhibition screening assays and can be used for screening of large number of compounds to identify new indications. In this study, new indication founds of previously used drugs, can be used after necessary validation and clinical trials. This method can be extended to screen more libraries of compounds for ACE and other targets.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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