Synthesis of oxazolidinone from enantiomerically enriched allylic alcohols and determination of their molecular docking and biologic activities

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ABSTRACT

Enantioselective synthesis of functionalized cyclic allylic alcohols via kinetic resolution in transesterification with different lipase enzymes has been developed. The influence of the enzymes and temperature activity was studied. By determination of ideal reaction conditions, byproduct formation is minimized; this made it possible to prepare enantiomerically enriched allylic alcohols in high ee's and good yields. Enantiomerically enriched allylic alcohols were used for enantiomerically enriched oxazolidinone synthesis. Using benzoate as a leaving group means that 1 mol % of potassium osmate is necessary and can be obtained high yields 98%. Inhibitory activities of enantiomerically enriched oxazolidinones (8, 10 and 12) were tested against human carbonic anhydrase I and II isoenzymes (hCA I and hCA II), acetylcholinesterase (AChE), and α-glycosidase (α-Gly) enzymes. These enantiomerically enriched oxazolidinones derivatives had Kᵢ values in the range of 11.6 ± 2.1–66.4 ± 22.7 nM for hCA I, 34.1 ± 6.7–45.2 ± 12.9 nM for hCA II, 16.5 ± 2.9 to 35.6 ± 13.9 for AChE, and 22.3 ± 6.0–70.9 ± 9.9 nM for α-glycosidase enzyme. Moreover, they had high binding affinity with −5.767, −6.568, −9.014, and −8.563 kcal/mol for hCA I, hCA II, AChE and α-glycosidase enzyme, respectively. These results strongly supported the promising nature of the enantiomerically enriched oxazolidinones as selective hCA, AChE, and α-glycosidase inhibitors. Overall, due to these derivatives' inhibitory potential on the tested enzymes, they are promising drug candidates for the treatment of diseases like glaucoma, leukemia, epilepsy; Alzheimer’s disease; type-2 diabetes mellitus that are associated with high enzymatic activity of CA, AChE, and α-glycosidase.

1. Introduction

Enzymatic kinetic resolution is stereo- method and highly a enantioselective in synthesis of organic compounds [1]. For the enzymatic kinetic resolution of alcohols can be operated as a transesterification by the enantiomers with dissimilar enantiomeric ratios [2]. This reaction selectively converts one of the enantiomers into the product, while leaving behind the enantiomerically enriched substrate. The yield of enantiomerically pure compounds never exceeding 50% is a disadvantage of the enzymatic reaction. The synthesis of enantiomerically enriched molecules with new methods is an important issue in organic chemistry [3,4]. The chemo-enzymatic reactions for the synthesis of enantiomerically pure compounds have been gradually increasing in synthetic studies, while the biocatalysts as chiral compounds have been widely used in preparative organic synthesis. In recent years, it is known in the literature that lipase enzymes catalyze esterification [5,6] and enantioselective hydrolysis [7] for many substrates. This feature has attracted the attention of organic chemists because lipase can be easily obtained and used (see Scheme 1 and Table 3).

The widespread presence of optically pure oxazolidinones in pharmaceuticals [8], natural products [9], synthetic intermediates [10] and biologically active compounds requires an efficient and reliable methodology due to their functional groups. As a result, many methods for enantiopure preparation continue to be in high demand [11]. The oxazolidinones are a new class of synthetic antimicrobial agents unrelated to any other antibacterial drug class as Linezolid [12,13].

Carbonic anhydrase (CA, E.C.4.2.1.1) is a metalloenzyme that contains zinc ions (Zn²⁺) in its active site. Zn²⁺ ion interacts with the substrate molecules directly and cause catalytic effect [14–16]. This enzyme family catalyzes the reversible conversion of carbon dioxide (CO₂) and water (H₂O) to bicarbonate (HCO₃⁻) and protons (H⁺) [17]. They are grouped into seven different gene families, namely α-, β-, γ-, δ-, ϵ-, ζ-, and the last

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recently discover θ-CAs isoenzymes in prokaryotes and eukaryotes. The
gene families do not have similarities with regard to amino acid sequences
[18,19]. Human carbonic anhydrases (hCAs) are included in the class of α-
CAs. Up to now, sixteen α-CA isoforms have been well defined in humans
[20–23]. Among them, CaS I, II, III, VII and XIII are cytosolic isoenzymes,
CaS IV, IX, XII, XIV and XV are membrane-bound isoenzymes, CaS VA and
VB are mitochondrial isoenzymes, and Ca VI is a secreted isoenzyme
[24,25]. The common property of all CA isoforms is that they have zinc
ions (Zn²⁺) bound to histidine amino acids. Zn²⁺ directly interact with
the substrate molecules and increase the catalytic activity of the enzyme,
thus producing a powerful hydrolysis of water to H⁺ and a highly reactive
Zn-OH molecule [26,27]. Carbonic anhydrase inhibitors (CAIs) are in
clinical use for the treatment of various activities like diuretics, anti-tu-
mour and anti-metastatic agents, anti-glaucoma, and anti-epilepsy
[28,29]. For this purpose, development of novel CAIs had a quite im-
portance.

Alzheimer’s disease (AD) affects mostly the aged people and above
resulting in impaired memory and behavior. This disorder clinically
involves the progressive degeneration of brain tissue that is influenced
by the deficit in acetylcholine (ACh) [30,31]. Acetylcholinesterase
(AChE, AChE; E.C.3.1.1.7), as a major element of the cholinergic
system in the peripheral and central nervous system is able to convert
acetylcholine (ACh) to acetate (CH₃COO⁻) and choline (Ch) [32,33]. It
is caused majorly by environmental and genetic influences. Cerebral
amyloid-β aggregation, a deficit in ACh and a deficit in cholinergic
nerve transmission, was observed in patients with AD [34,35]. Because
of serious side effects of the available AChE, there is the need to search
for newer effective and safe AChE to treat neurodegenerative disorders.
AChE inhibitors (AChEIs) or anticholinesterases inhibit cholinesterase,
increasing the level and length of ACh action [36]. A variety of uses of
AChEIs are common in medicine. As a result, a number of AChEIs have
been thought for the treatment of AD. They have been used in
clinical trials, including natural substances [37,38].

α-Glycosidase (E.C.3.2.1.20) release from intestine cells and hy-
drolases oligosaccharides and polysaccharide to monosaccharide units,
such as glucose and fructose in small intestine [39,40]. In human, α-
glycosidase inhibitors (α-GIs) had a great importance for controlling
of type-2 diabetes mellitus (T2DM) and hyperglycemia [41]. Recently,
two main chemical classes of N-comprising α-Glycosidase inhibitors (α-
GIs) contain sugar-based inhibitors [42]. α-GIs can reduce the uptake of
dietary carbohydrates and repress postprandial T2DM and hypergly-
ecemia. Thus, these α-GIs are endowed with sugar molecule such as
compete and moieties with the oligosaccharides for binding to the ac-
tive site of the enzyme, hence effectively reducing the postprandial
glucose amounts in T2DM [43-45].

In this study, we investigated the inhibition profiles of novel syn-
thetized enantiomerically enriched oxazolidinones (8, 10 and 12)
against human carbonic anhydrase isoenzymes, acetylcholinesterase,
and α-glycosidase enzymes and compared to standard inhibitors.

2. Results and discussions

2.1. Chemistry

We have started our study by finding the optimal enzyme from the
lipase enzymes for kinetic resolution and determining the best working
conditions for these enzymes such as Candida cylindracea lipase (CPL)
and Candida rugosa lipase (CRL). The CPL and CRL enzymes were tested
for the transesterification of cyclohex-2-en-1-ol (1) employing vinyl
acetate as acyl donor and solvent (Table 1).

The first bioconversion was performed by CPL and the reaction
mixed at 15°C. The conversion was monitored by TLC and ¹H NMR.
After 8h, 50:50 conversions were determined. The resulting products
were then purified with column chromatography. Enantiomerically
enriched compounds 1a and 2 were observed in 47% yield with [α]D
12.5 (c 1.0, CH₂Cl₂) and in 42% yield with [α]D: −4.1 (c 1.0, CH₂Cl₂)
respectively (entry 1 in Table 1). Similar decomposition reactions were
observed at 0°C, which 1a and 2 in 46% yield with [α]D: 16.8 (c 1.0,
CH₂Cl₂) and in 39% yield with [α]D: −4.2 (c 1.0, CH₂Cl₂), respectively
(entry 2 in Table 1). These results were not good; therefore, the same
conditions as the CRL enzyme were tested. Second bioconversion was
performed by CRL at 0°C. The conversion was monitored by TLC and
¹H NMR. After 10 h, 50:50 conversions were determined. The resulting
products were then purified with column chromatography. En-
tantiomerically enriched compounds 1a and 2 respectively were ob-
served in 41% yield with [α]D: 10.6 (c 1.0, CH₂Cl₂) and in 45% yield with
[α]D: −60.2 (c 1.0, CH₂Cl₂) respectively (entry 4 in Table 1).

Among the lipase enzymes studied, CRL was proved to be suitable for
the enantiomerically enriched esterification of substrate 1, which
showed high optical rotation

Since the acetate (2) has a higher optical rotation than the alcohol 1a,
we hydrolyzed the acetate (2, (−)-Cyclohex-2-en-1-ol (1b) was observed in
92% yield with [α]D: −86.7 (c 1.0, CH₂Cl₂) [Lit. (−)-Enantiomer [α]D:
+110.8 (c 1.2, CH₂Cl₂), ≥99 ee %] [46]. So we synthesized the en-
tantiomerically riched allylic alcohol. (−)-Cyclooct-2-en-1-ol (3b) was
hydrolyzed the acetate (1a) in 41% yield with [α]D: 25.8 (c 1.0, CH₂Cl₂)
and in 45% yield with [α]D: −52.4 (c 1.46, CH₂Cl₂), ≥99 ee % [46] and (−)
2,2-dimethyl-3a,4,5,7a-tetrahydronenzol[1,3]
dioxol-5-ol (5b) [α]D: 21.2 (c 1.0, CH₂Cl₂) [Lit. (−)-Enantiomer [α]D:
−25.3 (c 0.4, CHCl₃), 48% ee Mosher’s ester formation] [47] were syn-
thesized by CRL enzyme at 0°C in the same way as enantiomerically en-
riched allylic alcohols as see Table 2.

The enantiomeric purities of allylic alcohol (1b, 3b and 5b) in
principle cannot be easily determined by known asymmetric synthesis
methods. The absolute configurations of allylic alcohols (1b, 3b and
5b) were determined by comparison of their chiroptical data with those
reported in the literature [46,47] (see ).

Compound 5b was synthesized as described in the literature [48].
The stereochemistry of this compound was discussed in the literature
and the OH and ketal groups in the molecule were determined to be
trans with ¹H NMR and ¹³C NMR studies. Compound 8 was synthesized
in the literature by the same method as racemic [49]. Stereochemistry
of reaction was confirmed by spectroscopy data in the literature. But in
this study we synthesized enantiomerically enriched oxazolidinones (8,
10 and 12).

Previously, the preparation of (benzoyloxy)carbamate, from the
 corresponding enantiomerically enriched alcohols, was performed
using hydroxylamine hydrochloride (HONH₂.HCl) and carbonyldimi-
dazole (CDI) in pyridine at 40° C. After the reaction mixture was cold,
it was added the benzoyl chloride in small portions and stirred for 4h
0°C. The reaction mixture was then acidic work-up and purified by
column chromatography. This reaction is carried out in different stages
in the literature, but we proved it in one pot. To a solution of ben-
zoyloxy carbamate in t-butanol and water (4:1) was added dropwise
a solution of potassium osmate dihydrate in water. The reaction was
quenched by addition of sodium sulphite and the solvent was evapo-
rated. The crude product was purified by column chromatography on
silica gel to afford the respective title compound.

2.2. Bioorganic studies

Human carbonic anhydrase inhibition has been the subject of sev-
eral investigations since the discovery of the biological importance of

Scheme 1. Hydrolysis of the enantiomerically enriched acetate compound.
this enzyme in several living organisms [50]. In recent years, many compounds and derivatives have been approved as main classes of hCA inhibitors including hCA I and II isoenzymes [51]. Considering the fact that enantiomerically enriched oxazolidinones are found as effective CAIs, we synthesized novel enantiomerically enriched oxazolidinones to explore their possible hCA I, and II, AChE and α-glycosidase enzymes inhibition effects. The enzyme inhibition data are summarized in Table 4, along with those referred to acetazolamide (AZA), used as standard inhibitor for both hCA isoenzymes [52]. Acarbose was shown as α-glycosidase inhibitor. On the other hand, tacrine (TAC) was used as standard inhibitor for AChE. In order to evaluate the effect of enantiomerically enriched oxazolidinones against the metabolic enzymes, the following results has been delineated:

(i) The physiologically relevant hCA I is found at the highest level in erythrocytes and is also expressed in normal colorectal mucosa [53,54]. As for CA I, all enantiomerically enriched oxazolidinones (8, 10 and 12) showed K_i values in the low nanomolar range that gave K_i values ranging from 11.6 ± 2.1 nM to 66.4 ± 22.7 nM. Among the novel synthesized compounds, enantiomerically enriched oxazolidinone 12, which posses dimethyl, amine (−NH), hydroxyl (−OH) and carbonyl (−C=O) groups, showed the best inhibition (K_i: 19.58 ± 2.87 nM) when compared to AZA (Table 4). Novel synthesized compounds of 8 and 10 demonstrated noncompetitive inhibition against hCA I isoenzyme, however, compounds of 8 showed competitive inhibition against this iso-enzyme. On the other hand, AZA, which is positive control and used as a clinical drug, demonstrated a K_i value of 141.2 ± 50.8 nM. However, there is a 6-fold inhibition effects observed between enantiomerically enriched oxazolidinones 12 and 8, which possessed less functional groups. It is well known that grafting of electron-withdrawing groups in an inhibitor enhanced the CA isoenzymes inhibition effects. In contrast to this inhibition effects, incorporation of electron-donating groups resulted in a decreased activity [53].

(ii) Human CA II is physiologically dominant and highly active cytosolic isoform [52]. As shown in Table 4, the inhibition profile of the considered novel enantiomerically enriched oxazolidinones against dominant cytosolic hCA II revealed to be quite similar to that shown towards CA II. The enantiomerically enriched oxazolidinones demonstrated K_i values between 34.1 ± 6.7–45.2 ± 12.9 nM. As can be seen in the inhibition against hCA I, the most inhibition effects of hCA II observed by enantiomerically enriched oxazolidinone 12 that possessed K_i value of 34.1 ± 6.7 nM. All novel synthesized compounds demonstrated noncompetitive inhibition against cholinergic enzyme of AChE, however, compounds of 8 showed competitive inhibition against this enzyme. On the other hand AZA, which used to treat glaucoma, altitude sickness, epilepsy, periodic paralysis, heart failure and idiopathic intracranial hypertension [54,55] had a K_i value of 22.2 ± 0.7 nM against hCA II.

(iii) One of biochemical estimations is the AChE inhibition effect of novel enantiomerically enriched oxazolidinones. AChE enzyme inhibition properties were recorded according to the procedure of Ellman et al. [56] as described previously [57]. Novel enantiomerically enriched oxazolidinones (8, 10 and 12) had K_i values in ranging from 16.5 ± 2.9 to 35.6 ± 13.9 nM for cholinergic enzyme of AChE. Like hCA I isoenzyme, novel synthesized compounds of 8 and 10 demonstrated noncompetitive inhibition against cholinergic enzyme of AChE, however, compounds of 8 showed competitive inhibition against this enzyme. On the other

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**Table 1**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Enzyme</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
<th>[α]_D^25</th>
<th>YieldOAc (%)</th>
<th>[α]_D^25</th>
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<tr>
<td>1</td>
<td></td>
<td>CCL</td>
<td>8</td>
<td>15</td>
<td>47</td>
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<td>CCL</td>
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<td>16.8</td>
<td>39</td>
<td>−4.2</td>
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<tr>
<td>3</td>
<td></td>
<td>CRL</td>
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<td>15</td>
<td>42</td>
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<td>43</td>
<td>−36.4</td>
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<td>4</td>
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<td>8</td>
<td>0</td>
<td>41</td>
<td>10.6</td>
<td>45</td>
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<tr>
<td>5</td>
<td></td>
<td>CCL</td>
<td>8</td>
<td>0</td>
<td>39</td>
<td>3.6</td>
<td>40</td>
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</tr>
<tr>
<td>6</td>
<td></td>
<td>CRL</td>
<td>8</td>
<td>0</td>
<td>40</td>
<td>7.2</td>
<td>43</td>
<td>−27.6</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>CRL</td>
<td>10</td>
<td>15</td>
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<tr>
<td>9</td>
<td></td>
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<td>0</td>
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<td>8.4</td>
<td>46</td>
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<tr>
<td>10</td>
<td></td>
<td>CRL</td>
<td>10</td>
<td>15</td>
<td>42</td>
<td>7.5</td>
<td>44</td>
<td>−13.2</td>
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</tbody>
</table>

*a Reaction conditions: Substrate (0.2 mmol) and enzyme (20 mg/mmol) stirred in vinyl acetate (1 mL).

**Table 2**

Enantiomerically enriched allylic alcohols (1b, 3b and 5b).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Yield (%)</th>
<th>[α]_D^25</th>
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<tr>
<td>1</td>
<td>1b</td>
<td>92</td>
<td>−86.7</td>
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<tr>
<td>2</td>
<td>3b</td>
<td>90</td>
<td>−25.8</td>
</tr>
<tr>
<td>3</td>
<td>5b</td>
<td>86</td>
<td>−21.2</td>
</tr>
</tbody>
</table>

*a Isolated yield.

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hand, tacrine had $K_i$ value of $5.9 \pm 1.8 \text{nM}$ toward AChE. All evaluated novel enantiomerically enriched oxazolidinones (8, 10 and 12) showed effective inhibition against AChE, but enantiomerically enriched oxazolidinone 12, which possesses dimethyl, amine ($\text{eNH}$), hydroxyl ($\text{eOH}$) and carbonyl ($\text{eC}=\text{O}$) groups, showed perfect inhibition effect against AChE enzyme ($K_i: 16.5 \pm 2.9 \text{nM}$).

(iv) On the other hand, for the $\alpha$-glycosidase, which presents on cells lining the intestine, hydrolyzing monosaccharides to be absorbed through the intestine, novel enantiomerically enriched oxazolidinones (8, 10 and 12) exhibited $K_i$ values of between $22.3 \pm 6.0$–$70.9 \pm 9.9 \text{nM}$ (Table 4). Novel synthesized compounds of 8 and 12 demonstrated noncompetitive inhibition against digestive enzyme of $\alpha$-glycosidase, however, compounds of 10 showed competitive inhibition against this enzyme. The results obtained from $\alpha$-glycosidase assay showed that all novel enantiomerically enriched oxazolidinones (8, 10 and 12) had effective $\alpha$-glycosidase inhibition profiles than that of acarbose ($\text{IC}_{50}: 22.800 \text{mM})$ as standard $\alpha$-glycosidase inhibitor [58]. Also, highly effective $K_i$ values were obtained for novel enantiomerically enriched oxazolidinone 10 with $K_i$ value of $22.3 \pm 6.0 \text{nM}$. The inhibition of digestive enzyme of $\alpha$-glycosidase had great importance due treating and preventing diabetes, postprandial glucose amounts and hyperglycemia [59].

### Table 3

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Yield (%)$^a$</th>
<th>$\delta^b$</th>
<th>Products</th>
<th>Yield (%)$^a$</th>
<th>$\delta^b$</th>
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<td><img src="image2.png" alt="Image" /></td>
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<td>$-58.5$</td>
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<tr>
<td>3</td>
<td><img src="image5.png" alt="Image" /></td>
<td>65</td>
<td>$-28.5$</td>
<td><img src="image6.png" alt="Image" /></td>
<td>72</td>
<td>$-24.2$</td>
</tr>
</tbody>
</table>

$^a$ Isolated yield.

$^b$ (c 1.0, CH$_2$Cl$_2$).

### Table 4

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{IC_{50}}$ (nM)</th>
<th>$K_i$ (nM)</th>
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<tbody>
<tr>
<td></td>
<td>hCA I</td>
<td>hCA II</td>
</tr>
<tr>
<td>8</td>
<td>65.4</td>
<td>0.9737</td>
</tr>
<tr>
<td>10</td>
<td>34.6</td>
<td>0.9825</td>
</tr>
<tr>
<td>12</td>
<td>45.6</td>
<td>0.9829</td>
</tr>
<tr>
<td>AZA</td>
<td>113.79</td>
<td>0.9952</td>
</tr>
<tr>
<td>TAC$^*$</td>
<td>$-\text{—}$</td>
<td>$-\text{—}$</td>
</tr>
<tr>
<td>ACR$^**$</td>
<td>$-\text{—}$</td>
<td>$-\text{—}$</td>
</tr>
</tbody>
</table>

$^*$ Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and II isoenzymes.

$^*$ Acarbose (ACR) was used as a standard inhibitor for $\alpha$-glycosidase enzyme taken from Ref. [58].
predicted that the most active compounds have low IC50 value for logHERG, logBB, MDCK, and logKhsa. For this reason, it has been Lipinski’s rule of five. They are also in acceptable ranges with values of partition coefficient than 5, the compounds are utterly compatible with less hydrogen bond donors (DHB) than 10, and less octanol/water partition coefficient than 5, the compounds are utterly compatible with Lipinski’s rule of five. They are also in acceptable ranges with values of logHERG, logBB, MDCK, and logKhsa. For this reason, it has been predicted that the most active compounds have low IC50 value for HERG K+ channel blockage, penetrate blood-brain barrier and cell membrane, and are transported by serum albumin through blood. Moreover, these compounds are orally absorbed. ADME tests result clearly demonstrated that the compounds have physicochemical and pharmacokinetic properties compatible with those of 95% of known drugs [60].

2.3.2. Catalytic active site

Binding sites of the receptors were detected using SiteMap toll. Also, SiteScore and Dscore for binding site on the receptors were calculated. SiteScore leading to the identification of catalytic active sites on the receptor, was calculated as 1.063, 0.971, 1.090, and 1.016 for hCA I, hCA II, AChE, and α-glycosidase, respectively. Dscore of the binding sites were calculated as 1.061, 0.945, 1.113, and 0.961 for hCA I, hCA II, AChE, and α-glycosidase enzymes, respectively. These results shown that identified binding sites exhibited catalytic active sites properties. Ligand bonded catalytic sites surface have been presented in Fig. 1. The predicted catalytic active sites were selected as target in molecular docking process. Moreover, the sites were used at evaluation of docking hits.

2.3.3. Molecular docking

The most active compounds were docked into identified catalytic active sites of hCA I, hCA II, AChE, and α-glycosidase receptors with induced fit docking (IFD) methodology. The IFD methodology gives more accurate results than other docking methodology by providing flexibility both ligand and receptor. We started by verifying the docking methodology before most active compounds were docked into receptors. Docking validation was performed by re-docking inhibitor co-crystallized ligand. The result shown that the IFD process was performed with reasonable accuracy. Following docking validation, we docked most active compounds and reference inhibitors into the receptors with IFD methodology. After that, results were analyzed on the basis of binding energies of ligand-receptor complex, interactions between ligand and receptor, efficiency of ligand-binding site, and ligand-binding pose. Calculated binding energies of the most active compound and the reference inhibitors were presented as IFD Glide scores (kcal/mol) in Table 6. The result shown that most active compounds exhibited high binding affinity against their receptors. The most active compounds and the reference inhibitors, which have most negative binding energies, were selected as best-posed ligands complexed with the receptor. 2D interaction diagram of the best-posed ligands was shown in Fig. 3. 8-Hydroxy of compound 12 formed hydrogen bond with Glu92 residue of the hCA I (Fig. 3a). Dioxolo moiety of compound 12 formed hydrogen bond with Asn67 and Glu92 residues of the hCA II. Moreover, 8-hydroxy of compound 12 formed hydrogen bond with Thr200 and Pro201 residues of the hCA II (Fig. 3b). 8-hydroxy and oxazole moiety of compound 12 formed hydrogen bond with Tyr124 residue and dioxolo moiety of compound 12 formed hydrogen bond with Phe295 residue of the hCA II (Fig. 3c). Hydroxy and oxazole moiety of compound 10 formed hydrogen bond with Asp443, Apr526, Trp539, and His600 residues of the α-glycosidase (Fig. 3d). The compound 10 exhibited very similar interaction compared to the reference inhibitors (Fig. 3h). We have also observed that the most active compounds similarly inhibited to inhibition mechanisms of the reference inhibitors on the basis of surrounding residues (Fig. 3a–h).

We also analyzed superimposed pose and detailed binding mode of the most active compounds as seen in Figs. 1 and 4. The compounds very well located into the catalytic active site of the receptors. Hydrophobic residues of hCA I receptor surrounded dioxolo moiety of compound 12. Zn atom and oxygen of ketone very close. Hydroxyl and oxazole moiety were surrounded by hydrophilic residues of hCA I receptor (Fig. 1a). Hydrophilic residues of hCA II receptor surrounded dioxolo moiety of compound 12, unlike hCA I. The other moieties were surrounded by similar sites with hCA I (Fig. 1b). The compound 12 was surrounded by hydrophobic sites of AChE receptor (Fig. 1c). The compound 10 was embedded into hydrophilic sites of α-glycosidase receptor (Fig. 1d).

3. Conclusion

In summary, we have developed an efficient synthesis of enantiomerically enriched oxazolidinones (8, 10 and 12), known as protected 1,2-amino alcohols, with 1 mol% of osmium catalysts in high yield and they were evaluated for their inhibitory potentials on the hCA I, hCA II, AChE and α-glycosidase enzymes whose high and uncontrolled activities have been associated with the same diseases. Our compounds were found potent inhibitors against the tested enzymes.
including hCA I, hCA II, AChE, and α-glycosidase. They exerted better inhibitory potential than the conventional inhibitors that were purchased commercially enzymes. The enantiomerically enriched oxazolidinones represent a promising structural scaffold that can be further explored in order to generate other synthetics with enhanced inhibitory potential as well as selectivity against these enzymes. Reported molecules constitute drug candidates against the diseases associated with the aberrant activity of the tested enzymes. These diseases can be listed as glaucoma, epilepsy, altitude sickness, periodic paralysis, idiopathic intracranial hypertension, and heart failure (as CA inhibitors), to treat myasthenia gravis, postural tachycardia syndrome, AD (as cholinergic enzyme inhibitor) and treatment of T2DM (as α-glycosidase inhibitors). In our future studies, we will be designing and synthesizing similar molecules with potent biological activities and further dissecting these molecules efficiencies and biological activities by using in vitro and in vivo disease models.

**Fig. 1.** Catalytic active sites; (a) 12-hCA I, (b) 12-hCA II, (c) 12-AChE, and (d) 10-α-glycosidase. Catalytic active sites are represented as grey mesh, hydrophilic site is represented as green surface, hydrophobic site is represented as yellow surface, and metal binding site is represented as purple surface.

**Fig. 2.** Docking validation. (a) 3TV-hCA I, (b) 51J-hCA II, (c) 1YL-AChE, and (d) DSK-α-glycosidase. The poses of co-crystallized ligands are represented in grey color ball and stick modelling while that of docked ligands is represented in green color ball and stick mode.
4. Material and methods

4.1. Experimental

4.1.1. General

Enzymatic reactions were carried out in a Environmental shaker. Infrared spectra were obtained from KBr pellets on a Mattson 1000 FT-IR spectrophotometer. The 1H and 13C NMR spectra were recorded on Bruker and Varian 400 MHz spectrometers. Optical rotations were measured in a 1 dm cell using an ADP220 Bs Polarimeter at 25°C. Candida cylindracea lipase (CCL) and Candida rugosa lipase (CRL) were purchased from Aldrich. Elemental analyses are performed on LECO CHNS-932 apparatus. TLC was carried out on Merck 0.2-mm silica gel 60 F254 analytical aluminum plates. Column chromatography was performed on silica gel (60 mesh, Merck).

4.2. General procedure for kinetic resolution

To a stirred solution of 500 mg substrate in 5 mL vinyl acetate, 10 mg of CRL or CCL was added in one portion and the reaction mixture was shacked at 0°C (1H NMR and TLC monitoring). The reaction mixture was then filtered with EtOAc and the vinyl acetate and ethylacetate were evaporated. The products were purified by column chromatography (EtOAc/ hexane 1:1).

4.2.1. Kinetic resolution of racemic Cyclohex-2-enol (1a)

4.2.1.1. With CCL enzyme at 0°C

(+)-Cyclohex-2-enol (1a) Colorless liquid (232 mg, 46% yield); [α]D25 = 16.8 (c 1.0, CH2Cl2). 1H NMR (400 MHz, CDCl3, ppm): δ = 1.54–2.02 (m, 6H), 4.15 (m, 1H), 5.70 (m, 1H), 5.78 (m, 1H). 13C NMR (100 MHz, CDCl3, ppm): δ = 19.2, 25.2, 32.2, 65.7, 130.2, 130.6. IR (CHCl3, cm−1): 3026, 2861, 2859, 2684, 2668, 1650, 1437, 1365, 1288, 1181, 1003, 928.

(−)-Cyclohex-2-en acetate (2) (281 mg, 39% yield); [α]D25 = −4.2 (c 1.0, CH2Cl2), 1HNMR (400 MHz, CDCl3, ppm): δ = 5.90–5.96 (m, 1H), 5.62–5.67 (m, 1H), 5.22–5.26 (m, 1H), 2.02 (s, 3H), 1.99–1.57 (s, 6H). 13C NMR (100 MHz, CDCl3, ppm): δ = 171.1, 132.9, 125.8, 68.3, 28.5, 25.1, 21.6. 19.0, IR (CHCl3, cm−1): 2939, 2869, 1729, 1434, 1371, 1242, 1030.

4.2.1.2. With CRL enzyme at 0°C

(+)-Cyclohex-2-enol (1a) Colorless liquid (205 mg, 41% yield); [α]D25 = 10.6 (c 1.0, CH2Cl2), (−)-Cyclohex-2-en acetate (2) (324 mg, 45% yield); [α]D25 = −60.2 (c 1.0, CH2Cl2).

4.2.1.3. Synthesis of (−)-Cyclohex-2-en-1ol (1b)

To a solution of acetate 0.4 g (−)-Cyclohex-2-en-1ol (1b) in methanol (5 mL), K2CO3 (10 mg) was added. The mixture was stirred at 0°C for 3 h, and the solvent was evaporated and crude yield 0.257 g (92%), colorless oil. [α]D25 = −56.7 (c 1.0, CH2Cl2), Lit. [α]D22 = +110.8 (c 1.2, CH2Cl2) [46]. 1HNMR (400 MHz, CDCl3, ppm): δ = 1.54–2.02 (m, 6H), 4.15 (m, 1H), 5.70 (m, 1H), 5.78 (m, 1H). 13C NMR (100 MHz, CDCl3, ppm): δ = 19.2, 25.2, 32.2, 65.7, 130.2, 130.6. IR (CHCl3, cm−1): 3026, 2861, 2859, 2684, 2668, 1650, 1437, 1365, 1348, 1288, 1181, 1003, 928. Elemental Analysis: C, 73.43; H, 10.27; Found: C, 73.28; H, 10.55;

4.2.1.4. Kinetic resolution of racemic Cyclooct-2-enol with CRL enzyme (3a) at 0°C

(+)-Cyclooct-2-enol (3a) Colorless liquid (205 mg, 41% yield); [α]D25 = 10.6 (c 1.0, CH2Cl2), (−)-Cyclooct-2-en acetate (4) (286 mg, 43% yield); [α]D25 = −26.7 (c 1.0, CH2Cl2). 1H NMR (400 MHz, CDCl3, ppm): δ = 5.63–5.67 (m, 2H), 5.40–5.56 (m, 1H), 2.27–2.01 (m, 2H), 2.0 (s, 3H), 1.99–1.21 (m, 8H). 13C NMR (100 MHz, CDCl3, ppm): δ = 170.6, 130.9, 129.9, 72.5, 35.3, 28.9, 26.5, 26.0, 23.5, 21.6, IR (CHCl3, cm−1): 3395, 2839, 2858, 2815, 2858, 2858, 1710, 1664, 1452, 1359, 1282, 1038.

4.2.2. With CRL enzyme at 0°C

(+)-Cyclooct-2-enol (3a) Colorless liquid (202 mg, 40% yield); [α]D25 = 7.2 (c 1.0, CH2Cl2). 1H NMR (400 MHz, CDCl3, ppm): δ = 5.63–5.67 (m, 2H), 5.40–5.56 (m, 1H), 2.27–2.01 (m, 2H), 2.0 (s, 3H), 1.99–1.21 (m, 8H). 13C NMR (100 MHz, CDCl3, ppm): δ = 19.2, 25.2, 32.2, 65.7, 130.5, 130.6. 15N NMR (100 MHz, CDCl3, ppm): δ = 171.1, 132.9, 125.8, 68.3, 28.5, 25.1, 21.6, 19.0, IR (CHCl3, cm−1): 3026, 2861, 2859, 2684, 2668, 1650, 1450, 1437, 1365, 1348, 1288, 1181, 1003, 928. Elemental Analysis: C, 73.43; H, 10.27; Found: C, 73.28; H, 10.55;
4.2.1.5. Synthesis of (−)-Cyclooct-2-en-1-ol (3b). To a solution of acetate 0.4 g (−)-Cyclooct-2-en acetate (4) in methanol (5 mL), K₂CO₃ (10 mg) was added. The mixture was stirred at 0°C for 3 h, and the solvent was removed and crude yield 0.271 g (90%), colorless oil. [α]_D° = −25.8 (c 1.0, CH₂Cl₂), Lit. [α]_D° = −52.4 (c 1.46, CH₂Cl₂) [46]. ¹H NMR (400 MHz, CDCl₃, ppm): δ = 1.3–2.17 (m, 10H), 4.64–4.72 (m, 1H), 5.58–5.65 (m, 2H). ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 23.9, 26.1, 26.5, 29.3, 28.8, 69.7, 128.7, 135.2. IR (CHCl₃, cm⁻¹): 3028, 2859, 2683, 2662, 1651, 1449, 1420, 1184, 1021, 933. Elemental Analysis: C, 76.14; H, 11.18; Found: C, 76.28; H, 11.07.

4.2.1.6. Kinetic resolution of 2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol with CRL enzyme (5) at 0°C. 2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (5a) Colorless liquid (224 mg, 40% yield); [α]_D° = −20.6 (c 1.0, CH₂Cl₂). (−)-2,2-Dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl acetate (5b) (289 mg, 46% yield); [α]_D° = −25.8 (c 1.0, CH₂Cl₂); Lit. [α]_D° = −20.6 (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, ppm): δ = 1.21 (s, 3H); ¹³C NMR (100MHz, CDCl₃, ppm): 170.6, 129.9, 128.8, 109.1, 72.7, 71.6, 63.1, 53.5, 37.9, 26.9, 25.6, 21.4. IR (CHCl₃, cm⁻¹): 2984, 1732, 1644, 1370, 1250, 1150, 1040.

4.2.1.7. Synthesis of (−)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (5b). To a solution of acetate 0.4 g (−)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl acetate (6) in methanol (5 mL), K₂CO₃ (10 mg) was added. The mixture was stirred at 0°C for 3 h, and the solvent was removed and crude yield 0.275 g (86%), colorless oil. [α]_D° = −21.2 (c 1.0, CH₂Cl₂), Lit. [α]_D° = −25.3 (c 0.4, CHCl₃) [47]. ¹H NMR (400 MHz, CDCl₃, ppm): δ = 5.88 (d, 1H, J = 10.5 Hz), 5.66 (d, 1H, J = 10.5 Hz), 4.40–4.46 (m, 2H), 2.45–2.51 (m, 1H), 1.65–1.73 (m, 1H), 1.31 (d, 6H, J = 4.8 Hz). ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 134.4, 127.0, 109.1, 72.7, 71.6, 63.1, 35.3, 27.9, 26.9, 25.6, IR (CHCl₃, cm⁻¹): 3255, 3003, 2252, 1712, 1420, 1363, 1223, 1062, 918. Elemental Analysis: C, 63.51; H, 8.29; Found: C, 63.35; H, 8.46.

4.3. General Procedure I

The enantiomerically enriched alcohol (0.3 g, 1 eq) was added to N,N-carbonyldimidazole (1.5 eq) in pyridine (15 mL). Hydroxylamine hydrochloride (2.5 eq) was added after 3 h at 40°C and the reaction was stirred for 24 h at 40°C. After the reaction mixture was cold, it was added the benzyl chloride (1.2 eq) in small portions and stirred for 4 h at 0°C. The reaction mixture was quenched with HCl (1.0 M aq. sol., 25 mL) and the aqueous layer was extracted with Et₂O (3 × 15 mL). The combined organic layers were washed sequentially with water (20 mL) and NaHCO₃ (aq. sat. sol., 20 mL), dried (Na₂SO₄), filtered and concentrated in vacuum. The crude product was purified by column chromatography using hexane and ethyl acetate to afford the respective title compound.

4.4. General Procedure II

To a solution of O-substituted benzoyloxycarbamate (0.25 g, 1 eq) in t-BuOH and water (4:1, 20 mL/mmol) was added dropwise a solution of potassium osmate dihydrate (4 mg, 1 mol%) in water (0.5 mL). The reaction was quenched by addition of sodium sulphite (100 mg) and the solvent was evaporated. The crude product was purified by column chromatography on silica gel to afford the respective title compound.

4.4.1. Synthesis of (−)-Cyclohex-2-enyl benzoyloxycarbamate (7)

Following General Procedure I, the title compound (0.54 g, 68%) was obtained after purification by column chromatography on silica gel. [α]_D° = −72.3 (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, ppm): δ = 1.60–2.07 (m, 6H), 5.30 (m, 1H), 5.77 (m, 1H), 5.98 (m, 1H), 7.46 (m, 2H), 7.63 (m, 1H), 8.09 (m, 2H), 8.33 (bs, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 18.6, 24.8, 28.2, 71.0, 124.9, 126.8, 128.7, 130.0, 133.6, 134.2, 156.4, 165.9. IR (CHCl₃, cm⁻¹): 3252, 2944, 1784, 1741, 1653, 1525, 1426, 1329, 1184, 1102, 1060. Elemental Analysis: C, 64.36; H, 5.79; N, 5.36; Found: C, 64.17; H, 5.43; N, 5.47.

4.4.2. Synthesis of (−)-4-hydroxyhexahydrobenzo[de]ioxazol-2(3H)-one (8)

Following General Procedure II, the title compound (0.115 g, 79%) was obtained after purification by column chromatography on silica
gel. \( \delta_{13C} = -58.5 \) (c 1.0, CH\(_2\)Cl\(_2\)), \( \delta_{1} \) NMR (400 MHz, CDCl\(_3\), ppm): \( \delta = 1.25-2.18 \) (m, 6H), 3.83-3.87 (m, 1H), 3.91-3.96 (m, 1H), 4.70-4.73 (m, 1H). \(^{13}C\) NMR (100 MHz, CDCl\(_3\), ppm): \( \delta = 160.4, 76.2, 68.4, 55.8, 27.2, 26.0, 16.3 \). IR (CHCl\(_3\), cm\(^{-1}\)): 3380, 2929, 1732, 1071. Elemental Analysis: C, 53.49; H, 7.05; N, 8.91; Found: C, 53.24; H, 6.83; N, 8.62.

4.4.3. Synthesis of (−)-Cyclooct-2-yl benzoyloxycarbamate (9)

Following General Procedure I, the title compound (522 mg, 70%) was obtained after purification by column chromatography on silica gel. \( \delta = -33.4 \) (c 1.0, CH\(_2\)Cl\(_2\)), \(^{1}H\) NMR (400 MHz, CDCl\(_3\), ppm): \( \delta = 8.15-8.18 \) (m, 1H), 7.53-7.42 (m, 4H), 5.61 (dd, 2H, J = 9.88, 17.2 Hz), 5.2 (dd, 1H, J = 7.32, 10.25 Hz), 2.16-1.12 (m, 10H). \(^{13}C\) NMR (100 MHz, CDCl\(_3\), ppm): \( \delta = 167.4, 164.2, 134.6, 132.5, 130.7, 130.5, 129.0, 128.7-128.4, 77.4, 34.8, 28.8, 26.5, 25.8, 23.2. \) IR (CHCl\(_3\), cm\(^{-1}\)): 3532, 2929, 2857, 1774, 1755, 1714, 1451, 1216, 1226, 1012, 945. Elemental Analysis: C, 66.42; H, 6.62; N, 4.84; Found: C, 66.64; H, 6.48; N, 4.75.

4.4.4. Synthesis of (−)-4-hydroxoyctahydrocycloocta[d]oxazol-2(3H)-one (10)

Following General Procedure I, the title compound (124 mg, 78%) was obtained after purification by column chromatography on silica gel. \( \delta = -28.1 \) (c 1.0, CH\(_2\)Cl\(_2\)), \(^{1}H\) NMR (400 MHz, CDCl\(_3\), ppm): \( \delta = 6.7 \) (bs, 1H), 4.90-4.98 (m, 1H), 3.92-3.99 (m, 1H), 3.81-3.85 (m, 1H), 1.85–1.24 (m, 10H). \(^{13}C\) NMR (100 MHz, CDCl\(_3\), ppm): \( \delta = 161.2, 76.9, 76.8, 61.7, 34.0, 29.5, 26.6, 24.9, 19.5. \) IR (CHCl\(_3\), cm\(^{-1}\)): 3380, 2927, 1730, 1452, 1262, 1124, 1035, 980. Elemental Analysis: C, 53.49; H, 7.05; N, 8.91; Found: C, 53.24; H, 6.83; N, 8.62.

4.5. Biochemical studies

4.5.1. hCA inhibition studies

In this work, both hCA I, and II isozymes were purified from human erythrocytes by Sepharose-4B-Tyrosine-sulfanilamide affinity chromatography [61,62]. In this affinity technique, Sepharose-4B-Tyrosine-sulfanilamide was used as an affinity column for selective retention of CA isoenzymes [63]. CA activity determination was spectrophotometrically measured according to Verpoorte et al. [64] as described previously [65]. p-Nitrophenylacetate (PNA) was consumed as substrate for both isoenzymes and enzymatically transformed to p-nitrophenolate [66]. One CA isoenzyme activity unit is the amount of enzyme, which had absorbance change at 348 nm of PNA to 4-nitrophenolate over a period of 3 min at 25°C [67]. Bradford technique was used for the investigation of protein amount during the purification stages [68]. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for fixation of both isoenzymes [69] as described in previous studies [70,74]. Bovine serum albumin was used as the standard protein [71]. For determining the inhibition parameters of each enantiomerically enriched oxazolidinones, an activity (%) [Oxazolidinones] graph was drawn [72]. To calculate K_i values, three different concentrations of novel enantiomerically enriched oxazolidinones were tested [73,74].

4.5.2. AChE inhibition studies

The inhibitory effect of novel enantiomerically enriched oxazolidinones (8, 10 and 12) on AChE activity was performed according to Ellman’s method [56] as described previously [75,76]. Acetylthiocholine iodide (AChI) was used as substrate for the cholinergic reaction. In brief, an aliquot (100 µL) of Tris/HCl buffer (pH 8.0, 1.0 M) and different concentration of sample solutions (10–30 µg/mL) were added to 50 µL of AChE enzyme solution (5.32 × 10^{-3} EU). The solutions were incubated at 20°C for 10 min. An aliquot (50 ± 0.5 µM) of 5,5'-dithio-bis(2-nitro-benzoic)acid (DTNB) and AChI was added to incubated mixture and enzymatic reaction was initiated. AChE activity was spectrophotometrically determined at 412 nm [77,78].

4.5.3. α-Glycosidase inhibition studies

α-Glycosidase inhibition effect of novel enantiomerically enriched oxazolidinones was evaluated according to the method of Tao et al. [58] as described previously [79]. Firstly, phosphate buffer (pH 7.4, 75 µL) was mixed with of 5 µL of the sample and α-glycosidase enzyme solution (20 µL), which prepared in phosphate buffer (0.15 U/mL, pH 7.4). After preincubation 50 µL of p-nitrophenyl-α-glycopyranoside (p-NPG) in phosphate buffer (5 mM, pH 7.4) was added and solution was re-incubated at 37°C. The absorbance o mixtures were recorded at 405 nm [79]. For the determination of K_i values, three different novel enantiomerically enriched oxazolidinones (8, 10 and 12) concentrations were used. Then, the Line-weaver-Burk graphs were drawn [80] as described previously [81].

4.6. In silico studies

Compounds which exhibited best-inhibition effect on the enzymes were selected as most active compound and in silico studies were performed with them to figure out drug-likeness properties and binding mechanism of the most active compounds on the enzymes using the Small Drug Discovery Suites package (Schrödinger 2017-2, LLC, USA).

4.6.1. Ligand and protein preparation

2D structures of the most active compounds were sketched and following their 3D structures were produced with Maestro 11.4. The 3D structures with correct molecular geometries and protonation state at pH 7.0 ± 0.2, was created using Epik module and OPLS-2005 force field at LigPrep toll of Schrodinger. X-Ray crystal structures of hCA I, hCA II, AChE, and α-glycosidase (PDB code: 4WR7, 5AML, 4MOE, and 5NNO, respectively) were selected because of their low they resolution (1.5Å, 1.36 Å, 2 Å, and 2.1 Å, respectively) and were received from http://www.rcsb.org/ web page (RCSB Protein Data Bank). In order to make it ready for use in, the crystal structures were repaired and prepared using protein preparation wizard toll as our previously reported study [82]. Briefly, bond order and charges bond and charges have been assigned and then missing hydrogen atoms and side chains have been filled to crystal structures. Side chain of amino acids has been ionized at physiological pH with the help of Propka software. Water molecules that were formed less than 3 contacts with the protein or ligand were removed. Finally, energy minimization and geometry optimization have also been performed using OPLC force field [19,83].
4.6.2. ADME test

ADME test was performed for the purpose of predicting pharmacokinetic properties of most active compounds with QikProp module in Maestro 11.4. The test ensures information about absorption, distribution, metabolism, and excretion of the compounds in comparison with 95% of known drugs. The drug-likeness of the most active compounds has been identified by checking against Lipinski’s rule of five [84].

4.6.3. Catalytic site prediction

Catalytic site of the receptors has been predicted with SiteMap tool in Maestro 11.4. SiteMap was run using the default parameter of top-ranked potential protein binding sites setting on prepared receptors. Catalytic site for each receptor was generated and Sitescore and Dscore were calculated with SiteMap process. The site with the highest Sitescore was selected as catalytic site and the catalytic active sites were used to produce centroid of the residues for IFD process [85].

4.6.4. Induced fit docking

In order to characterize binding affinity and interactions between the most active compounds and receptors, molecular docking studies were performed with IFD tool in Maestro 11.4 as our previously reported study [82]. Briefly, centroid of the residues has been indicated around the selected ligand in the catalytic site of the receptor. Side chains were automatically trimmed based on B-factor, closest residues to the ligand were refined within 3.4 Å of ligand pose. Reliability of molecular docking process has been decided by performing docking validation. Molecular docking validation was carried out with re-docking procedure by evoking inhibitor complexed in the crystal structure of the receptor. After molecular docking validation, most active compounds were docked into the receptors with the same process [86].

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https:// doi.org/10.1016/j.bioorg.2019.102980.

References


