



# 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  $\alpha$ -glycosidase ( $\alpha$ -Gly) enzymes. These enantiomerically enriched oxazolidinones derivatives had  $K_i$  values in the range of  $11.6 \pm 2.1$ – $66.4 \pm 22.7$  nM for hCA I,  $34.1 \pm 6.7$ – $45.2 \pm 12.9$  nM for hCA II,  $16.5 \pm 2.9$  to  $35.6 \pm 13.9$  for AChE, and  $22.3 \pm 6.0$ – $70.9 \pm 9.9$  nM for  $\alpha$ -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  $\alpha$ -glycosidase enzyme, respectively. These results strongly supported the promising nature of the enantiomerically enriched oxazolidinones as selective hCA, AChE, and  $\alpha$ -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  $\alpha$ -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 ( $Zn^{2+}$ ) in its active site.  $Zn^{2+}$  ion interacts with the substrate molecules directly and cause catalytic effect [14–16]. This enzyme family catalyzes the reversible conversion of carbon dioxide ( $CO_2$ ) and water ( $H_2O$ ) to bicarbonate ( $HCO_3^-$ ) and protons ( $H^+$ ) [17]. They are grouped into seven different gene families, namely  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -,  $\zeta$ -, n- and the last

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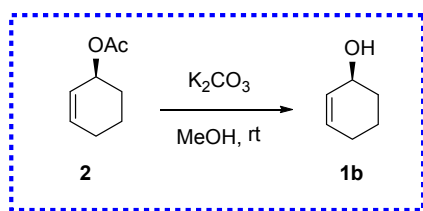
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Scheme 1. Hydrolysis of the enantiomerically enriched acetate compound.

recently discover  $\theta$ -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  $\alpha$ -CAs. Up to now, sixteen  $\alpha$ -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 isoenzymes is that they have zinc ions ( $Zn^{2+}$ ) bounded to histidine amino acids.  $Zn^{2+}$  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-tumour and anti-metastatic agents, anti-glaucoma, and anti-epilepsy [28,29]. For this purpose, development of novel CAIs had a quite importance.

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 ( $CH_3COO^-$ ) and choline (Ch) [32,33]. It is caused majorly by environmental and genetic influences. Cerebral amyloid- $\beta$  aggregation, a deficit in ACh and a deficit in cholinergic neurotransmission, 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 damages. AChE inhibitors (AChEIs) or anticholinesterases inhibit cholinesterase, increasing the level and length of ACh action [36]. A variety of usages 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].

$\alpha$ -Glycosidase (E.C.3.2.1.20) release from intestine cells and hydrolyses oligosaccharides and polysaccharide to monosaccharide units, such as glucose and fructose in small intestine [39,40]. In human,  $\alpha$ -glycosidase inhibitors ( $\alpha$ -GIs) had a great importance for controlling of type-2 diabetes mellitus (T2DM) and hyperglycemia [41]. Recently, two main chemical classes of N-comprising  $\alpha$ -Glycosidase inhibitors ( $\alpha$ -GIs) contain sugar-based inhibitors [42].  $\alpha$ -GIs can reduce the uptake of dietary carbohydrates and repress postprandial T2DM and hyperglycemia. Thus, these  $\alpha$ -GIs are endowed with sugar molecule such as compete and moieties with the oligosaccharides for binding to the active 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 synthesized enantiomerically enriched oxazolidinones (**8**, **10** and **12**) against human carbonic anhydrase isoenzymes, acetylcholinesterase, and  $\alpha$ -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 (CCL) and *Candida rugosa* lipase (CRL). The CCL 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 CCL and the reaction mixed at 15 °C. The conversion was monitored by TLC and  $^1H$  NMR. After 8 h, 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  $[\alpha]_D^{25}$ : 12.5 (c 1.0,  $CH_2Cl_2$ ) and in 42% yield with  $[\alpha]_D^{25}$ : -4.1 (c 1.0,  $CH_2Cl_2$ ) respectively (entry 1 in Table 1). Similar decomposition reactions were observed at 0 °C, which **1a** and **2** in 46% yield with  $[\alpha]_D^{25}$ : 16.8 (c 1.0,  $CH_2Cl_2$ ) and in 39% yield with  $[\alpha]_D^{25}$ : -4.2 (c 1.0,  $CH_2Cl_2$ ), 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. Enantiomerically enriched compounds **1a** and **2** respectively were observed in 41% yield with  $[\alpha]_D^{25}$ : 10.6 (c 1.0,  $CH_2Cl_2$ ) and in 45% yield with  $[\alpha]_D^{25}$ : -60.2 (c 1.0,  $CH_2Cl_2$ ) 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  $[\alpha]_D^{25}$ : -86.7 (c 1.0,  $CH_2Cl_2$ ) [Lit. (+)-Enantiomer  $[\alpha]_D^{25}$ : +110.8 (c 1.2,  $CH_2Cl_2$ ),  $\geq 99$  ee %] [46]. So we synthesized the enantiomerically rich allylic alcohol. (-)-Cyclooct-2-en-1-ol (**3b**)  $[\alpha]_D^{25}$ : -25.8 (c 1.0,  $CH_2Cl_2$ ) [Lit. (-)-Enantiomer  $[\alpha]_D^{25}$ : +52.4 (c 1.46,  $CH_2Cl_2$ ),  $\geq 99$  ee %] [46] and (-)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (**5b**)  $[\alpha]_D^{25}$ : -21.2 (c 1.0,  $CH_2Cl_2$ ) [Lit. (-)-Enantiomer  $[\alpha]_D^{25}$ : -25.3 (c 0.4,  $CHCl_3$ ), 48% ee Mosher's ester formation] [47] were synthesized by CRL enzyme at 0 °C in the same way as enantiomerically enriched 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 the 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  $^{13}C$  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**).

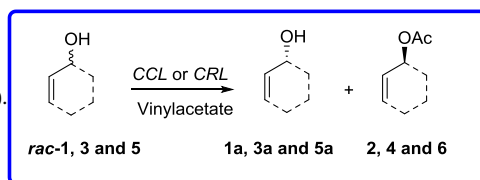
Previously, the preparation of (benzoyloxy)carbamate, from the corresponding enantiomerically enriched alcohols, was performed using hydroxylamine hydrochloride ( $HONH_2 \cdot HCl$ ) and carbonyldiimidazole (CDI) in pyridine at 40 °C. After the reaction mixture was cold, it was added the benzoyl chloride in small portions and stirred for 4 h 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 benzoyloxycarbamate 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 evaporated. The crude product was purified by column chromatography on silica gel to afford the respective title compound.

### 2.2. Biochemical studies

Human carbonic anhydrase inhibition has been the subject of several investigations since the discovery of the biological importance of

Table 1

Enzymatic resolution of cyclic allylic alcohols (1–3).



Entry	Substrate	Enzyme <sup>a</sup>	Time (h)	Temperature (°C)	Yield <sub>OH</sub> (%) <sup>b</sup>	$[\alpha]_D^{25}$ <sup>c</sup>	Yield <sub>OAc</sub> (%) <sup>b</sup>	$[\alpha]_D^{25}$ <sup>c</sup>
1		CCL	8	15	47	12.5	42	-4.1
2		CCL	10	0	46	16.8	39	-4.2
3		CRL	6	15	42	9.3	43	-36.4
4		CRL	8	0	41	10.6	45	-60.2
5		CCL	8	0	39	3.6	40	-15.4
6		CRL	8	0	40	7.2	43	-27.6
7		CRL	10	15	41	6.4	42	-14.3
8		CCL	8	0	43	5.2	45	-14.6
9		CRL	8	0	44	8.4	46	-20.6
10		CRL	10	15	42	7.5	44	-13.2

<sup>a</sup> Reaction conditions: Substrate (0.2 mmol) and enzyme (20 mg/mmol) stirred in vinyl acetate (1 mL).<sup>b</sup> Isolated yield.<sup>c</sup> (c 1.0, CH<sub>2</sub>Cl<sub>2</sub>).

Table 2

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

Entry	Substrate	Yield (%) <sup>a</sup>	$[\alpha]_D^{25}$ <sup>b</sup>
1		92	-86.7
2		90	-25.8
3		86	-21.2

<sup>a</sup> Isolated yield.<sup>b</sup> (c 1.0, CH<sub>2</sub>Cl<sub>2</sub>).

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  $\alpha$ -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  $\alpha$ -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:

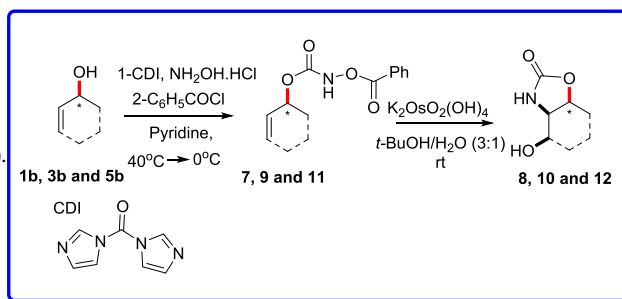
(i) The physiologically relevant hCA I is found at the highest level in erythrocytes and is also expressed in normal colorectal mucosa [53,54]. As for CA I, all enantiomerically enriched oxazolidinones (8, 10 and 12) showed  $K_i$  values in the low nanomolar range that gave  $K_i$  values ranging from  $11.6 \pm 2.1$  nM to  $66.4 \pm 22.7$  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 ( $K_i$ :  $19.58 \pm 2.87$  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 isoenzyme. On the other hand, AZA, which is positive control and used a clinical drug, demonstrated a  $K_i$  value of  $141.2 \pm 50.8$  mM. 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  $34.1 \pm 6.7$ – $45.2 \pm 12.9$  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  $34.1 \pm 6.7$  nM. All novel synthesized compounds demonstrated noncompetitive inhibition against hCA II isoenzyme. 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  $22.2 \pm 0.7$  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  $16.5 \pm 2.9$  to  $35.6 \pm 13.9$  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

Table 3

Synthesis of enantiomerically enriched oxazolidinones (**8**, **10** and **12**).

Entry	Substrate	Yield (%) <sup>a</sup>	$[\alpha]_D^{25b}$	Products	Yield (%) <sup>a</sup>	$[\alpha]_D^{25b}$
1		68	-72.3		79	-58.5
2		70	-33.4		78	-28.1
3		65	-28.5		72	-24.2

<sup>a</sup> Isolated yield.<sup>b</sup> (c 1.0, CH<sub>2</sub>Cl<sub>2</sub>).

hand, tacrine had  $K_i$  value of  $5.9 \pm 1.8$  nM toward AChE. All evaluated novel enantiomerically enriched oxazolidinones (**8**, **10** and **12**) showed effective inhibition against AChE, but enantiomerically enriched oxazolidinone **12**, which posses dimethyl, amine (-NH), hydroxyl (-OH) and carbonyl (-C=O) groups, showed perfect inhibition effect against AChE enzyme ( $K_i$ :  $16.5 \pm 2.9$  nM).

(iv) On the other hand, for the  $\alpha$ -glycosidase, which present 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  $22.3 \pm 6.0$ – $70.9 \pm 9.9$  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 ( $IC_{50}$ : 22.800 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  $22.3 \pm 6.0$  nM. The inhibition of digestive enzyme of  $\alpha$ -glycosidase had great importance due treating and preventing diabetes, postprandial glucose amounts and hyperglycemia [59].

Table 4

Enzyme inhibition results of enantiomerically enriched oxazolidinones (**8**, **10** and **12**) against human carbonic anhydrase I and II isoenzymes (hCA I and hCA II), acetylcholinesterase (AChE) and  $\alpha$ -glycosidase ( $\alpha$ -Gly).

Compounds	IC <sub>50</sub> (nM)		K <sub>i</sub> (nM)									
	hCA I	r <sup>2</sup>	hCA II	r <sup>2</sup>	AChE	r <sup>2</sup>	$\alpha$ -Gly	r <sup>2</sup>	hCA I	hCA II	AChE	$\alpha$ -Gly
<b>8</b>	65.4	0.9737	36.7	0.9735	30.4	0.9917	89.5	0.9846	66.4 ± 22.7	40.2 ± 18.6	35.6 ± 13.8	70.9 ± 9.9
<b>10</b>	34.6	0.9825	38.9	0.9684	61.3	0.9914	61.3	0.9614	29.8 ± 10.0	45.2 ± 12.9	71.9 ± 4.1	22.3 ± 6.0
<b>12</b>	45.6	0.9829	24.8	0.9637	58.7	0.9675	30.5	0.9647	11.6 ± 2.1	34.1 ± 6.7	16.5 ± 2.9	39.9 ± 3.6
AZA <sup>*</sup>	113.79	0.9932	31.79	0.9816	–	–	–	–	141.2 ± 50.8	22.2 ± 0.7	–	–
TAC <sup>**</sup>	–	–	–	–	5.97	0.9706	–	–	–	–	5.9 ± 1.8	–
ACR <sup>***</sup>	–	–	–	–	–	–	22,800	–	–	–	–	12600 ± 78

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

\*\* Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.

\*\*\* Acarbose (ACR) was used as a standard inhibitor for  $\alpha$ -glycosidase enzyme taken from Ref. [58].

**Table 5**  
Physically and pharmaceutically properties of the most active compounds.

Compound	<sup>a</sup> MW	<sup>b</sup> DHB	<sup>c</sup> AHB	<sup>d</sup> logPo/w	<sup>e</sup> logHERG	<sup>f</sup> logBB	<sup>g</sup> MDCK	<sup>h</sup> logKhsa	<sup>i</sup> % Hum. Oral Abs.
10	185.222	2	5.2	0.205	-2.489	-0.417	331.089	-0.543	78.995
12	229.232	2	6.7	0.019	-2.769	-0.542	251.298	-0.584	75.881

<sup>a</sup> Molecular weight (< 500 Da).

<sup>b</sup> Number of hydrogen bond donors (< 5).

<sup>c</sup> Number of hydrogen bond acceptors (< 10).

<sup>d</sup> Octanol/water partition coefficient (recommended range: -2.0 to 6.5).

<sup>e</sup> IC<sub>50</sub> value for blockage of HERG K<sup>+</sup> channels (acceptable range: above 25.0).

<sup>f</sup> B/blood partition coefficient (recommended range: -3.0 to 1.2).

<sup>g</sup> Cell permeability in nm/sec (< 25 is poor and > 500 is great).

<sup>h</sup> Binding to human serum albumin (acceptable range: 21.5-1.5).

<sup>i</sup> Percentage of human oral absorption (< 25% is poor and > 80% is high).

### 2.3. In silico studies

#### 2.3.1. Absorption, Distribution, Metabolism, and Excretion (ADME) test

Pharmaceutical properties of most active compounds have been identified with ADME test and were displayed in Table 5. In the light of Lipinski's rule of five results, which presented in Table 5, were evaluated whether they are eligible with pharmaceutical properties of commercial drugs. Because the most active compounds have less molecular weight than 500 Da, less hydrogen bond acceptors (AHB) than 5, 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 IC<sub>50</sub> value for HERG K<sup>+</sup> 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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 complexed into the crystal structure of the receptors. Docking validation results are shown in Fig. 2. According to these results, the re-docked ligands were similarly located same region of the receptors as co-crystallized ligand. The result shown that the IFD process was performed with reasonable accuracy.

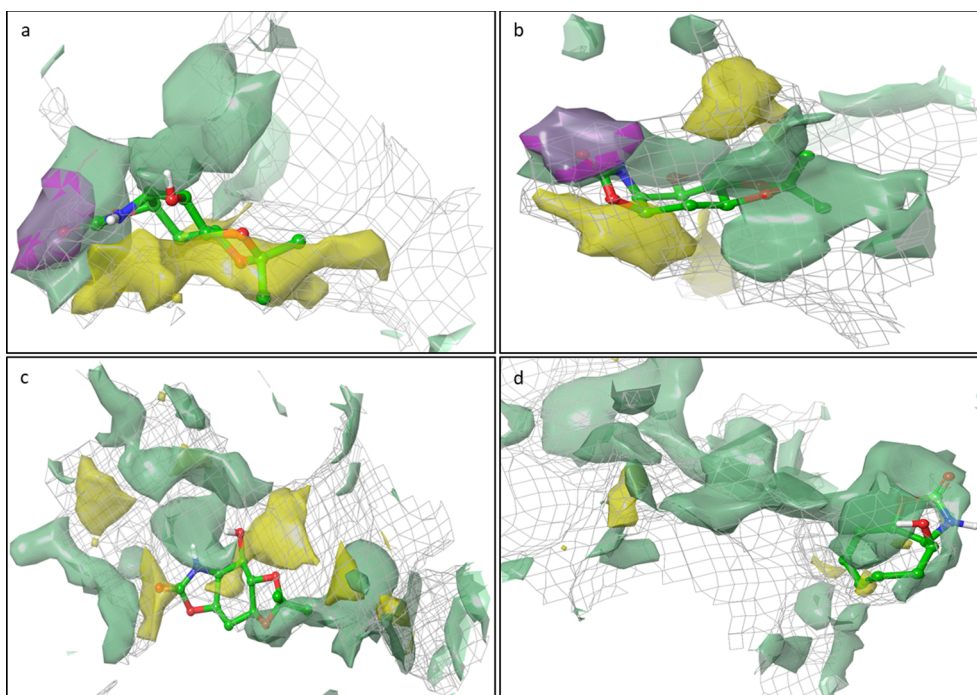
Following docking validation, we docked most active compounds and reference inhibitors into the receptors with IFD methodology. After that, results were analyzed on the basis of binding energies of ligand-receptor complex, interactions between ligand and receptor, efficiency of ligand-binding site, and 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 Gln92 residue of the hCA I (Fig. 3a). Dioxolo moiety of compound 12 formed hydrogen bond with Asn67 and Gln92 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, Agr526, Trp539, and His600 residues of the  $\alpha$ -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  $\alpha$ -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  $\alpha$ -glycosidase enzymes whose high and uncontrolled activities have been associated with the same diseases. Our compounds were found potent inhibitors against the tested enzymes

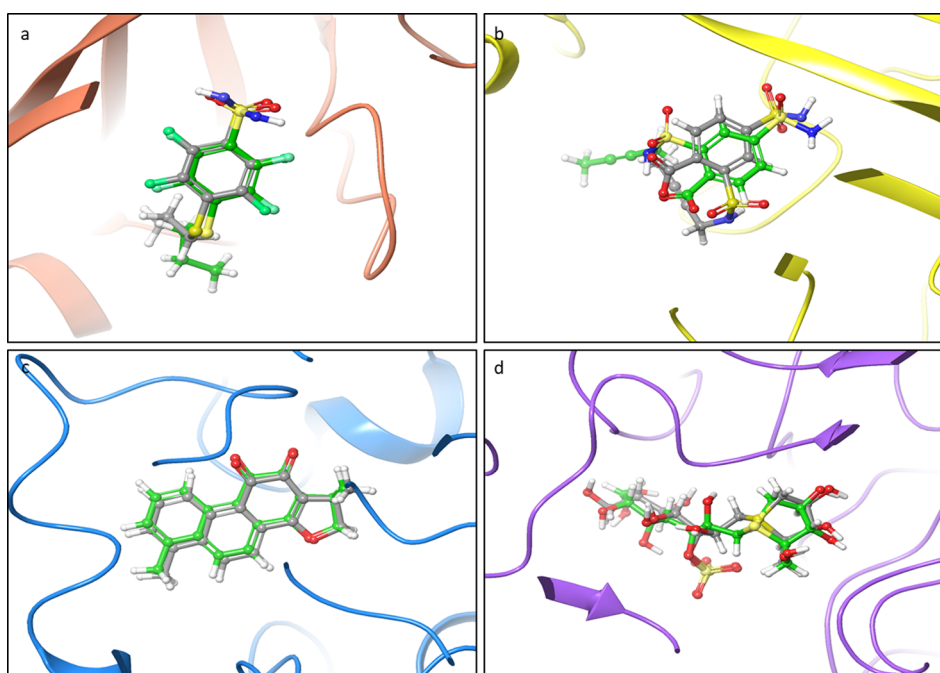




**Fig. 1.** Catalytic active sites; (a) 12-hCA I, (b) 12-hCA II, (c) 12-AChE, and (d) 10- $\alpha$ -glycosidase. Catalytic active sites are represented as grey mesh, hydrophilic site is represented as green surface, hydrophobic site is represented as yellow surface, and metal binding site is represented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

including hCA I, hCA II, AChE, and  $\alpha$ -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  $\alpha$ -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. 2.** Docking validation. (a) 3TV-hCA I, (b) 51J-hCA II, (c) 1YL-AChE, and (d) DSK- $\alpha$ -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.

**Table 6**

IFD Glide scores (kcal/mol) of the most active compounds in the catalytic sites of against human carbonic anhydrase I and II isoenzymes (hCA I and hCA II), acetylcholinesterase (AChE) and  $\alpha$ -glycosidase ( $\alpha$ -Gly).

Compounds	IFD Glide Score			
	hCA I	hCA II	AChE	$\alpha$ -Gly
10	-	-	-	-8.563
12	-5.767	-6.568	-9.014	-
AZA*	-9.016	-9.560	-	-
TAC**	-	-	-9.579	-
ACR***	-	-	-	-16.933

\* Acetazolamide (AZA) was used as a standard inhibitor for human carbonic anhydrase isoenzymes I, and II (hCA I, and II).

\*\* Tacrine (TAC) was used as a standard inhibitor for acetylcholinesterase enzyme (AChE).

\*\*\* Acarbose (ACR) was used as a standard inhibitor for  $\alpha$ -glycosidase enzyme taken from Ref. [58].

## 4. Material and methods

### 4.1. Experimental

#### 4.1.1. General

Enzymatic reactions were carried out in an Environmental shaker. Infrared spectra were obtained from KBr pellets on a Mattson 1000 FT-IR spectrophotometer. The  $^1\text{H}$  and  $^{13}\text{C}$  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 shaken at 0 °C ( $^1\text{H}$  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)

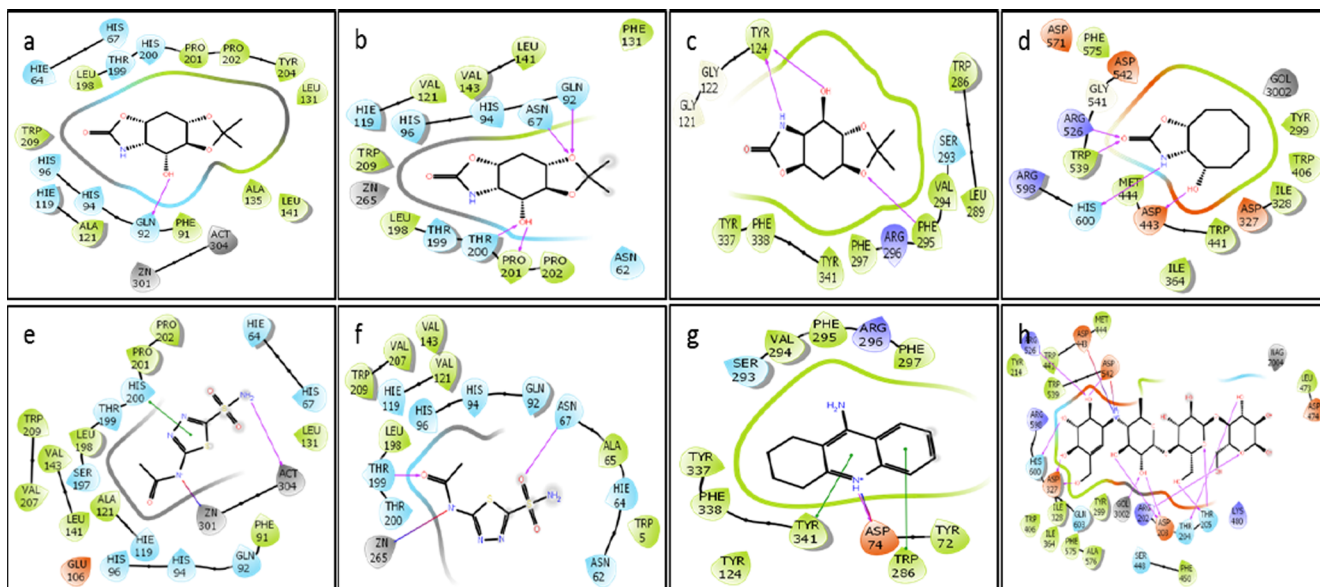
4.2.1.1. *With CCL enzyme at 0 °C.* (+)-Cyclohex-2-enol (1a) Colorless liquid (232 mg, 46% yield);  $[\alpha]_D^{25} = 16.8$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 1.54\text{--}2.02$  (m, 6H), 4.15 (m, 1H), 5.70 (m, 1H), 5.78 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 19.2, 25.2, 32.2, 65.7, 130.2, 130.6$ . IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ): 3026, 2861, 2859, 2684, 2668, 1650, 1450, 1437, 1365, 1348, 1288, 1181, 1003, 928.

(-)-Cyclohex-2-en acetate (2) (281 mg, 39% yield);  $[\alpha]_D^{25} = -4.2$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 5.90\text{--}5.96$  (m, 1H), 5.62–5.67 (m, 1H), 5.22–5.26 (m, 1H), 2.02 (s, 3H), 1.99–1.57 (s, 6H)  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 171.1, 132.9, 125.8, 68.3, 28.5, 25.1, 21.6, 19.0$ , IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ): 2939, 2869, 1729, 1434, 1371, 1242, 1030.

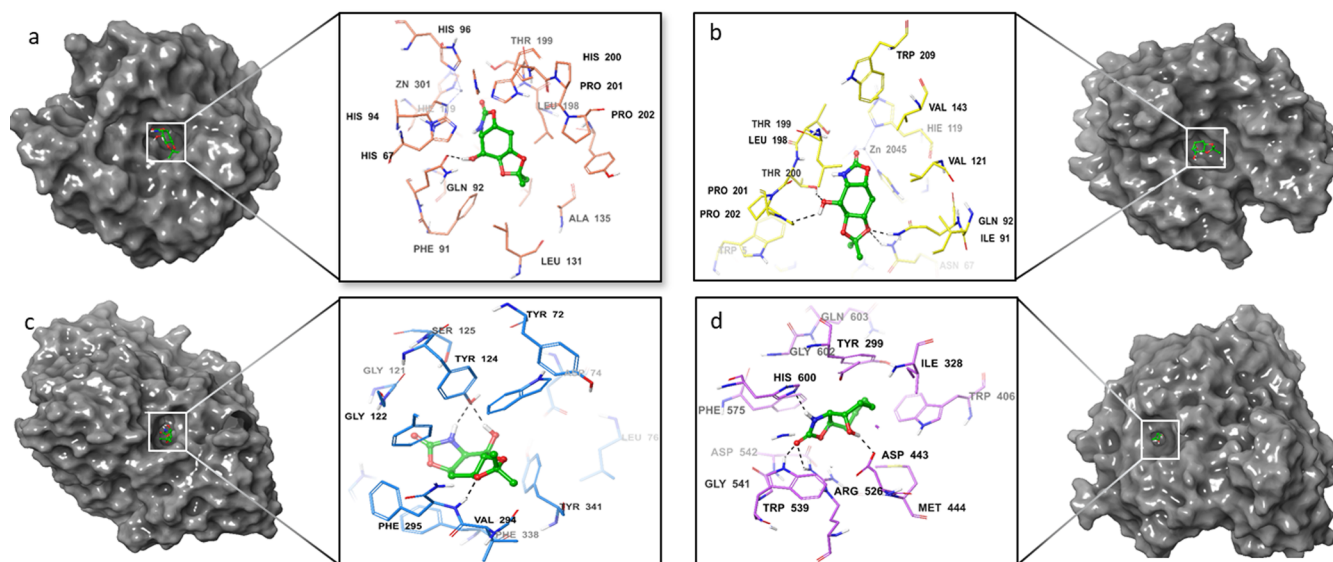
4.2.1.2. *With CRL enzyme at 0 °C.* (+)-Cyclohex-2-enol (1a) Colorless liquid (205 mg, 41% yield);  $[\alpha]_D^{25} = 10.6$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ), (-)-Cyclohex-2-en acetate (2) (324 mg, 45% yield);  $[\alpha]_D^{25} = -60.2$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),

4.2.1.3. *Synthesis of (-)-Cyclohex-2-en-1-ol (1b).* To a solution of acetate 0.4 g (-)-Cyclohex-2-en-1-ol (1b) in methanol (5 mL),  $\text{K}_2\text{CO}_3$  (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.  $[\alpha]_D^{25} = -56.7$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ), Lit.  $[\alpha]_D^{25} = +110.8$  (c 1.2,  $\text{CH}_2\text{Cl}_2$ ) [46].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 1.54\text{--}2.02$  (m, 6H), 4.15 (m, 1H), 5.70 (m, 1H), 5.78 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 19.2, 25.2, 32.2, 65.7, 130.2, 130.6$ . IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ): 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;

4.2.1.4. *Kinetic resolution of racemic Cyclooct-2-enol with CRL enzyme (3)* at 0 °C. (+)-Cyclooct-2-enol (3a) Colorless liquid (202 mg, 40% yield);  $[\alpha]_D^{25} = 7.2$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ). (-)-Cyclooct-2-en acetate (4) (286 mg, 43% yield);  $[\alpha]_D^{25} = -27.6$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 5.63\text{--}5.67$  (m, 2H), 5.40–5.56 (m, 1H), 2.27–2.01 (m, 2H), 2.0 (s, 3H), 1.99–1.21 (m, 8H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 170.6, 130.9, 129.9, 72.5, 35.3, 28.9, 26.5, 26.0, 23.5, 21.6$ , IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ): 3395, 2931, 2858, 1710, 1664, 1452, 1359, 1282, 1038



**Fig. 3.** 2D receptor-ligand interaction diagram; (a) 12-hCA I, (b) 12-hCA II, (c) 12-AChE, (d) 10- $\alpha$ -glycosidase, (e) AZA-hCA I, (f) AZA-hCA II, (g) TAC-AChE, and (h) ACR- $\alpha$ -glycosidase.



**Fig. 4.** Superimposed pose of the most active compounds into hCA I, hCA II, AChE, and  $\alpha$ -glycosidase receptors. (a) Best-pose of **12** into catalytic active site of hCA I, (b) Best pose of **12** into catalytic active site of hCA II, (c) Best-pose of **12** into catalytic active site of AChE, and (d) Best-pose of **10** into catalytic active site of  $\alpha$ -glycosidase. Receptors are depicted in the ribbon model which hCA I with orange color, hCA II with yellow color, AChE with blue color, and  $\alpha$ -glycosidase with purple color. Receptors are represented as grey solid surface. Compounds are represented in ball and stick modelling which green color carbon and residues are represented in thick tube modelling same color with receptors ribbon model.

**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),  $K_2CO_3$  (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.  $[\alpha]_D^{25} = -25.8$  (c 1.0,  $CH_2Cl_2$ ), Lit.  $[\alpha]_D^{25} = -52.4$  (c 1.46,  $CH_2Cl_2$ ) [46].  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta = 1.3$ –2.17 (m, 10H), 4.64–4.72 (m, 1H), 5.58–5.65 (m, 2H).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta = 23.9$ , 26.1, 26.5, 29.3, 28.8, 69.7, 128.9, 135.2. IR ( $CHCl_3$ ,  $cm^{-1}$ ): 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, 44% yield);  $[\alpha]_D^{25} = 8.4$  (c 1.0,  $CH_2Cl_2$ ). (–)-2,2-Dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl acetate (**6**) (289 mg, 46% yield);  $[\alpha]_D^{25} = -20.6$  (c 1.0,  $CH_2Cl_2$ ),  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta = 5.71$  (dm, A part of AB system,  $J = 10.8$  Hz, 1H), 5.65 (dm, B part of AB system,  $J = 10.8$  Hz, 1H), 5.28 (m, 1H), 4.32–4.46 (m, 2H), 2.31 (ddt, A part of AB system,  $J = 13.8$ , 5.5, 1.1 Hz, 1H), 1.65 (ddd, B part of AB system,  $J = 13.8$ , 9.1, 2.8 Hz, 1H), 1.92 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm): 170.6, 129.9, 128.8, 109.1, 72.3, 71.3, 66.5, 31.5, 28.0, 26.6, 21.4. IR ( $CHCl_3$ ,  $cm^{-1}$ ): 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),  $K_2CO_3$  (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.  $[\alpha]_D^{25} = -21.2$  (c 1.0,  $CH_2Cl_2$ ), Lit.  $[\alpha]_D^{25} = -25.3$  (c 0.4,  $CHCl_3$ ) [47].  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta = 5.88$  (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).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta = 134.4$ , 127.0, 109.1, 72.7, 71.6, 63.1, 35.3, 27.9, 26.5, IR ( $CHCl_3$ ,  $cm^{-1}$ ): 3505, 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*-carbonyldiimidazole (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 benzoyl chloride (1.2 eq) in small portions and stirred for 4 h 0 °C. The reaction mixture was quenched with HCl (1.0 M aq. sol., 25 mL) and the aqueous layer was extracted with  $Et_2O$  (3  $\times$  15 mL). The combined organic layers were washed sequentially with water (20 mL) and  $NaHCO_3$  (aq. sat. sol., 20 mL), dried ( $Na_2SO_4$ ), 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.  $[\alpha]_D^{25} = -72.3$  (c 1.0,  $CH_2Cl_2$ ),  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta = 1.60$ –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),  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta = 18.6$ , 24.8, 28.2, 71.0, 124.9, 126.8, 128.7, 130.0, 133.6, 134.2, 156.4, 165.9. IR ( $CHCl_3$ ,  $cm^{-1}$ ): 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[d]oxazol-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.  $[\alpha]_D^{25} = -58.5$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 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).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 160.4$ ,  $76.2$ ,  $68.4$ ,  $55.8$ ,  $27.2$ ,  $26.0$ ,  $16.3$ . IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ):  $3380$ ,  $2927$ ,  $1730$ ,  $1071$ . 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-enyl benzoyloxycarbamate (9)

Following General Procedure I, the title compound (522 mg, 70%) was obtained after purification by column chromatography on silica gel.  $[\alpha]_D^{25} = -33.4$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 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).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , 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 ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ):  $3527$ ,  $2929$ ,  $2857$ ,  $1774$ ,  $1755$ ,  $1714$ ,  $1451$ ,  $1316$ ,  $1242$ ,  $1126$ ,  $1012$ ,  $945$ . 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-hydroxyoctahydrocycloocta[d]oxazol-2(3H)-one (10)

Following General Procedure II, the title compound (124 mg, 78%) was obtained after purification by column chromatography on silica gel.  $[\alpha]_D^{25} = -28.1$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 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).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 161.2$ ,  $76.9$ ,  $76.8$ ,  $61.7$ ,  $34.0$ ,  $29.5$ ,  $26.6$ ,  $24.9$ ,  $19.5$ . IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ):  $3398$ ,  $2929$ ,  $1730$ ,  $1452$ ,  $1262$ ,  $1124$ ,  $1035$ ,  $980$ . Elemental Analysis: C, 58.36; H, 8.16; N, 7.56; Found: C, 58.70; H, 8.31; N, 7.64;

#### 4.4.5. Synthesis of (–)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl (benzoyloxy) carbamate (11)

Following General Procedure I, the title compound (390 mg, 65%) was obtained after purification by column chromatography on silica gel.  $[\alpha]_D^{25} = -28.5$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 8.06$ – $8.18$  (m, 2H),  $7.44$ – $7.69$  (m, 2H),  $5.92$  (d, 1H,  $J = 10.5$  Hz),  $5.82$  (d, 1H,  $J = 10.5$  Hz),  $5.48$  (m, 1H),  $4.46$  (m, 2H),  $2.50$ – $2.61$  (m, 2H),  $1.13$  (m, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 166.3$ ,  $155.4$ ,  $134.5$ ,  $130.2$ ,  $129.0$ ,  $128.8$ ,  $127.8$ ,  $124.9$ ,  $109.2$ ,  $72.2$ ,  $71.2$ ,  $69.6$ ,  $31.7$ ,  $30.5$ ,  $29.9$ . Elemental Analysis: C, 61.25; H, 5.75; N, 4.20; Found: C, 61.48; H, 5.83; N, 4.26.

#### 4.4.6. Synthesis of (–)-4-hydroxyoctahydrocycloocta[d]oxazol-2(3H)-one (12)

Following General Procedure II, the title compound (122 mg, 72%) was obtained after purification by column chromatography on silica gel.  $[\alpha]_D^{25} = -24.2$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 7.41$  (bs, 1H),  $5.93$ – $6.02$  (m, 1H),  $5.81$  (d, 1H,  $J = 10.4$  Hz),  $5.72$  (d, 1H,  $J = 10.4$  Hz),  $5.34$ – $5.42$  (m, 1H),  $4.61$ – $4.45$  (m, 3H),  $2.50$ – $2.61$  (m, 1H),  $1.71$ – $1.79$  (m, 1H),  $1.40$ – $1.22$  (m, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 160.2$ ,  $119.7$ ,  $80.6$ ,  $73.2$ ,  $72.6$ ,  $71.1$ ,  $66.4$ ,  $29.7$ ,  $28.8$ ,  $27.6$ . IR ( $\text{CHCl}_3$ ,  $\text{cm}^{-1}$ ):  $3367$ ,  $2928$ ,  $1715$ ,  $1600$ ,  $1574$ ,  $1379$ ,  $1236$ ,  $1219$ ,  $1062$ ,  $1032$ ,  $878$ . Elemental Analysis: C, 52.40; H, 6.60; N, 6.11; Found: C, 52.61; H, 6.45; N, 6.24.

### 4.5. Biochemical studies

#### 4.5.1. hCA inhibition studies

In this work, both hCA I, and II isoenzymes were purified from human erythrocytes by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography [61,62]. In this affinity technic, Sepharose-4B-L-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-nitrophenylate 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  $\mu\text{L}$ ) of Tris/HCl buffer (pH 8.0, 1.0 M) and different concentration of sample solutions (10–30  $\mu\text{g}/\text{mL}$ ) were added to 50  $\mu\text{L}$  of AChE enzyme solution ( $5.32 \times 10^{-3}$  EU). The solutions were incubated at 20 °C for 10 min. An aliquot (50  $\mu\text{L}$ , 0.5 mM) 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. $\alpha$ -Glycosidase inhibition studies

$\alpha$ -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  $\mu\text{L}$ ) was mixed with of 5  $\mu\text{L}$  of the sample and  $\alpha$ -glycosidase enzyme solution (20  $\mu\text{L}$ ), which prepared in phosphate buffer (0.15 U/mL, pH 7.4). After preincubation 50  $\mu\text{L}$  of *p*-nitrophenyl- $\beta$ -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 Lineweaver-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 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  $7.0 \pm 2.0$ , 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  $\alpha$ -glycosidase (PDB code: 4WR7, 5AML, 4MOE, and 5NNO, respectively) were selected because of their low they resolution (1.5 Å, 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 redocking procedure by evicting 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>.

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