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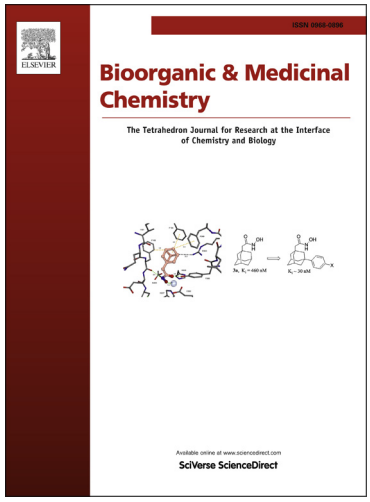

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N-Heteroarylmethyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid A Novel Scaffold for the Design of Uncompetitive α-Glucosidase Inhibitors

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Abstract:

The enzyme α-glucosidase has attracted interest owing to its involvement in the digestive process of carbohydrate, its role in intracellular glycoprotein trafficking, tumorigenesis and viral infection. In this study, several members of a new family of *N*-heteroarylmethyl substituted azasugars were synthesized and evaluated as α-glucosidase inhibitors. We systematically investigated the effect of different *N*- substituents as well as the role of hydroxyl and carboxylate moieties on the piperidine ring. The compounds N-heteroarylmethyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid emerged as potent α-glucosidase inhibitors. Unlike Acarbose and other clinically relevant α-glucosidase inhibitors, these compounds act through a reversible uncompetitive mechanism of inhibition which make them attractive candidates for drug development.

1. Introduction

Restriction of sugar intake through diet is often recommended as a measure to improve glycemic control in diabetics.[1, 2] Compounds affecting carbohydrate breakdown and absorption in the gut are frequently prescribed to poorly compliant patients, or as supplements to the dietary regimen. Drugs known as α-glucosidase inhibitors belong to this class. Three α-glucosidase inhibitors - Acarbose, Miglitol and Voglibose - are currently approved for the treatment of type 2 diabetes. Of the three, Acarbose is the most widely prescribed.[3] It is a pseudo-tetrasaccharide of microbial origin (Actinoplanes) that acts as reversible competitive inhibitor of extracellular α-glucosidase of the brush border of the small intestine in addition to pancreatic alpha-amylase.[4]

Owing to the success of α-glucosidase inhibitors as antidiabetic agents and the postulated involvement of this enzyme in tumorigenesis [5] and viral infection, [6] the interest in the discovery and development of novel, structurally different inhibitors is much alive.[7-10] Several of the compounds described in the literature are drawn from plants used in the folk medicine around the world as remedy against diabetes. [11] Fewer α-glucosidase inhibitors have originated through rational drug design and novel chemical synthesis. [12-14] None has supplanted the use of Acarbose or the other clinically tested inhibitors.

Iminosugars are a rich source of glycosidase inhibitors.[14-16] Since the discovery of nojirimicin,[17] the first identified natural α-glucosidase inhibitor, a number of synthetic mimetics based on the same scaffold of that of azasugars [18] and aminocyclitols [19], have been evaluated. As with nojirimycin, the prototypical iminosugar and enzymatic transition state analogue, a protonated nitrogen in these compounds is responsible for the formation of a stable electrostatic interaction with the critical carboxylate ion located within the catalytic pocket of the α-glucosidase. It is postulated that the ammonium ion by mimicking the positively charged oxycarbenium ion in the pyranose ring of the natural substrate during catalysis, accounts for the competitive mechanism of inhibition of the compounds.[11] Since most glycosidases utilize the carboxylate ion as a catalytic group, the

poor specificity of iminosugars and the related scaffolds toward α-glucosidase is a limiting factor for their development as drugs.

Additional experimentation has involved compounds where the protonable nitrogen is substituted or is replaced with another atom. Of these, Emiglitate, a synthetic analogue of deoxynojirimycin [4] and Salacinol, a naturally occurring thiasugar [20] display remarkable potency both in vitro and in vivo. Interestingly in these compounds the protonable center (either nitrogen or sulphur) carries a substituent harboring an electron rich region. A structural feature that is uncommon in natural and synthetic iminosugars or in α-glucosidase inhibitors in general. Also there is little information on the effect of replacing the hydroxyl residues on the iminosugar pyranose ring with different substituents. Such a substitution may drastically affect the inhibitory activity since key features of the natural substrate would be missing. However, the recent discovery [21] that 4,5 dihydroxy-1,2 cyclohexanedicarboxylic acids are endowed with α-glucosidase inhibitory activity challenges this view. We were intrigued by the presence of carboxylic acid, a feature that could lead to new molecules with binding mode that is different from that of the current drugs. Given these premises, we embarked in a chemical program aimed at synthesizing a novel series of azasugars embedding in their structure some of the non-canonical features discussed above. In particular the role of carboxylic acid on the azasugar ring and of a N-linked heteroaryl substituent bearing hydrogen bond donor/acceptors has been investigated. Here we report the finding that a series of N-heteroarylmethyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid compounds act as potent α-glucosidase inhibitors. Unlike Acarbose and the other clinically available α-glucosidase inhibitors, the compounds act through a reversible uncompetitive mechanism of inhibition which make them attractive as candidate for drug development.

2. Results and Discussions

2.1 Chemistry

The scaffold 1-heteroarylmethyl-4-hydroxy-piperidine-3-carboxylic acids **7a-d** (Fig.1) [22] was prepared with slight modifications of the literature procedures. In detail the commercially available ethyl 1-benzyl-4-oxo-3-piperidine carboxylate hydrochloride **1** (from Aldrich), after neutralization by TEA (triethylamine) was reduced by sodium borohydride to give an inseparable mixture of the corresponding alcohol **2** (mixture of *cis*- and *trans*- isomers).[23] This mixture was treated with *tert*-butyldimethylsilyl chloride (TBSCl) in the presence of imidazole in DMF to give the TBS-ether **5** and the *cis*-isomer of alcohol **2b**.[24] Column chromatography on silica gel gave the pure alcohol **2b**. Removal of benzyl protecting group by means of palladium on carbon (10%) in ethanol, followed by alkylation of the chlorohydrate, by means of Hüning base in acetonitrile [25] in the presence of alkylating halides, yielded the targets **7a-7d**. Sample **3** plus **4** was prepared by simple NaOH/MeOH mediated hydrolysis of sample **2**. Preparations of samples **10a-10f** were realized as outlined into Fig.2 [22] starting from commercially available arecoline hydrobromide **8**. Accordingly demethylation of arecoline was performed by known procedure.[26] Product **15** was obtained from **9** by its hydrolysis and tested as such. The demethylated product **9**, in turn, was alkylated by the suitable alkyl halide in the presence of bicarbonate [27], followed by hydrolysis with sodium hydroxide, to give the targets **10a-10d**. Targets **10e** and **10f** were synthesized by adopting the alkylating procedure (DIPEA, acetonitrile and the corresponding alkylating agent), followed by hydrolysis by $NaOH_{aq/MeOH}$. [25] In order to test the potential activity of other double bond isomer, product **14** was prepared *via* an alternative demethylation procedure of arecoline with chloroformiate followed by isomerization of the resulting carbamate **12** (Fig.2) with LDA, in the presence of hexametapol (HMPA), and SO_2Cl_2 mediated esterification with MeOH. The 5-hydroxy guvacine scaffold **20** was obtained by means of oxidation of product **17** (Fig.2) by meta-chloroperbenzoic acid followed by elimination to give intermediate **20**. After removal of *N*-Boc protecting group, the corresponding alkylation [25] yielded targets **21a-21e**. Hydrolysis of the said products with hydrogen chloride gave the end products **22a-22e** (Fig.2).

2.2 Enzyme inhibition and mechanism

2.2.1 Screening of the glycosidase activity

All the compounds underwent initial testing at 100 μM level. In addition to α-glucosidase, the compounds were also tested on β-glucuronidase and hyaluronidase. This was justified by the presence of a carboxylate moiety on the azasugar ring as in some analogues of siastatin B reported in the literature [14] displaying activity against β-glucuronidase. None of our compounds showed activity toward these two enzymes (Table 1). On the other hand, compounds **22b, 22c**, **22d**, and **22e** were identified as potent α-glucosidase inhibitors. The IC_{50} of these compounds fell between 2 and 13 μM when evaluated over the 0.17 to 100 μM concentration range. In particular compound **22c** displayed the highest inhibitory activity (IC_{50} 2.34 μM). An inspection of the structure of the active compounds highlights key features required for activity. Beside the critical presence of the 5-hydroxy residue (which cannot be replaced by the same substituent in 4 as in compounds **7a-c)** a 3-carboxylate residue on the 1,2,5,6-tetrahydropyridine ring appears crucial. Specifically its esterification leads to loss of activity as seen in compounds **21b-e**,. Also critical is the presence of an *N*-substituent carrying an electron-rich region. Indeed, when the pyridine or the 1,3-diazacyclohexan-2,6-dione rings are replaced by a benzene ring (as in compound **22a**), the activity is lost. The remarkable inhibitory effect of these compounds in spite of the reduced protonability of the tertiary amine and as well as other major structural changes such as a reduced hydroxylation of the tetrahydropyridine ring, leads us to hypothesize alternative mechanism of inhibition to that of the canonical transition state analogues of the D-glucosyl carbocation. The modality through which the novel inhibitors affect α-glucosidase kinetically, was therefore investigated.

2.2.2 Modality of enzymatic inhibition

A study of the mechanism of enzymatic inhibition was carried out on the active compounds. The inhibitory kinetics were investigated in the presence of increasing concentrations (0.1 to 1.5 mM) of the 4-nitrophenyl-α-glucopyranoside (pNPG) substrate. For all the compounds, both K_m and V_{max}, calculated from Michaelis-Menten plots of the results (see Supp. Fig A), decreased at increasing inhibitor concentrations. In order to gain insight into the modality of inhibition, the results were analyzed according to Dixon

[28] (Fig. 3A-D). In all cases, families of 1/V vs [I] regression lines displaying nearly identical slope were computed strongly suggesting the compounds acted as uncompetitive inhibitors. This same pattern was seen when data where plotted according to Lineweaver-Burk (1/V vs 1/[S]) (Supp. Fig A) thus strengthening the previous conclusion. Because of the identical slopes, K_i values could not be derived from the Dixon plot and were thus calculated according to Cornish–Bowden [29] (Fig. 3E-H). For the four compounds tested, the K_i values ranged between 1.2 and 12.5 uM.

To further corroborate our findings, the enzymatic kinetic for compound **22c** was re-examined using maltose as substrate. The results shown in Fig. 4, are in agreement with the conclusions reached from the experiments using pNPG as substrate. Recently Yang et al. [30] have proposed a different method for the analysis of the kinetic data that overcomes possible misinterpretation of the kinetic results.[30] In particular this problem applies to uncompetitive inhibitors that may be erroneously assigned to the mixed inhibitors category. According to this procedure K_m and V_{max} are plotted against the inhibitor concentration and two new kinetic constants, K_{ik} and K_{iv}, are derived. The found K_{ik}/K_{iv} ratios comprised between 1 and 2 at all the inhibitor's concentrations tested (see Table 2), fits in well with Yang's computed parameter for uncompetitive inhibitors.[30]

Uncompetitive inhibitors of α-glucosidase have rarely been reported and the structural features underlying their mode of action have not been systematically investigated. Cornish-Bowden [31] has discussed as to why uncompetitive inhibition is rarely seen in metabolic system by comparing the "catastrophic" response of a metabolic pathway to the presence of an uncompetitive inhibitor to the much milder response elicited by a competitive inhibitor. Based on his conclusions, uncompetitive inhibitors are to be regarded as superior for drug development when compared to competitive and non-competitive inhibitors and are expected to display better *in-vivo* efficacy. Westley et al. [32] reached similar conclusions, and further suggested that specific enzymic reaction products - rather than analogs of substrates, provide a promising basis for the systematic design of uncompetitive inhibitors. Given the paucity of structure-activity relationship

data on uncompetitive inhibitors, the issue of the identity of the interacting site in yeast α-glucosidase involved in binding of the novel inhibitors, remains at present unresolved and must await further investigation including computer-assisted docking studies.

To gain information on the specificity of action, a survey of the literature was conducted to identify compounds with structural features that are shared by the active azasugars we report. A series of 1-benzyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid derivates has been reported as platelet antiaggregants.[33] However in these compounds the tetrahydropyridine ring is lacking both hydroxyl and N-linked heteroaromatic substituents. A series of tertiary and quaternary methyl and propargyl N-substituted 1,2,5,6-tetrahydropyridine-3-carboxylic acids derivatives has been reported to be endowed with muscarinic activity.[34] In these compounds the tetrahydropyridine ring is not hydroxylated and the replacement of the N-alkyl group by larger substituents results in loss of activity. A number of siastatin B derivatives also bears partial resemblance to our compounds. [14] While harboring a 3-carboxyl azasugars backbone, their activity against β-glucuronidase, heparanase or neuraminidase [35-37], is critically dependent upon the presence of a 2-animoacetyl substituent which is absent in our products. None of the structure surveyed display activity against α-glucosidase of the same order as our compounds. In summary, the structures described in this paper differ from previously reported compounds in incorporating on the same structure three key features: 1) a carboxylic acid at position 3 attached to a sp2 carbon atom, 2) an hydroxyl group at position 5 and, 3) an heteroaryl substituent at position 1 bearing hydrogen bond donor/acceptors.

3. Conclusions

In the present investigation, a series of *N*-heteroarylmethyl-5-hydroxy-1,2,5,6 tetrahydropyridine-3-carboxylic acid compounds was found endowed with potent α-glucosidase inhibitory activity. Unlike Acarbose and the other clinically available inhibitors, the compounds displayed uncompetitive modality of enzyme inhibition which makes them good candidates for *in vivo* testing. The results from the biological

evaluation of compounds, whose structure is comprised within a rather limited chemical space, allowed for the identification of structural features that are critical for the activity of this novel class of α-glucosidase inhibitors. A carboxylate and a hydroxyl residues properly positioned on the azasugar ring, were found both necessary and sufficient for activity when coupled with a suitable aglycone attached to the protonable nitrogen. This simple scaffold and the significant inhibitory activity it affords, are good starting points for further structural investigations. The data gathered reduce, at least in part, the paucity of information on the structure-activity relationship of uncompetitive α-glucosidase inhibitors. They should serve as a basis for the design and development of novel therapeutics for the treatment of diabetes mellitus and other diseases where α-glucosidase is involved.

4. Experimental

4.1 Chemistry: Material and Methods

All starting materials, unless otherwise stated, were purchased and used without any further purification. The starting material for the 4-hydroxy-piperidine-3-carboxylic acid scaffold was the commercially available ethyl 1-benzyl-4-oxopiperidine-3-carboxylate from Sigma Aldrich. For the compounds reported in Fig.2 the starting material was the commercially available Arecoline hydrobromide from Qingdao Sicemo, LTD, Qingdao, China. Solvents were distilled and dried according to standard procedures. Column chromatography was performed using Merck KGaA Silicagel 60 (230-400 Mesh-ASTM). Melting points were obtained using a Stuart Scientific SMP3 Melting Point apparatus. IR spectra were recorded on a Nicolet 380 FT-IR infrared spectrometer. NMR spectroscopy was performed on a Varian-Mercury 400 spectrometer using the residual signal of the solvent as the internal standard. The chemical shifts are reported in ppm and coupling constants (*J*) in Hz. GC–MS spectra were recorded using Agilent Technologies 6850 and 5975 GC-Mass instrumentation. LC–MS spectra were obtained using an Agilent Technologies MSD1100 single-quadrupole mass spectrometer. Elemental analyses were obtained at Laboratorio Scienze Ambientali (Ravenna, Italy) using a Flash 2000, series CHNS/O Analyzer (Thermo Scientific).

Preparation of ethyl 1-benzyl-4-hydroxypiperidine-3-carboxylate 2

Ethyl 1-benzyl-4-oxo-3-piperidine carboxylate hydrochloride **1** (5 g, 16.8 mmol) was dissolved into 130 ml methanol. NEt_3 (2.3 ml, 16.8 mmol) was dropped into this solution at 0 ^oC and stirred for 10 min at this temperature. $NaBH_4$ (2.1 g, 3 eq) was added into the reaction mixture in portions. The reaction mixture was kept at 0 ^oC for 2hrs. HCl_{aq} (5 M) was added to adjust the pH =2~3. The solvent was partially removed to small volume under vacuum and the residue was neutralized by saturated $NaHCO_3$ solution. This mixture was extracted by CH_2Cl_2 (30 ml×3). The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum to obtain 3.64 g of yellow liquid as a mixture of two diastereomers which was purified by flash chromatography (AcOEt: cyclohexane =3:2) to give product **2** (3.64 g, ratio: *trans/cis*=4/7, yield: 82.7%).

Spectra for (3S*,4S*)-ethyl 1-benzyl-4-hydroxypiperidine-3-carboxylate 2a

IR (film): 1731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.18 (t, *J*=7.2 Hz, 3H), 1.57 (m, 1H), 1.87 (m, 1H), 2.03 (m, 2H), 2.53 (m, 1H), 2.78 (m, 1H), 3.04 (m, 1H), 3.45 (q_{AB}, *J*=13.6 Hz, 2H), 3.71 (m, 1H), 4.08 (m, 2H), 7.15-7.27 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.12, 32.57, 49.64, 51.33, 53.41, 60.88, 62.24, 69.67, 127.8, 128.28, 128.96, 173.38; MS (EI) m/z =263 [M]; Elemental Analysis: Calcd. for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32; O, 18.23; Found: C, 68.64; H, 8.07; N, 5.34

Spectra for (3S*,4R*)-ethyl 1-benzyl-4-hydroxypiperidine-3-carboxylate 2b

IR (film): 1731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.16 (t, *J*=7.2 Hz, 3H), 1.71 (m, 1H), 1.80 (m, 1H), 2.41 (m, 2H), 2.58 (m,1H), 2.68 (m, 2H), 3.41 (q_{AB}, *J*=13.2 Hz, 2H), 4.01 (m, 1H), 4.08 (m, 2H), 7.15-7.26 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.08, 31.51, 46.17, 50.62, 60.76, 62.70, 77.50, 127.19, 128.24, 129.08, 173.70; MS (EI) m/z = 263 [M].

Preparation of sodium 1-benzyl-4-hydroxypiperidine-3-carboxylates 3 and 4

Compound **2** (150 mg, 0.57 mmol) was mixed with $NaOH_{aq}$ (0.32 ml, 2M) and MeOH (3 ml) for 3 hrs at r.t. Once the reaction was complete, the solvent was removed under vacuum. The residue was treated with anhydrous MeOH and filtered. The filtrate was

concentrated under vacuum to obtain the mixture of two diastereomers (**3** and **4)** (140 mg, ratio: *trans*/*cis*= 2:3, yield: 95%)

Spectral data of mixture of compounds 3 and 4)

Sodium (3*S,4*S**)-1-benzyl-4-hydroxypiperidine-3-carboxylate 3**

IR (film): 1644 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 1.57 (dq, J_1=4.0 Hz, J_2=10.8 Hz, 1H), 1.88 (m, 1H), 2.05 (m, 2H), 2.31 (ddd, J_1=4.0 Hz, J_2=1.2 Hz, J_2=10.0 Hz,1H), 2.88 (m, 1H), 3.15 (m, 1H), 3.54 (q_{AB}, J=12.8 Hz, 2H), 3.61 (dt, J_1=4.8 Hz, J_2=10.8 Hz, 1H), 7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 33.94, 49.43, 53.01, 55.88, 63.68, 71.89, 128.32, 129.25, 130.75, 138.61, 181.01; MS (ESI) m/z =234 $[M-Na]^-$; Elemental Analysis: Calcd. for $C_{13}H_{16}NNaO_3$: C, 60.69; H, 6.27; N, 5.44; Na, 8.94; O, 18.66; Found:C, 60.85; H, 6.29; N,5.45.

Sodium (3*S,4*R**)-1-benzyl-4-hydroxypiperidine-3-carboxylate 4 (for the spectra of corresponding acid derivative see product 7d)**

IR (film): 1644 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ=1.75 (m, 2H), 2.44(m, 1H), 2.52 (m, 3H), 2.86 (m, 1H), 3.54 (q_{AB}, J=12.8 Hz, 2H), 4.11 (m, 1H), 7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 32.16, 49.63, 52.57, 55.87, 64.22, 66.87, 128.26, 129.21, 130.65, 138.55, 181.56; MS (ESI) m/z =234 $[M-Na]^-$.

Preparation of (*3S*, 4R)-ethyl 1-benzyl-4-hydroxypiperidine-3-carboxylate 2b and (*3S*, 4S**)-ethyl 1-benzyl-4-(*ter*-butyldimethylsilyloxy)piperidine-3-carboxylate 5.**

The inseparable mixture of two diastereomers **2** (2.5g, 9.5 mmol) was treated with imidazole (0.36 g, 5.2 mmol) and TBDMSCl (0.72g, 4.75) in anhydrous DMF (16 ml) at r.t. After stirring for 5hrs the reaction mixture was decomposed with ice/water and extracted with CH_2Cl_2 (30 ml x 3). The organic phase was washed by brine, dried by sodium sulfate and the solvent removed under vacuum to obtain a yellow oil (3.3g). Purification by flash chromatography (Ether : Cyclohexane=1:1) gave **2b** (1.17g) and **5** (1.31 g) in 85% overall yields. Spectral data as follow:

(*3S*, 4R)-ethyl 1-benzyl-4-hydroxypiperidine-3-carboxylate 2b（for spectral data see compound 2）**

(*3S*, 4S)-ethyl 1-benzyl-4-(ter-butyldimethylsilyloxy)piperidine-3-carboxylate 5**

IR (film): 1732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 0.01 (s, 3H), 0.04 (s, 3H), 0.85 (s, 9H), 1.23 (t, *J*= 7.2 Hz, 3H), 1.65 (m, 1H), 1.84 (m, 1H), 2.16 (t, *J*=12.0 Hz, 1H), 2.28 (t, *J*=12.0 Hz, 1H), 2.62 (dt, J_1=2.8 Hz, J_2=12.0 Hz, 1H), 2.80(m, 1H), 2.89 (d, *J*=10.8 Hz, 1H), 3.52 (s, 2H), 3.90 (ddd, J_1=4.0 Hz, J_2=4.4 Hz, J_3=5.6 Hz, 1H), 4.09 (m, 2H), 7.20-7.31(m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.13, 20.26, 32.57, 44.42, 46.92, 60.80, 66.08, 173.82; MS (EI) m/z =377 [M]; Elemental Analysis: Calcd. for $C_{21}H_{35}NO_3Si$: C, 66.80; H, 9.34; N, 3.71; O, 12.71; Si, 7.44; Found: C,66.98; H, 9.37; N, 3,72.

Preparation of (*3S*,4R)-ethyl 4-hydroxypiperidine-3-carboxylate hydrochloride 6**

Product **2b** (1.03 g, 3.9 mmol), Pd/C 10% (1g) and EtOH (100 ml) were mixed together and left to react in Parr apparatus for 1.5 hrs under hydrogen pressure (15 psi). The reaction mixture was filtered through celite. The filtrate was concentrated under vacuum to obtain compound **6** as a yellow oil (0.65 g, yield: 96%). Spectral data were superimposable with literature data. [38]

Synthesis of (3*S*,4R)-4-hydroxy-1-(heteroarylmethyl)piperidine-3-carboxylic acids:**

Preparation of (3*S*,4R)-4-hydroxy-1-(pyridin-2-yl-methyl)piperidine-3-carboxylic acid 7a as general procedure.**

Compound **6** (87 mg, 0.5 mmol), 2-(bromomethyl)-pyridine hydrobromide (126 mg, 0.5 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (0.35 ml, 2.0 mmol) and CH_3CN (10 ml) were mixed together at 0 ^{o}C. The reaction was kept at r.t. for 5 hrs. Once the reaction was completed (t.l.c. test) the solvent was concentrated under vacuum and the residue was dissolved in 20 ml CH_2Cl_2. The organic phase was washed by saturated $NaHCO_3$. The aqueous phase was extracted by CH_2Cl_2 (15 ml×2). The organic phase was collected, dried with sodium sulphate and the solvent removed under vacuum to afford a residue which was purified by flash chromatograph (AcOEt: MeOH=1:1) to obtain pure compound **7a** (100 mg, yield: 75.8%) identified as ethyl ester. Spectral data as follow

Ethylester of (3*S*,4R)-4-hydroxy-1-(pyridin-2-yl-methyl)piperidine-3-carboxylic acid 7a**

IR (film): 1728 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.17 (t, J=6.4 Hz, 3H), 1.81 (m, 2H), 2.51 (m, 2H), 2.71(m, 2H), 3.38 (bs, 1H, OH), 3.64 (q_{AB}, J=14.4 Hz, 2H), 4.11(m, 3H), 7.11(m, 1H), 7.34 (d, J=8.0 Hz, 1H), 7.59 (dt, J_1=2.0 Hz, J_2=7.6 Hz, 1H), 8.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.05, 31.71, 46.30, 47.13, 50.32, 60.61, 64.26, 65.74, 121.98, 122.98, 136.35, 149.10, 158.49, 173.56; MS (EI) m/z =264 [M]; Elemental Analysis: $C_{14}H_{20}N_2O_3$ C, 63.62; H, 7.63; N, 10.60; O, 18.16; Found: C, 63.81; H, 7.65; N, 10.63.

Product **7a** was obtained by treatment of the corresponding ester (100 mg, 0.43 mmol) with $NaOH_{aq}$ (0.32 ml, 2 M) and MeOH (2.5 ml) at r.t. for 5 hrs. The solvent was removed under *vacuum* to obtain a crude mixture which was purified by flash chromatography (AcOEt: acetone: H_2O: CH_3COOH= 5:3:2:2) to obtain pure **7a** (87 mg, yield: 92%).

IR (film): 1658 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 1.79 (m, 2H), 2.48 (m, 1H), 2.55 (m, 2H), 2.70 (bs, 2H), 3.61 (q_{AB}, J=13.6 Hz, 2H), 4.05 (bs, 1H), 7.33 (m, 1H), 7.30 (m, 1H), 7.54 (d, J=7.6 Hz, 1H), 7.81 (dt, J_1=1.2 Hz, J_2=7.6 Hz, 1H), 8.48 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 32.57, 49.63, 49.85, 53.10, 65.09, 66.74, 123.78, 125.20, 138.60, 149.70, 159.44, 181.52; MS (EI) m/z =235 $[M-H]^-$; Elemental Analysis: Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86; O, 20.32; Found: C, 61.15; H, 6.85; N, 11.89.

Preparation of (3*S,4*R**)-4-hydroxy-1-(pyridin-3-ylmethyl)piperidine-3-carboxylic acid 7b.**

This product was prepared following the reported procedure for compound **7a**. Starting from compound **6** (87 mg, 0.5 mmol), 3-(bromomethyl)-pyridine hydrobromide (126 mg, 0.5 mmol), the ethyl ester of **7b** was obtained after the flash chromatography (AcOEt: MeOH=1:1) (95 mg, yield: 72%).

IR (film): 1728 m^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.17 (t, J=7.2 Hz, 3H), 1.76 (m, 1H), 1.82 (m, 1H), 2.46 (m, 2H), 2.67 (m, 2H), 3.50 (bs, 1H), 3.50 (q_{AB},J=13.6 Hz, 2H), 4.10 (m, 3H), 7.21 (dd, J_1=4.8 Hz, J_2=7.6 Hz,1H), 7.62 (d, J=7.6 Hz, 1H), 8.46 (d, J=14.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.05, 31.76, 46.33, 48.12, 50.93, 59.95, 60.66, 65.94, 123.28, 133.77, 136.59, 148.42, 150.19, 173.41; MS (EI) m/z =264 [M];

Elemental Analysis: Calcd. for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60; O, 18.16; Found: C, 63.80; H, 7.65; N, 10.63.

Following the same procedure for the preparation of compound **7a**, the ethyl ester of compound **7b** (95 mg, 0.41 mmol) was treated with $NaOH_{aq}$ (0.31 ml, 2M) and MeOH (2.5 ml). After chromatography (AcOEt: acetone: H_2O: CH_3COOH= 5:3:2:2) **7b** was obtained (85 mg, yield: 94.4%).

IR (film): 1654 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ= 1.75 (m, 2H), 2.49 (m, 3H), 2.73 (m, 1H), 3.58 (q_{AB}, J=13.6 Hz, 2H), 4.10 (m, 1H), 7.40 (dd, J_1=5.6 Hz, J_2=8.0 Hz, 1H), 7.84 (dt, J_1=1.6 Hz, J_2=8.0 Hz, 1H), 8.43 (dd, J_1=1.2 Hz,J_2=4.8 Hz, 1H), 8.51 (d, J=1.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 32.49, 49.43, 49.63, 51.32, 51.32, 61.05, 66.78, 125.03, 125.07, 135.60, 139.35, 148.92, 151.00, 181.46; MS (EI) m/z =235 $[M-H]^-$; Elemental Analysis: Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86; O, 20.32 ; Found: C, 61.16; H, 6.85; N, 11.89.

Preparation of (3*S,4*R**)-4-hydroxy-1-(pyridin-4-ylmethyl)piperidine-3-carboxylic acid 7c.**

This product was prepared following the above reported procedure for compound **7a**. Starting from compound **6** (87 mg, 0.5 mmol), 4-(bromomethyl)-pyridine hydrobromide (126 mg, 0.5 mmol), ethyl ester of **7c** was obtained after the flash chromatography (AcOEt: MeOH=1:1) (100 mg, yield: 75.8%).

IR (film): 1728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.17 (t, J=7.2 Hz, 3H), 1.79 (m, 2H), 2.45 (m, 2H), 2.66 (m, 2H), 3.48 (q_{AB}, J=14.8 Hz, 2H), 3.66 (bs, 1H, OH), 4.08 (q, J=7.2 Hz, 2H), 4.13(m, 1H), 7.21 (d, J=6.0 Hz, 2H), 8.45 (dd, J_1=1.2 Hz, J_2=4.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.06, 31.86, 46.41, 48.02, 51.03, 60.63, 61.47, 65.11, 123.67, 147.87, 149.50, 149.54, 173.29; MS (EI) m/z =264 [M]; Elemental Analysis: Calcd. for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60; O, 18.16; Found: C, 63.79; H, 7.65; N, 10.63.

Following the same procedure for the preparation of compound **7a**, and starting from the corresponding ethyl ester of **7c** (100 mg, 0.43 mmol), $NaOH_{aq}$ (0.33 ml, 2M) and MeOH

(2.5 ml), compound **7c** was obtained after chromatography (AcOEt: acetone: H_2O: CH_3COOH= 5:3:2:2) (80 mg, yield: 84.6%).

IR (film): 1658 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ= 1.77 (m, 2H), 2.58 (m, 4H), 3.57 (s, 2H), 4.06 (s, 1H), 7.40 (m, 2H), 8.40 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ= 32.59, 49.45, 49.66, 52.83, 62.58, 66.81, 125.80, 125.84, 149.94, 150.02, 150.42, 181.49; MS (EI) m/z =235 $[M-H]^-$; Elemental Analysis: Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86; O, 20.32 ; Found: C, 61.14; H, 6.85; N, 11.89.

Preparation of (3*S,4*R**)-1-benzyl-4-hydroxypiperidine-3-carboxylic acid 7d**

Compound **7d** was obtained by hydrolysis of compound **2b** (100 mg, 0.43 mmol), $NaOH_{aq}$ (0.33 ml, 2M) and MeOH (2.5 ml), removal of the solvent and flash chromatography of the residue (AcOEt: acetone: H_2O: CH_3COOH= 5:3:1:1) (88 mg, yield: 93.6%).

IR (film): 1644 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ= 1.76 (m, 2H), 2.44 (m, 1H), 2.53 (m, 3H), 2.83 (m, 1H), 3.54 (q_{AB}, J=13.2, 2H), 4.10 (m, 1H), 7.22-7.35 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ= 34.77, 51.86, 52.08, 52.29, 66.90. 69.82, 130.94, 131.89, 133.46, 141.24, 184.22; MS (EI) m/z = 234 $[M-H]^-$; Elemental Analysis: Calcd. for $C_{13}H_{17}NO_3$:C, 66.36; H, 7.28; N, 5.95; O, 20.40; Found: C, 66.58; H, 7.30; N, 5.97.

Preparation of methyl 1,2,5,6-tetrahydropyridine-3-carboxylate hydrochloride 9

The pH of a solution of Arecoline hydrobromide **8** (Qingdao Sicemo, LTD, Qingdao, China) (15g) in water (30 ml) was adjusted to pH=13~14 by saturated Na_2CO_3 solution. This solution was extracted by ether (50 ml×3). The yellow oil (Arecoline, 9.8g) was obtained after drying and removing the solvent.

Arecoline (1.55g, 10 mmol) was dissolved into 35 ml CH_2Cl_2. A mixture of ACE-Cl (1.2 ml, 11 mmol) and $ClCH_2CH_2Cl$ (2 ml) was dropped into the reaction mixture at 0 °C and stirred for 15 min. The temperature was increased to 100 °C and the mixture refluxed for 6 hrs (monitor by TLC). The solvent was removed under vacuum. The residue was dissolved into 25 ml methanol. This reaction mixture was kept refluxing at 110 °C for 6 hrs until the reaction completed (monitor by TLC: AcOEt : Cyclohexane=3:2). Then the

solvent was removed to obtain compound **9** (0.825 g, two steps overall yield: 58.5%). This product is directly used in the next step without further purification.

IR (film): 1710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.72 (s, 1H, NH), 2.19 (m, 2H), 2.87 (t, *J*=5.7 Hz, 2H), 3.49 (m, 2H), 3.68 (s, 3H), 6.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.15, 41.83, 44.02, 51.40, 130.26, 138.22, 166.41; MS (EI) m/z =141 [M-HCl]; Elemental Analysis: Calcd. for $C_7H_{12}ClNO_2$: C, 47.33; H, 6.81; Cl, 19.96; N, 7.89; O, 18.01; Found: C, 47.48; H, 6.83; N, 7.92.

Synthesis of sodium-1-(heteroarylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylates:

Preparation of sodium-1-benzyl-1,2,5,6-tetrahydropyridine-3-carboxylate 10a as general procedure.

$NaHCO_3$ (179 mg, 2.24 mmol) and EtOH (20 ml) were mixed together and stirred at 0 °C for 5 min. Product **9** (100 mg, 0.56 mmol) and (bromomethyl)-benzene (96.6 mg, 0.56 mmol) were then added to the mixture at 0° C. The reaction mixture was refluxed at 110 °C for 5 hrs until the reaction was completed (t.l.c. test). The solvent was removed under vacuum and the residue was dissolved with 20 ml CH_2Cl_2. The organic phase was washed by saturated $NaHCO_3$. The aqueous phase was extracted by CH_2Cl_2 (15 ml×3). The organic phase was collected, dried with sodium sulfate and concentrated under vacuum to obtain crude product. This was purified by flash chromatography (AcOEt: cyclohexane = 1:4) to obtain **10a-methyl ester** (50 mg, yield: 55.7%) which was identified by its spectral data.

IR (KBr): 1722 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.33 (m, 2H), 2.54 (t, *J*= 5.6 Hz, 2H), 3.24 (m, 2H), 3.66 (s, 2H), 3.73 (s, 3H), 7.02 (m, 1H), 7.25-7.37 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.55, 48.30, 51.47, 51.62, 62.44, 127.12, 128.28, 129.05, 138.00, 166.35;MS (EI) m/z =231 [M]; Elemental Analysis: Calcd. for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06; O, 13.83; Found: C, 72.90; H, 7.43; N, 6.08.

Methyl ester derivative of **10a** (45 mg) was dissolved in 1 ml of MeOH. Then 0.145 ml of $NaOH_{aq}$ (2 M) was added. This reaction mixture was kept at 50 °C for 2.5 hrs. After completion, the solvent was removed under vacuum to obtain the crude product which

was dissolved into anhydrous MeOH and filtered. The filtrate was concentrated under vacuum to obtain the pure **10a** (40 mg, yield: 85%).

IR (film): 1700 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.63 (m, 2H), 3.31 (m, 4H), 4.44 (s, 2H), 7.21 (m, 1H), 7.44-7.59 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ= 23.91, 60.79, 125.41, 130.26, 130.39, 131.29, 132.41, 138.05, 138.08, 166.65; MS (ESI) m/z = 216 $[M\text{-}Na]^-$; Elemental Analysis: Calcd. for $C_{13}H_{14}NNaO_2$: C, 65.26; H, 5.90; N, 5.85; Na, 9.61; O, 13.37; Found: C, 65.48; H, 5.92; N, 5.87.

Preparation of sodium 1-(pyridin-2-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 10b

Following the same procedure for the preparation of compound **10a**, starting from $NaHCO_3$ (239 mg, 2.84 mmol), EtOH (20 ml), product **9** (126 mg, 0.71 mmol) and 2-(bromomethyl)pyridine hydrobromide (179 mg, 0.71 mmol), compound **10b-methyl ester** was obtained after flash chromatography (AcOEt : MeOH =9 :1) (127 mg, yield: 77.0%)

IR (film): 1722 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.36 (m, 2H), 2.61 (t, J= 5.6 Hz, 2H), 3.28 (q_{AB}, J= 2.8 Hz, 2H), 3.71 (s, 3H), 3.80 (s, 1H), 7.02 (m, 1H), 7.17 (m, 1H), 7.42 (d, J= 8.0 Hz, 1H), 8.44 (dt, J_1= 1.2 Hz, J_2= 7.6 Hz, 1H), 8.57 (d, J= 4.8 Hz , 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.46, 48.73, 51.62, 63.96, 122.07, 122.99, 128.93, 136.45, 137.94, 149.25, 158.48, 166.79; MS (EI) m/z =232[M]; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06; O, 13.78; Found: C, 67.46; H, 6.96; N, 12.10.

Following the same procedure for the preparation of compound **10a**, starting from the corresponding ester of **10b** (87 mg, 0.38 mmol), and 0.28 ml of $NaOH_{aq}$ (2M), compound **10b** was obtained (72 mg, yield: 80%).

IR (film): 1662 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.29 (m, 2H), 2.57 (t, J=5.6 Hz, 2H), 3.27 (dd, J_1=2.8 Hz, J_2=4.8 Hz,2H), 3.79 (s, 2H), 6.69 (m, 1H), 7.32 (dddd, J_1=1.2 Hz, J_2=4.8 Hz,J_3=7.6 Hz,1H), 7.56 (dt, J_1=7.6 Hz, J_2=1.2 Hz,1H), 7.83 (dt, J_1= 2.0 Hz, J_2=7.6 Hz, 1H), 8.50 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 26.85, 50.30, 54.39, 64.61, 123.92, 125.24, 132.14, 135.78, 138.70, 149.65, 159.21, 174.70; MS (ESI) m/z =

217 [M-Na]$^-$; Elemental Analysis: Calcd. for $C_{12}H_{13}N_2NaO_2$: C, 60.00; H, 5.45; N, 11.66; Na, 9.57; O, 13.32; Found: C, 60.17; H, 5.47; N, 11.69.

Preparation of sodium 1-(pyridin-3-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 10c

Following the same procedure for the preparation of compound **10a**, starting from $NaHCO_3$ (239 mg, 2.84 mmol), EtOH (20 ml), product **9** (126 mg, 0.71 mmol) and 3-(bromomethyl)pyridine hydrobromide (179 mg, 0.71 mmol), compound (**10c-methyl ester**) was obtained after flash chromatography (AcOEt : MeOH =98:2) (70 mg, yield: 42.4%).

IR (film): 1712 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.31 (m, 2H), 2.53 (t, J= 5.6 Hz, 2H), 3.20 (m, 2H), 3.63 (s, 2H), 3.70 (s, 3H), 7.00 (m, 1H), 7.24 (m, 1H), 7.67 (d, J= 8.0 Hz, 1H), 8.50 (d, J= 4.4 Hz, 1H), 8.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.44, 48.35, 51.53, 51.56, 59.58, 123.36, 128.79, 133.52, 136.56, 137.90, 148.68, 150.30, 166.17; MS (EI) m/z =232 [M]; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06; O, 13.78; Found: C,67.38; H, 6.96; N,12.09.

Following the same procedure for the preparation of compound **10a**, starting from the **10c -methyl ester** (70 mg, 0.30 mmol), and 0.23 ml of $NaOH_{aq}$ (2 M), compound **10c** was obtained (60 mg, yield: 83%)

IR (film): 1663 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.30 (m, 2H), 2.60 (t, J=5.6 Hz, 2H), 3.21 (dd, J_1=2.4 Hz, J_2=4.4 Hz, 2H), 3.70 (s, 2H), 6.69 (m, 1H), 7.41 (dddd, J_1=0.8 Hz,J_2=5.2 Hz, J_3=7.6 Hz,1H), 7.89 (dt, J_1=1.6 Hz, J_2=8.0 Hz, 1H), 8.45 (dd, J_1=1.6 Hz, J_2=4.8 Hz, 1H), 8.53 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 26.80, 50.11, 54.11, 60.57, 125.12, 125.14, 132.04, 132.16, 135.36, 135.61, 139.51, 148.99, 151.12, 174.59; MS (ESI) m/z =217 [M-Na]$^-$; Elemental Analysis: Calcd. for $C_{12}H_{13}N_2NaO_2$: C, 60.00; H, 5.45; N, 11.66; Na, 9.57; O, 13.32; Found: C, 60.19; H, 5.47; N, 11.70.

Preparation of sodium 1-(pyridin-4-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 10d

Following the same procedure for the preparation of compound **10a**, starting from $NaHCO_3$ (239 mg, 2.84 mmol), EtOH (20 ml), product **9** (126 mg, 0.71 mmol) and 4-

(bromomethyl)pyridine hydrobromide (179 mg, 0.71 mmol), compound **10d-methyl ester** was obtained after flash chromatography (AcOEt : MeOH =8:1) (80 mg, yield: 48.5%)

IR (film): 1710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.35 (m, 2H), 2.52 (m, 2H), 3.22 (m, 2H), 3.64 (s, 2H), 3.72 (s, 3H), 7.02 (m, 1H), 7.28 (d, *J*= 6.0 Hz, 2H), 8.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.44, 48.59, 51.51, 51.65, 61.12, 123.67, 128.81, 137.88, 147.50, 149.83, 165.15; MS (EI) m/z =232 [M]; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06; O, 13.78; Found: C, 67.41; H, 6.96; N, 12.09.

Following the same procedure for the preparation of compound **10a**, starting from **10d - methyl ester** (150 mg, 0.56 mmol), and 0.70 ml of $NaOH_{aq}$ (2 M), compound **10d** was obtained (114 mg, yield: 85%)

IR (film): 1666 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.31 (m, 2H), 2.55 (t, *J*=6.0 Hz, 2H), 3.22 (dd, J_1=2.4 Hz,J_2=4.8 Hz, 2H), 3.70 (s, 2H), 6.69 (m, 1H), 7.46 (d, *J*=7.2 Hz, 2H), 8.46 (d, *J*=7.2 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ= 26.84, 50.31, 54.30, 62.24, 125.93, 125.96, 132.03, 132.14, 135.68, 149.99, 150.07, 174.61; MS (EI) m/z =217 $[M-Na]^-$; Elemental Analysis: Calcd. for $C_{12}H_{13}N_2NaO_2$: C, 60.00; H, 5.45; N, 11.66; Na, 9.57; O, 13.32; Found: C, 60.18; H, 5.47; N,11.69.

Preparation of sodium 1-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 10e

Product **9** (150 mg, 0.85 mmol), 6-(chloromethyl)uracil (137 mg, 0.85 mmol) and diisopropyl amine (0.739 ml, 4.25 mmol) were mixed in 15 ml of CH_3CN. This reaction mixture was kept at r.t. for 3 hrs. After the reaction completed (t.l.c testing), the solvent was removed under vacuum to obtain the crude product. This crude product was purified after flash chromatography (AcOEt : MeOH =3:1) to obtain **10e-methyl ester** (150 mg, yield: 66.7%).

IR (film): 1733, 1716, 1700, 1653 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.40 (m, 2H), 2.58 (t, *J*=6.0 Hz, 2H), 3.23 (q_{AB}, *J*= 2.8 Hz, 2H), 3.42 (d, *J*= 1.2 Hz , 2H), 3.73 (s, 3H), 4.35 (s, 1H), 7.03 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 27.19, 41.27, 48.37, 52.21, 58.66, 100.40, 129.61, 139.33, 155.35, 167.64; MS (EI) m/z = 266 [M+1]; Elemental

Analysis: Calcd. for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84; O, 24.13; Found: C, 54.43; H, 5.71; N, 15.87.

Compound **10e-methyl ester** (50 mg, 0.18 mmol) was treated with $NaOH_{aq}$ (2 M) (0.43 ml) at 50°C for 3 hrs. After the hydrolysis was complete, the solvent was removed under vacuum. The residue was dissolved in anhydrous MeOH (5 ml). The mixture was filtered. The solution was concentrated under vacuum to give **10e** (25 mg, yield: 48 %)

IR (film): 1715, 1670 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ= 2.50 (m, 2H), 3.03 (m, 2H), 3.59 (m, 2H), 3.85 (m, 2H), 5.92 (m, 1H), 6.77 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ= 23.61, 48.36, 51.35, 56.09, 102.44, 130.12, 133.00, 149.98, 152.81, 166.53, 172.85; MS (ESI) m/z =274 [M+H]; Elemental Analysis: Calcd. for $C_{11}H_{12}N_3NaO_4$: C, 48.36; H, 4.43; N, 15.38; Na, 8.41; O, 23.42; Found: C, 48.46; H, 4.44; N, 15.41.

Preparation of sodium 1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 10f

Following the same procedure for the preparation of **10e**, starting from **9** (150 mg, 0.85 mmol), 2-(chloromethyl)-benzimidazole (142 mg, 0.85 mmol) and diisopropyl amine (0.443 ml, 2.55 mmol), compound **10f-methyl ester** (50 mg, yield: 21.7%) was obtained after flash chromatography (AcOEt : cyclohexane = 3:1)

IR (film): 1709 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 2.33 (m, 2H), 2.64 (t, J=5.2 Hz, 2H), 3.31 (q_{AB}, J=2.8 Hz, 2H), 3.70 (s, 3H), 3.96 (s, 2H), 7.01 (m, 1H), 7.22 (m, 2H), 7.57(m, 2H), 10.42 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 26.30, 48.90, 51.63, 51.72, 55.85, 12.38, 137.82, 152.09, 166.05; MS (EI) m/z = 240 [M-OCH_3]; Elemental Analysis: Calcd. for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49; O, 11.79; Found: C, 66.57; H, 6.34; N, 15.53.

Following the same procedure for the preparation of compound **10e**, starting from **10f-methyl ester** (50 mg, 0.18 mmol), and 0.14 ml of $NaOH_{aq}$ (2 M), compound **10f** was obtained (30 mg, yield: 60%)

IR (film): 1663 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ= 2.42 (m, 2H), 2.96 (m, 2H), 3.55 (m, 2H), 4.22 (m, 2H), 6.70 (m, 1H), 7.32 (m, 2H), 7.61 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ= 23.73, 47.94, 51.06, 52.63, 115.02, 123.30, 130.54, 132.58, 147.06, 173.10; MS

(ESI) m/z =280 [M+H]; Elemental Analysis: Calcd. for $C_{14}H_{14}N_3NaO_2$: C, 60.21; H, 5.05; N, 15.05; Na, 8.23; O, 11.46; Found: C, 60.43; H, 5.07; N,15.10.

Preparation of 1-ethyl 3-methyl 5,6-dihydropyridine-1,3(2*H*)-dicarboxylate 11

The pH of a solution of Arecoline hydrobromide **8** (Qingdao Sicemo, LTD, Qingdao, China) (15g) in water (30 ml) was adjusted to 13~14 by saturated Na_2CO_3 solution. This solution was extracted by ether (50 ml×3). The yellow oil (Arecoline, 9.8g) was obtained after drying and removing the solvent.

Arecoline (6.59g, 42.5 mmol) was dissolved into 80 ml toluene at 0 ^oC. A mixture of ClCOOEt (12.24 ml, 11 mmol) and toluene (20 ml) was dropped into the reaction mixture at 0 ^oC in 1 hour and kept stirring for 15 min at this temperature. Then the temperature was increased to 100 ^oC and the mixture refluxed for 2.5 hrs. This reaction was decomposed with ice water and the pH adjusted to 2-3 with HCl_{aq} (1 M). The mixture was extracted by AcOEt (20 ml×3). The organic phase was washed by NaHCO3 and brine, dried and the solvent removed under vacuum to obtain **11** as yellow oil (5.8g, yield: 64%)

IR (film): 1704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.26 (t, J=7.1 Hz, 3H), 2.31(m, 2H), 3.52 (t, J=5.6 Hz, 2H), 3.75(s, 3H), 4.16 (m, 4H), 7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.65, 25.37, 39.19, 42.49, 51.69, 61.44, 128.96, 137.78, 155.54, 165.64; MS (EI) m/z =213 [M]; Elemental Analysis: Calcd. for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57; O, 30.01; Found: C, 56.54; H, 7.12; N,6.59.

Preparation of 1-(ethoxycarbonyl)-1,2,5,6-tetrahydropyridine-3-carboxylic acid 12

Product **11** (462 mg, 2.0 mmol) was dissolved into 10 ml toluene at 0 °C. A solution of $NaOH_{aq}$ (0.6 ml, 5 M) was dropped into the reaction mixture at 0 °C. Then the temperature was increased to 50 °C for 5.5 hrs. The reaction mixture was decomposed with ice water, adjusted the pH to 2-3 with HCl_{aq} (1 M) and extracted with AcOEt (25 ml×3). The organic phase was collected, dried with Na_2SO_4 and the solvent removed under vacuum to obtain compound **12** (390 mg, yield: 98%). This product was directly used for the next step without any further purification.

IR (film): 1705, 1687 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.28 (t, *J*=8.8 Hz, 3H), 2.32 (m, 2H), 3.53 (t, *J*=5.6 Hz, 2H), 3.76 (m, 4H), 7.08(s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.60, 25.51, 42.24, 48.11, 61.74, 127.87, 140.23, 157.89, 169.91; MS (EI) m/z =199 [M]; Elemental Analysis: Calcd. for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03; O, 32.13; Found: C, 54.44; H, 6.60; N,7.05.

Preparation of 1-ethyl 3-methyl 5,6-dihydropyridine-1,3(4H)-dicarboxylate 14

BuLi (2.2 ml, 2.5M in THF, 5.5 mmol) was added into a solution of diisopropyl amine (0.70 ml, 5.0 mmol) in THF (20 ml) at -78 °C. The temperature was increased to -10 °C and 4 ml of HMPA were added at this temperature. This reaction mixture was kept at this temperature for 30 min and then cooled down to -78 °C again. A solution of compound **12** (500 mg, 2.5 mmol) in THF (5 ml) was dropped into the base solution at -78 °C. This reaction mixture was kept at -78 °C for 3 hrs, then poured into a cold saturated NH_4Cl_{aq} (20 ml). The pH was adjusted to pH=2-3 and the mixture was extracted with AcOEt (30 ml×3). The organic phase was washed with HCl (1M) and brine, dried with Na_2SO_4 and evaporated to obtain crude product which was purified by flash chromatography (AcOEt : Cyclohexane =3:1) to obtain a mixture of *ene*-isomers **13**. This mixture was processed in the next step without any purification.

The mixture of isomers **13** (360 mg, 1.81 mmol) was directly dissolved into 1 ml of MeOH. This solution was added into a solution of SO_2Cl_2 (2.88 ml) in MeOH (20 ml) at 0 ^{o}C. This reaction mixture was kept at 0 ^{o}C for 1hr. After the reaction completed, the solvent was removed to obtain the crude product which was purified by column chromatography (AcOEt : cyclohexane= 1:4) to give pure compound **14**.

IR (film): 1724, 1700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.34 (t, *J*=6.8 Hz, 3H), 1.85 (m, 2H), 2.32 (dt, *J*=1.2 Hz, *J*=6.0 Hz, 2H), 3.61 (m, 2H), 3.74(s, 3H), 4.27 (q, *J*=6.8 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 14.45, 20.63, 20.89, 42.16, 51.32, 62.76, 102.12, 135.47, 153.36, 167.96; MS (EI) m/z =213 [M]; Elemental Analysis: Calcd. for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57; O, 30.01; Found: C, 56.50; H, 7.11; N, 6.59.

Preparation of 1,4,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 15

Product **9** (500 mg, 2.82 mmol) was dissolved into 10 ml toluene at 0 °C. A solution of $NaOH_{aq}$ (8.6 ml, 2 M) was dropped into the reaction mixture at 0 °C. Then the temperature was brought to 110 °C and kept for 6 hrs (monitoring by tlc: AcOEt/ acetone/ water/ acetic acid=5:3:2:2). The reaction mixture was decomposed with ice water and the pH adjusted to 2-3 with HCl_{aq} (3 M). The solvent was removed under vacuum. The residue was treated with ethereal hydrogen chloride and filtered to obtain pure **15** [38, 39] (330 mg, yield: 92%).

Mp: 305 °C (Decomp). IR (film): 1665 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ= 2.27 (m, 2H), 2.92 (t, J=6.0 Hz, 2H), 3.56 (m, 2H), 6.75 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ= 24.32, 41.00, 44.13, 130.86, 134.50, 173.12.

Preparation of 1-*tert*-butyl 3-methyl 5,6-dihydropyridine-1,3(2H)-dicarboxylate 16

Compound **9** (5.49 g, 31.0 mmol) was mixed with triethylamine (5.65 ml, 40.3 mmol) and di-*tert*-butyl dicarbonate (10.14g, 46.5 mmol) in 60 ml of anhydrous methanol at r.t. The reaction mixture was kept overnight. After removing the solvent under vacuum, 60 ml of water were added to the residue. The pH of the mixture was adjusted to 4-5 and extracted with ethyl acetate (80 ml×3). The collected organic phase were dried and evaporated to obtain compound **16** (11.08 g, yield: 79%). Spectral data were superimposable with literature data.[40]

Preparation of 1-*tert*-butyl 3-methyl 2,3-dihydropyridine-1,3(6*H*)-dicarboxylate 17

BuLi (8.96 ml, 2.5 M in THF) was added into a solution of diisopropyl amine (3.15 ml, 22.41 mmol) in THF (35 ml) at -78°C. Then the temperature was increased to -10 °C for 30 min and then cooled down to -78°C. A solution of compound **16** (3g, 12.45 mmol) in THF (10 ml) was dropped into the base solution and kept for 3 min. The reaction mixture was poured into a cold NH_4Cl solution (1M, 80 ml) and extracted with ether (60 mlx3). The organic phase was collected, dried and evaporated to obtain a crude product which was purified by flash chromatography (AcOEt : Cyclohexane =1:4) to give pure compound **17** (1.48 g. yield 49.3%). Spectral data were superimposable with literature data.[40]

Preparation of (1*S,5*S**,6*R**)-3-*tert*-butyl 5-methyl 7-oxa-3-azabicyclo[4.1.0]heptane-3,5-dicarboxylate 18 and (1*R**,5*S**,6*S**)-3-*tert*-butyl 5-methyl 7-oxa-3-azabicyclo[4.1.0]heptane-3,5-dicarboxylate 19**

Product **17** (250 mg, 1.03 mmol) was dissolved into anhydrous CH_2Cl_2 (20 ml). 3-Chloroperbenzoic acid (MCPA, 357 mg, 2.06 mmol) was added. The reaction mixture was kept at r.t for 3 hrs, then an extra 1 eq. of MCPA (179 mg, 1.03 mmol) was added and the mixture left overnight. The reaction was decomposed with $Na_2S_2O_5$ and extracted with AcOEt (15 ml × 3). The organic phase was collected, washed with $Na_2S_2O_5$, brine, dried with Na_2SO_4 and evaporated to obtain a crude mixture of **18** and **19** which was purified by flash chromatography (AcOEt : cyclohexane =1:4) (175 mg, overall yield: 66%).

An aliquot of the mixture was further chromatographed to obtain pure isolated **18** and **19**. Spectral data as follow

18 White solid. Mp: 70-74 ^{o}C; IR (film): 1738, 1698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.45 (s, 9H), 3.00 (m, 2H), 3.34 (m, 1H), 3.42 (d, *J*= 15.2 Hz 1H), 3.61 (d, *J*= 4.0 Hz, 1H), 3.76 (s, 3H), 3.84-4.20 (m, 2H); ^{13}C NMR (100 MHz,$CDCl_3$) δ= 28.33, 38.10, 38.92, 41.28, 50.89, 51.22, 52.17, 80.30, 154.32, 170.88. MS (EI) m/z =242 [M-CH_3]; Elemental Analysis: Calcd. for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44; O, 31.09; Found: C, 56.15; H, 7.46; N, 5.45.

19 Oil. IR (film): 1738, 1698 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ=1.41 (s, 9H), 3.03 (d, *J*= 19.2 Hz 1H), 3.23 (d, *J*= 20.8 Hz 1H), 3.44 (m, 1H), 3.48 (dd, J_1= 4.0 Hz, J_2= 1.2 Hz, 1H), 3.57 (m, 1H), 3.71(s, 3H), 3.78 (m, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ= 28.26, 39.80, 40.35, 42.03, 50.00, 50.90, 52.14, 80.13,154.75, 171.48. MS (EI) m/z =242 [M-CH_3]; Elemental Analysis: Calcd. for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44; O, 31.09; Found: C, 56.17; H, 7.46; N, 5.46.

Preparation of 1-*tert*-butyl 3-methyl 5-hydroxy-5,6-dihydropyridine-1,3(2*H*)-dicarboxylate 20.

A mixture of compounds **18** and **19** (500 mg, 2.02 mmol) and KOH solid (226 mg, 4.04 mmol) were dissolved in 20 ml of MeOH. The reaction mixture was kept at r.t for 30

min. The pH of the reaction was adjusted to 6-7 by HCl_{aq} (2 M). The solvent was removed under vacuum. The residue was mixed with 20 ml of water and extracted with AcOEt (15 ml × 3). The organic phase was collected, dried with Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (AcOEt : cyclohexane =2:3) to obtain pure compound **20** (350 mg, yield: 67%).

White solid. Mp: 78-85 ^oC; IR (film): 1699 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ=1.47 (s, 9H), 3.49-3.51(m, 1H), 3.65-3.73 (m, 1H), 3.78 (s, 3H), 3.97 (dt, J_1= 18.4 Hz, J_2= 2.4 Hz, 1H), 4.18 (dt, J_1= 18.4 Hz, J_2= 2.0 Hz, 1H), 4.26 (m, 1H), 6.92 (m, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ= 28.43, 42.78, 46.16, 52.05, 63.46, 80.72, 129.34, 139.79, 154.95, 165.66. MS (EI) m/z =257 [M]; Elemental Analysis: Calcd. for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44; O, 31.09; Found: C, 56.17; H, 7.46; N, 5.46.

Synthesis of methyl 1-(heteroarylmethyl)-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylates:

Preparation of methyl 1-benzyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylate 21a and 1-benzyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 22a (as general procedure).

Compound **20** (100 mg, 0.39 mmol) was dissolved in 3 ml of CH_2Cl_2. TFA (3 ml) was added into this solution at 0 ^oC. The reaction mixture was kept at 0 ^oC for 20 min. The solvent was removed under vacuum. The crude residue was dissolved in 10 ml of CH_3CN. (Bromomethyl)benzene (67 mg, 0.389 mmol) and diisopropyl amine (0.27 ml, 1.56 mmol) were added into the solution at 0 ^oC. This reaction mixture was kept at r.t. for 3hrs. After the reaction completed, the solvent was removed under vacuum. The residue was dissolved with CH_2Cl_2 and washed with brine. The organic phase was separated, dried and the solvent was removed. This crude product was purified by flash chromatography (AcOEt : cyclohexane = 2:3) to obtain pure compound **21a** (20 mg, yield: 21%).

IR (film): 1718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 2.36 (bs, 1H, OH), 2.53 (dd, J_1= 11.6 Hz, J_2= 3.2 Hz, 1H), 2.75 (dd, J_1= 11.6 Hz, J_2= 3.6 Hz, 1H), 2.99 (d, J= 17.2 Hz, 1H), 3.43 (d, J= 16.8 Hz, 1H), 3.68 (q_{AB}, J= 12.8 Hz, 2H), 3.75 (s, 3H), 4.21 (m, 1H), 7.31 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 51.69, 51.85, 56.39, 62.00, 63.97, 127.51,

128.45, 129.09, 131.29, 137.50, 166.03. MS (EI) m/z =247 [M]; Elemental Analysis: Calcd. for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; O, 19.41; Found: C, 68.18; H, 6.95; N, 5.67.

Preparation of 22a

Compound **21a** (20 mg) was dissolved into 2 ml of HCl_{aq} (1 M). After refluxing for 4.5 hrs at 110 ^{o}C, the solvent was removed under vacuum to obtain compound **22a** (20 mg, yield: 91%).

IR (film): 1708 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ= 3.31-3.62 (m, 2H), 3.76-4.25 (m, 2H), 4.50 (m, 2H), 4.67 (m, 1H), 7.11(m, 1H), 7.55 (m, 5H) ; ^{13}C NMR (100 MHz, D_2O) δ= 48.91, 54.14, 59.63, 60.08, 126.53, 127.88, 129.33, 129.49, 130.36, 131,13, 135.87, 166.76. MS (ESI) m/z = 269 [M]; Elemental Analysis: Calcd. for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98; Cl, 13.14; N, 5.19; O, 17.80; Found: C, 58.07; H, 6.00; N, 5.21.

Synthesis of methyl 5-hydroxy-1-(pyridin-2-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 21b and the corresponding acid 5-hydroxy-1-(pyridin-2-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 22b

Following the procedure of **21a** and starting from **20** (90 mg, 0.35 mmol), 2-(bromomethyl)-pyridine hydrobromide (87 mg, 0.35 mmol) and diisopropyl amine (0.24 ml, 1.4 mmol) compound **21b** was obtained (36 mg, yield: 41%) after flash chromatography (AcOEt :MeOH=5:1).

IR (film): 1710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.56 (d, J= 11.6 Hz, 1H), 2.71 (dd, J_1= 11.6 Hz, J_2= 3.6 Hz, 1H), 2.99 (d, J= 16.8 Hz, 1H), 3.38 (d, J= 16.8 Hz, 1H), 3.66 (s, 3H), 3.74 (m, 2H), 4.20 (m,2H), 4.70 (s, 1H, OH), 6.90 (m, 1H), 7.12 (m, 1H), 7.30 (d, J= 4.8 Hz, 1H), 7.60 (m, 1H), 8.45 (d, J= 4.4 Hz, 1H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ= 51.00, 51.53, 56.57, 62.87, 63.93, 122.19, 122.86, 131.15, 136.71, 139.82, 149.11, 158.41, 165.51; MS (ESI) m/z =249 $[M+1]^+$; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28; O, 19.33; Found: C, 63.09; H, 6.52; N, 11.32.

Preparation of 22b

Compound **21b** (15 mg) was dissolved into 2 ml of HCl_{aq} (1 M). The reaction was refluxed for 3 hrs at 110 ^{o}C. The solvent was removed under vacuum to obtain compound **22b** (15 mg, yield: 81%).

IR (film): 1708 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ= 3.52 (m, 2H), 4.00 (m, 2H), 4.74 (m, 2H), 7.13 (m, 1H), 8.00 (m, 2H), 8.45 (m, 1H), 8.83 (d, J=5.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ= 49.50, 54.54, 57.75, 60.47, 126.37, 126.72, 127.74, 136.92, 143.61, 146.11, 146.14, 166.92; MS (EI) m/z = 269 $[M\text{-}HCl\text{-}H]^{-}$; Elemental Analysis: Calcd. for $C_{12}H_{16}Cl_2N_2O_3$: C, 46.92; H, 5.25; Cl, 23.08; N, 9.12; O, 15.63; Found: C, 47.04; H, 5.26; N, 9.14.

Synthesis of methyl 5-hydroxy-1-(pyridin-3-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 21c and the corresponding acid 5-hydroxy-1-(pyridin-3-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 22c

Following the procedure of **21a** and starting from **20** (90 mg, 0.35 mmol), 3-(bromomethyl)-pyridine hydrobromide (87 mg, 0.35 mmol) and diisopropylamine (0.24 ml, 1.4 mmol), compound **21c** was obtained after flash chromatography (AcOEt :MeOH=5:1)(75 mg, yield: 87%).

IR (film): 1685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.56 (dd, J_1= 11.2 Hz, J_2= 2.4 Hz, 1H), 2.76(dd, J_1= 3.6 Hz, J_2= 11.2 Hz, 1H), 2.99 (d, J= 16.4 Hz, 1H), 3.42 (d, J= 16.8 Hz, 1H), 3.70 (q_{AB}, J= 13.6 Hz, 2H), 3.76 (s, 3H), 4.24 (m, 1H), 4.76(s, 1H, OH), 6.98 (m, 1H), 7.72 (dd, J_1=11.2 Hz, J_2= 3.6 Hz, 1H), 8.57 (m, 2H), 8.60 (s, 1H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ= 51.93, 52.07, 57.36, 59.62, 65.13, 124.19, 130.58, 134.65, 137.32, 140.87, 149.40, 151.10, 166.49; MS (ESI) m/z =249 [M+1]; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28; O, 19.33; Found: C,63.02; H,6.51; N,11.30.

Preparation of 22c

Compound **21c** (20 mg) was dissolved into 2 ml of HCl_{aq} (1 M). This reaction was refluxed for 5 hrs at 110 ^{o}C. The solvent was removed under vacuum to obtain compound **22c** (23 mg, yield:92%).

IR (film): 1707 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ= 3.55 (dd, J_1=4.0Hz, J_2=12.4Hz, 1H), 3.62 (dd, J_1=4.4Hz, J_2=12.8Hz, 1H), 4.02 (dt, J_1=2.4Hz, J_2=16.8Hz, 1H), 4.17 (d, J=15.6Hz, 1H), 4.86 (m, 2H), 7.14 (m, 1H), 8.25 (dd, J_1=5.6Hz, J_2=8.0Hz, 1H), 8.88 (dt, J_1=1.2Hz, J_2=8.4Hz, 1H), 8.99 (d, J=5.6Hz, 1H), 9.12 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ= 49.21, 54.19, 55.67, 60.07, 126.02, 128.04, 128.78, 136.55, 143.22, 143.79, 149.33, 166.91; MS (EI) m/z =269 $[M\text{-}HCl\text{-}H]^-$; Elemental Analysis: Calcd. for $C_{12}H_{16}Cl_2N_2O_3$:C, 46.92; H, 5.25; Cl, 23.08; N, 9.12; O, 15.63; Found: C, 47.03; H, 5.26; N, 9.14.

Synthesis of methyl 5-hydroxy-1-(pyridin-4-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylate 21d and the corresponding acid 5-hydroxy-1-(pyridin-4-ylmethyl)-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 22d

Following the procedure of **21a** and starting from **20** (100 mg, 0.37 mmol), 4-(bromomethyl)-pyridine hydrobromide (94 mg, 0.37 mmol) and diisopropyl amine (0.27 ml, 1.56 mmol), compound **21d** was obtained after flash chromatography (AcOEt :MeOH=5:1) (70 mg, yield: 78%).

IR (film): 1685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.57 (dd, J_1= 12.0 Hz, J_2= 2.8 Hz, 1H), 2.74 (dd, J_1= 4.0 Hz, J_2= 12.0 Hz, 1H), 3.01 (d, J= 16.4 Hz, 1H), 3.42 (d, J= 16.8 Hz, 1H), 3.68 (q_{AB}, J= 14.0 Hz, 2H), 3.76 (s, 3H), 4.25 (m, 1H), 4.76 (bs, 1H, OH), 6.98 (m, 1H), 7.32 (m, 2H), 8.59 (m, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ= 51.93, 52.19, 57.50, 61.06, 65.18, 124.48, 130.64, 140.77, 148.59, 150.56, 166.47; MS (ESI) m/z =249 [M+1]; Elemental Analysis: Calcd. for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28; O, 19.33; Found: C, 63.10; H, 6.52; N, 11.32.

Preparation of 22d

Compound **21d** (20 mg) was dissolved into 2 ml of HCl_{aq} (1 M). This reaction was refluxed for 5 hrs at 110 ^{o}C. The solvent was removed under vacuum to obtain compound **22d** (17 mg, yield: 68%).

IR (film): 1707 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ= 3.51 (dd, J_1=4.0Hz, J_2=12.8Hz, 1H), 3.63 (dd, J_1=4.0Hz, J_2=12.8Hz, 1H), 4.05 (dt, J_1=1.2Hz, J_2=16.4Hz, 1H), 4.02 (d, J=16.4 Hz, 1H), 4.91 (m, 2H), 7.17 (m, 1H), 8.33 (d, J=6.4 Hz,1H), 8.98 (d, J=6.4Hz,

1H); ^{13}C NMR (100 MHz, D_2O) δ= 49.80, 54.62, 57.52, 60.04, 125.92, 129.14, 136.64, 142.35, 148.66, 166.77; MS (EI) m/z =269 $[M\text{-}HCl\text{-}H]^-$; Elemental Analysis: Calcd. for $C_{12}H_{16}Cl_2N_2O_3$: C, 46.92; H, 5.25; Cl, 23.08; N, 9.12; O, 15.63; Found: C, 47.05; H, 5.26; N, 9.15.

Synthesis of methyl 1-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methyl)-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylate 21e and the corresponding acid 1-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methyl)-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride 22e

Following the procedure of **21a** and starting from **20** (105 mg, 0.39 mmol), 6-(chloromethyl) uracil (63 mg, 0.39 mmol) and diisopropyl amine (0.20 ml, 1.17 mmol), compound **21e** was obtained after flash chromatography (AcOEt : MeOH=9:1) (50 mg, yield: 46%).

IR (film): 1716 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ= 2.64 (dd, J_1=4.4 Hz, J_2=11.6 Hz, 1H), 2.78 (dd, J_1= 4.4 Hz, J_2= 11.6 Hz, 1H), 3.11 (d, J= 16.4 Hz, 1H), 3.30 (d, J= 16.4 Hz, 1H), 3.56 (q_{AB}, J= 15.2 Hz, 2H), 3.71 (s, 3H), 4.32 (m, 1H), 4.42 (d, J= 1.2 Hz, 1H, OH), 5.63 (m, 1H), 6.89 (m, 1H), 10.02 (m, 2H, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ= 51.75, 52.03, 57.56, 58.15, 64.67, 97.38, 100.23, 130.68, 139.65, 152.66, 165.11, 166.42. MS (ESI) m/z =282 [M+H]; Elemental Analysis: Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38; N, 14.94; O, 28.44; Found: C, 51.42; H, 5.40; N,15.00.

Preparation of 22e

Compound **21e** (22 mg) was dissolved into 2 ml of HCl_{aq} (1 M). The reaction was refluxing for 4 hrs at 110 ^{o}C. The solvent was removed under vacuum to obtain compound **22e** (20 mg, yield: 69%).

IR (film): 1712 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ= 3.45 (m, 2H), 3.89 (dt, J_1=2.0 Hz, J_2=16.4 Hz, 1H), 4.06 (d, J=16.8 Hz, 1H), 4.23 (m, 3H), 6.22 (s, 1H), 7.19 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ= 49.58, 54.80, 55.19, 59.98, 105.22, 125.62, 136.76, 144.15, 152.34, 165.85, 166.56; MS (EI) m/z = 398 $[M+Na]^+$; Elemental Analysis: Calcd. for $C_{11}H_{16}Cl_3N_3O_5$: C, 35.08; H, 4.28; Cl, 28.24; N, 11.16; O, 21.24; Found: C, 35.17; H, 4.29; N,11.19.

4.2 Biology Material and Methods

The newly synthesized compounds were weighted and dissolved in DMSO as 100 mM stock solution and aliquots stored at -80°C up to the time of use.

4.2.1 Chemicals and Reagents

The chemical reagents, the enzymes α-glucosidase (EC 3.2.1.20) from Saccharomyces cerevisiae, β-glucuronidase (E.C. 3.2.1.31) from bovine liver and hyaluronidase (E.C. 3.2.1.35) from bovine testes were purchased from Sigma (St. Louis, MO). From the same supplier were obtained the chromogenic substrates 4-Nitrophenyl derivative of α-glucopyranoside (pNPG), phenolphthalein β-glucuronide, hyaluronic acid from bovine vitreous humor. The glucose assay kit (HK/G6P-DH) was from Megazyme (Wicklow, Ireland).

4.2.3 In vitro enzymatic assays

α-glucosidase activity was measured through kinetic end-point assays [41]. A mix consisting of 50 µL deionizated water, 0.35 µL of α-glucosidase (0.175 U/ml) and 1 µL of the properly diluted compound's stock solution in DMSO, was pre-incubated 20 min at 37 °C followed by the addition of 50 µL phosphate buffered saline (140 mM) pH 7.2, 1.35 µL reduced glutathione (9 mM) and 12 µL 4-nitrophenyl-α-glucopyranoside (pNPG) (0.9 mM) as substrate. The cocktail was further incubated 45 min at 37°C and the color development monitored at 400 nm on a Bio-Rad Benchmark Plus plate reader. The percent effect of the compound on the α-glucosidase enzymatic activity was calculated by the following formula: % Inhibition/Activation = [(AC - AS)/AC] ×100, where AC is the absorbance of the control (samples with DMSO alone) and AS is the absorbance of the tested sample. Each experiment was performed in triplicates, along with appropriate blanks.

The same protocol was followed for the experiments reported in Figs 3 and Supp. Fig. A, except that the substrate and the inhibitor concentrations were varied as indicated. When maltose was used as substrate (Fig. 4), at the end of the incubation with α-glucosidase,

the reaction was quenched by heating at 95 ^{o}C 3 min, and the liberated glucose measured following the assay kit instructions for the 96 well plate format.

β-glucuronidase activity was assayed by monitoring the quantity of phenolphthalein liberated by alkalinization of the reaction mix at the end of the incubation. Hyaluronidase was assayed by a nephelometric procedure. The enzyme supplier recommended protocols were followed in both cases.

4.2.4. Enzyme Inhibition Kinetic

To assess the inhibitory potency and the enzymatic kinetic, the compounds were tested at increasing doses ranging from 0.17 to 100 µM. The results were analyzed using Graph Pad Prism 5.0. (GraphPad Software, San Diego, CA) to calculate the concentration required to achieve 50% inhibition (IC_{50}). The enzyme inhibition modality was investigated in experiments using incremental concentrations (0.1 to 1.5 mM) of the substrate and by testing the compound at different concentrations as indicated. The Michaelis-Menten constant K_m and maximal velocity V_{max} were determined trough a nonlinear regression analysis using GraphPad. The inhibition constant (K_i) was determined according to Dixon [28] and Cornish–Bowden [29]. K_{ik} and K_{iv} rate constants were calculated according to Yang et al [30]. Linear regression analysis were performed with Excel.

Legends to the Tables and Figures

Figure 1: Reagents and Conditions. *i*: NEt_3, $NaBH_4/CH_3OH$. *ii*: $NaOH_{aq}$2M/MeOH (1/10). *iii*:TBDMSCl/Imidazole/DMF. *iv*: Pd/C (10%), EtOH; *v*: CH_3CN, base, $ArCH_2X$; and then *ii*.

Figure 2: Reagents and Conditions. *i:* ACE-Cl, Toluene then MeOH; *ii*: (a) $NaOH_{aq}$ 2M/ Toluene, reflux. (b) Ethereal saturated HCl. *iii:* (*for* **10a-10d)** $NaHCO_3$, EtOH, $ArCH_2X$. *iv:* $NaOH_{aq}$ 2M/MeOH (1/10); (for **10e-10f**) CH_3CN, Di-isopropylethylamine (DIPEA), $ArCH_2X$. *iv:* $NaOH_{aq}$ 2M/MeOH (1/10). *v*: ClCOOEt, Toluene, reflux. *vi*: $NaOH_{aq}$ 5M/ Toluene, 50°C. *vii*: LDA, THF, HMPA (mixture of 2-3, 3-4, 4-5 *ene*-isomers **13**). *viii*: HCl_{aq} (1N). *ix:* $SOCl_2$/MeOH. *x*: Flash chromatography. *xi*: $NaOH_{aq}$ (5eq), $(Bu^tOCOO)O_2$, Bu^tOH. *xii:* LDA, THF.[38, 40] *xiii*: MCPA/DCM. *xiv*: Methanolic KOH (0.2 M). *xv*: (a) TFA, DCM, (b) ACN, DIPEA, $ArCH_2X$. *xvi*: HCl_{aq} (1N).

Figure 3: Enzymatic kinetics as a function of substrate [S] and inhibitor [I] concentrations. *Panels A-D*: Dixon plot (1/V vs [I]); *Panels E-H*: Cornish-Bowden plot ([S]/V vs [I]). *Panels A,E*: compound 22b. *Panels B,F*: 22c. *Panels C,G*: 22d. *Panels D,H*: 22e. Plotted values at various [S] are marked as follows: ○ 0.1 mM; ● 0.3 mM; ◇ 0.5 mM; ◆ 0.75 mM; △ 1 mM; ▲ 1.5 mM. On the [S]/V vs [I] graph, the absolute [I] value at the intercept point of the regression lines, correspond to Ki. Each plotted point is the average of the results from three independent determinations.

Figure 4: Enzymatic kinetics for compound 22c using maltose as substrate. *Panels A*: Michaelis-Menten (V vs [S]); *Panel B*: Lineweaver-Burk plot (1/V vs 1/[S]); *Panel C*: Dixon plot (1/V vs [I]); *Panel D*: Cornish-Bowden plot ([S]/V vs [I]). In panels A,B the plotted values at various [I] are marked as follows: ▲ none; △ 1.2 uM; ◆ 1.6 uM; ◇ 2 uM; ● 2.4 uM; ○ 4 uM; ■ 8 uM. In panels C,D the plotted values at various [S] are marked as follows: ○ 0.1 mM; ● 0.3 mM; ◇ 0.5 mM; ◆ 0.75 mM; △ 1 mM; ▲ 1.5

mM. Each plotted point is the average of the results from three independent determinations.

Supp. Figure A: Enzymatic kinetics as a function of substrate [S] and compound [I] concentrations. *Panels A-D*: Michaelis-Menten plot (V vs [S]); *Panels E-H*: Lineweaver-Burk plot (1/V vs 1/[S]). *Panels A,E*: compound 22b. *Panels B,F*: 22c. *Panels C,G*: 22d. *Panels D,H*: 22e. In panels *A,E* the plotted values at various [I] are marked as follows: ▲ none; △ 2 uM; ◆ 4 uM; ◇ 6 uM; ● 8 uM; ○ 12 uM; ■ 20 uM. In panels *B,F*: ▲ none; △ 1.2 uM; ◆ 1.6 uM; ◇ 2 uM; ● 2.4 uM; ○ 4 uM; ■ 8 uM. In panels *C,D,G,H*: ▲ none; △ 3 uM; ◆ 6 uM; ◇ 9 uM; ● 12 uM; ○ 18 uM; ■ 30 uM. Each plotted point is the average of the results from three independent determinations.

Table 1: ***Effect of a series of compounds tested at 100 µM on a set of glycolytic enzymes.*** The compounds were added dissolved in DMSO not to exceed 1% of the total volume. The results were computed as percent of the enzymatic activity measured in the presence of DMSO alone. Details on the individual assays are given in *4.2.3*.

Table 2: ***Effect of compound 22c at different concentrations on V_{max}, K_m, and the K_{ik} to K_{iv} ratio using pNPG and maltose as substrates.*** V_{max} and K_m values were calculated according to Lineweaver-Burk from the data shown in Supp. Fig. A and Fig. 4. The K_{ik}/K_{iv} ratio was calculated according to Yang et al. [30]. Details on the individual assays are given in *4.2.3*.

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Table 1

Compound ID	*α-glucosidase* S.cerevisiae	*β-glucuronidase* Helix pomatia	*Hyaluronidase* Bovine testes
2	100	95	103
3,4	82	102	100
7a	95	103	100
7b	97	104	100
7c	85	108	100
7d	82	102	100
10a	97	89	100
10b	95	96	101
10c	102	102	99
10d	99	95	99
10e	97	100	102
10f	93	100	100
14	109	96	100
21a	92	93	99
21b	97	103	100
21c	105	102	99
21d	94	105	99
21e	95	97	100
22a	95	97	101
22b	**2**	103	101
22c	**2**	100	102
22d	**3**	103	104
22e	**3**	102	100

Table 2

Inhibitor (22c)	*Substrate*					
	pNPG			*maltose*		
[I] uM	*Vmax*	*Km*	*Kik/Kiv*	*Vmax*	*Km*	*Kik/Kiv*
0	2.333	1.226	//	2.262	1.85	//
1.2	1.821	0.95	1.031	1.429	1.19	1.051
1.6	1.575	0.8	1.089	1.141	0.915	1.039
2	1.419	0.745	0.998	0.976	0.837	1.089
2.4	1.136	0.664	1.245	0.786	0.671	1.069
4	0.837	0.558	1.494	0.567	0.53	1.201
8	0.455	0.36	1.718	0.315	0.356	1.473

Figure 1

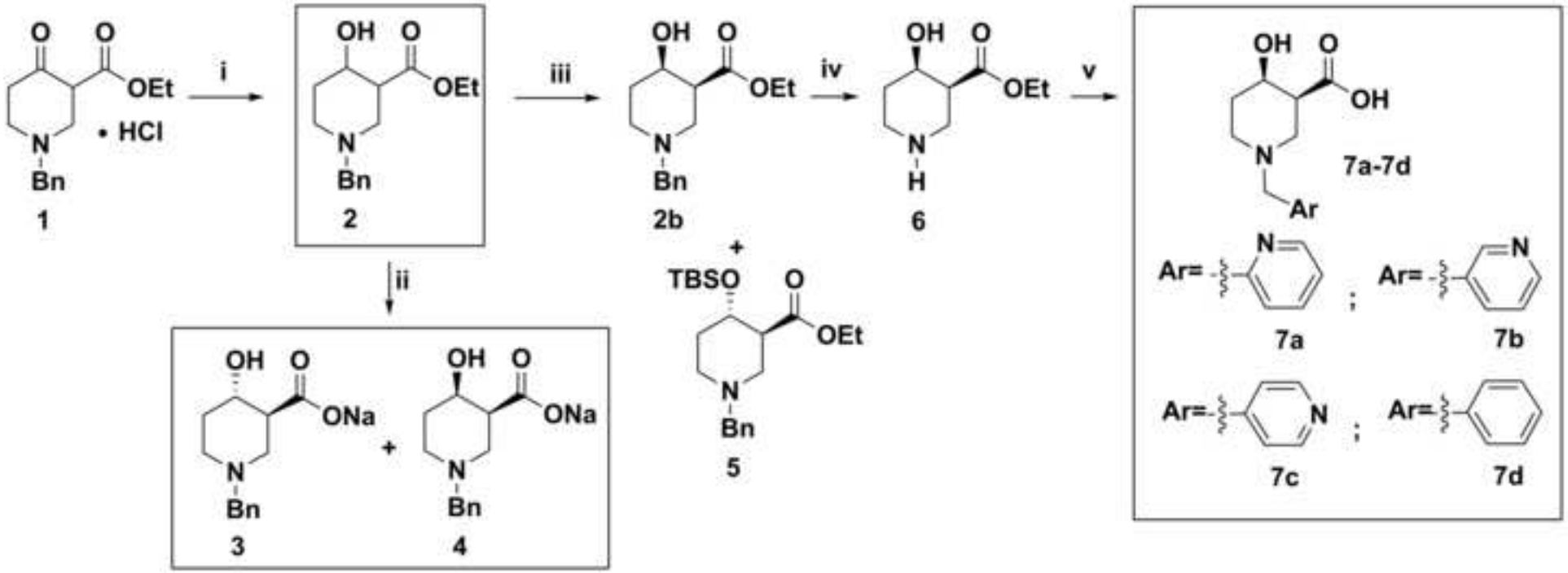

Figure 2

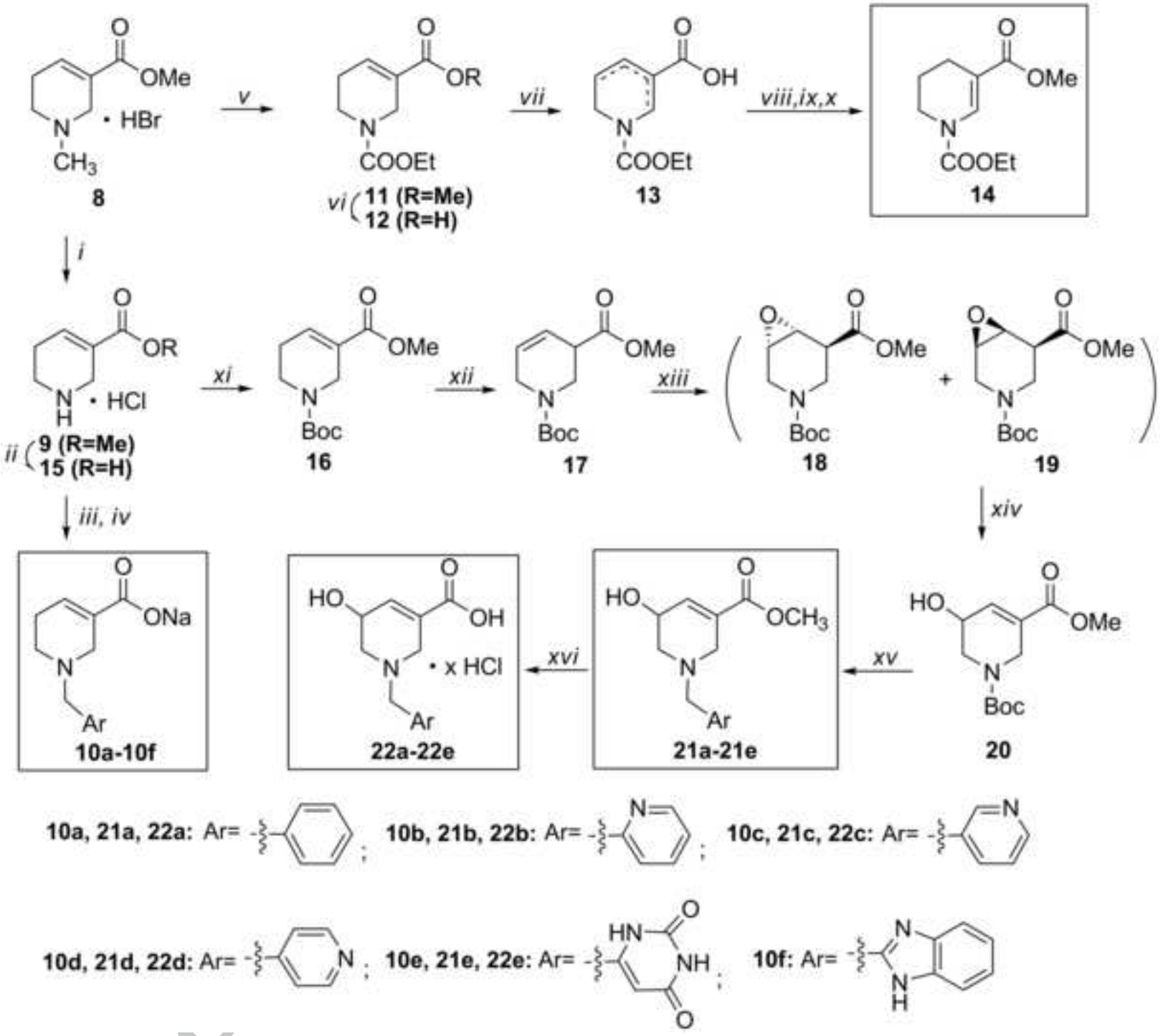

Figure 3

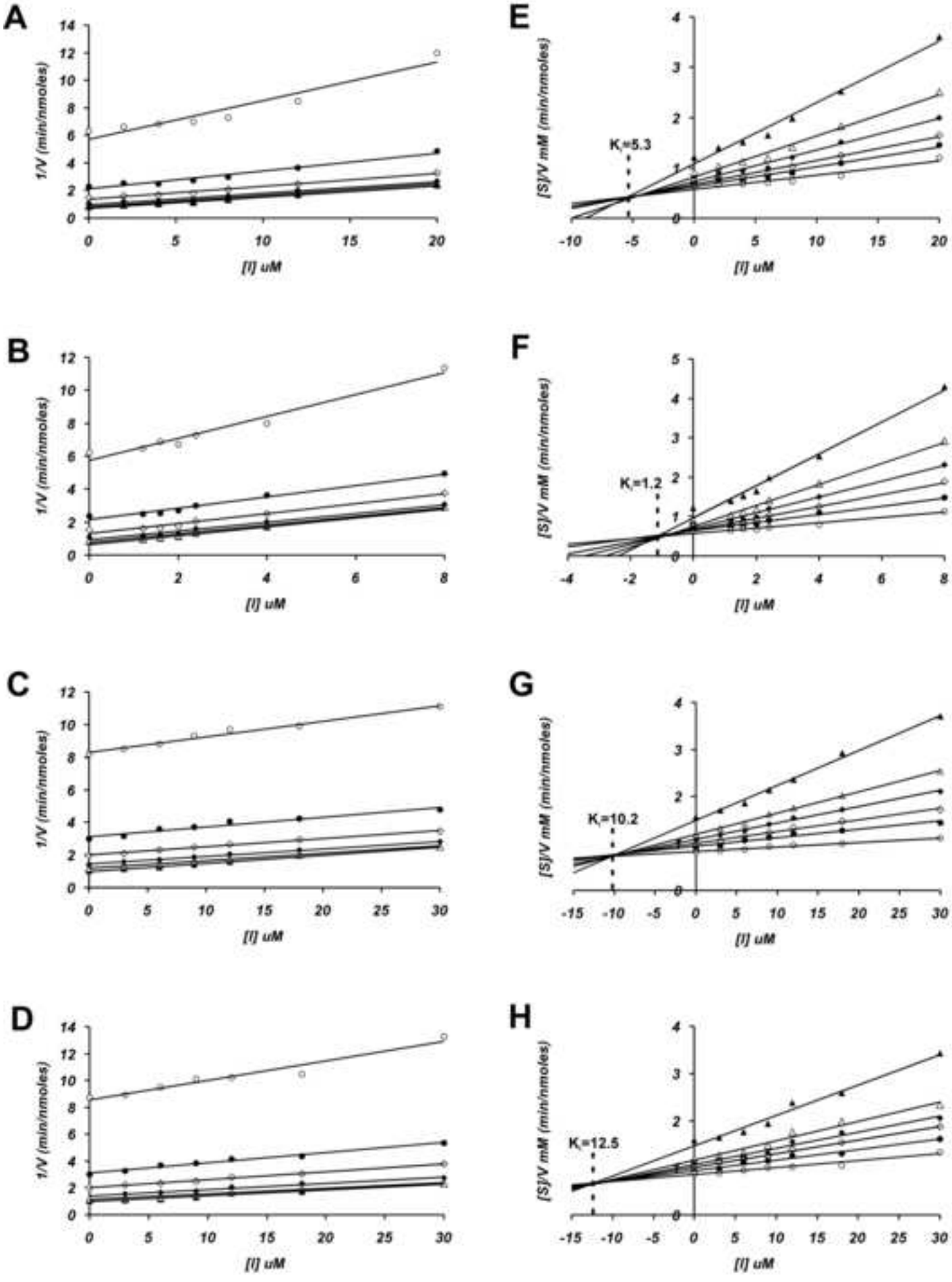

Figure 4