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Original article

Synthesis, biological assay *in vitro* and molecular docking studies of new imidazopyrazinone derivatives as potential dipeptidyl peptidase IV inhibitors

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ABSTRACT

A series of novel imidazopyrazinone derivatives were synthesized and evaluated with regard to their ability to inhibit dipeptidyl peptidase IV (DPP-IV) *in vitro*. Of these compounds (2*R*)-4-oxo-4-[2-(3-carbamoylbenzyl)-hexahydro-3-oxoimidazo [1,5-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid (**17h**, $IC_{50} = 78$ nM) was shown to effectively inhibit the activity of the dipeptidyl peptidase IV enzyme. Molecular docking studies were also performed to illustrate the binding mode of compounds **15c** and **17h**. Favorable interactions were identified from the binding of inhibitor **15c** with DPP-IV. By analogy to the binding mode of compound **15c**, it seems that the introduction of a substituted benzyl moiety onto the imidazopyrazinone could remarkably improve the inhibitory activity of compound **17h**.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a severe and increasingly prevalent disease. According to the IDF Diabetes Atlas, the estimated number for 2010 is about 285 million [1]. T2DM is the most prevalent abnormality related to glucose homeostasis. Individuals with T2DM have a relative insulin deficiency due to insulin resistance or abnormalities. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are two important incretin hormones released from the intestine in response to nutrient ingestion. Both peptides stimulate insulin secretion in a strictly glucose-dependent manner [2]. In addition, GLP-1 also suppresses the secretion of glucagon, slows gastric emptying, reduces food intake, and stimulates the regeneration and differentiation of islet β-cells [2,3]. Unfortunately, GLP-1 can be deactivated rapidly (<2 min) by plasma DPP-IV via the cleavage of two amino acids from its N-terminus [4]. The inhibition of DPP-IV leads to increased levels of endogenous GLP-1 and GIP [2]. Therefore, DPP-IV has become a validated target for the treatment of type 2 diabetes, and several inhibitors of DPP-IV are currently launched in certain countries or undergoing late-stage clinical trials [5].

In the past few years, we have endeavored to synthesize novel dipeptide surrogates containing a piperazine mimic. These dipeptide analogues have exhibited strong inhibition of DPP-IV. These observations prompted us to explore the new DPP-IV inhibitors that contain our basic piperazine structure. Herein, we describe the synthesis, *in vitro* and molecular docking simulations of several novel imidazopyrazinone compounds for used as potential DPP-IV inhibitors.

2. Synthesis

The imidazopyrazinone analogues described in this report as DPP-IV inhibitors were synthesized using standard peptide couplings of β-amino acids with fused heterocycles [6].

The Merck researchers provided two excellent routes to synthesis β-amino acid **7** [7,8], in this report, another flexible route without crystallization was described, which could conveniently apply to lab investigation. Our initial synthetic efforts were focused on producing the β-amino acids (Scheme 1). 2,4,5-Trifluorobenzoic acid was converted to acetyl chloride, then the mono-anion of bis [trimethylsilyl] malonate was synthesized from bis[trimethylsilyl] malonate and *n*-BuLi, and added to acetyl chloride to produce β-keto acid **1** according to a known method [9]. The enantioselective reduction of **1** to β-hydroxy acid **2** was carried out using (+)-diisopinocampheylchloroborane ((+)-DIP-Cl) in 64% yield and 92% ee.

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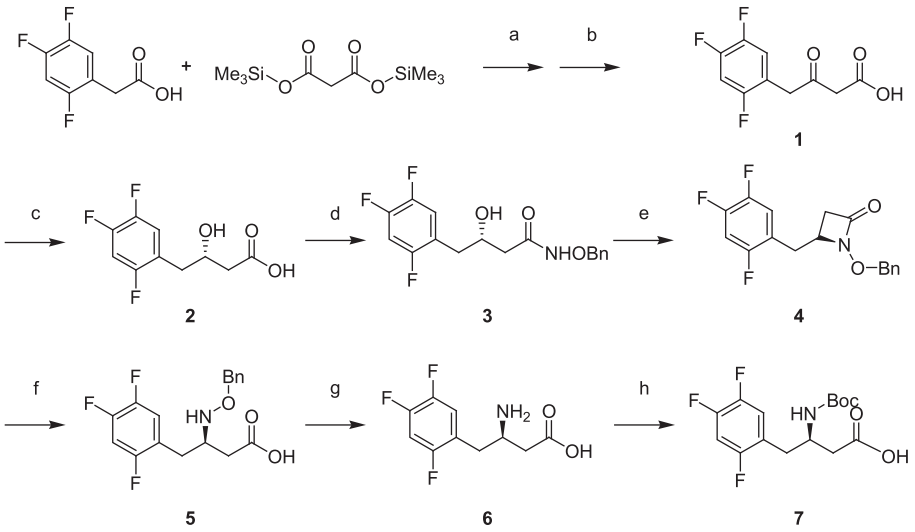
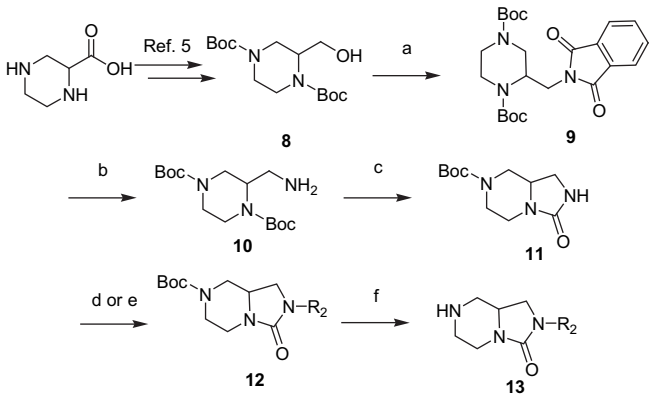


Scheme 1. Synthesis of β-amino acid **7**. (a) ClCOCOCl, DMF, CH_2Cl_2, rt; (b) *n*-BuLi, THF, −60 °C, 40%; (c) (+)-DIP-Cl, TEA, CH_2Cl_2, 0 °C, 64%; (d) $BnONH_2$, EDC, HOBT, THF, rt, 60%; (e) PPh_3, DIAD, THF, rt, 72%; (f) LiOH, H_2O, THF, MeOH, 0 °C, 93%; (g) 10% Pd/C, H_2, MeOH, rt, 92%; (h) Boc_2O, TEA, H_2O, THF, rt, 91%.

Compound **3** was then prepared from β-hydroxy acid **2** and *O*-benzyl hydroxylamine using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimidehydrochloride (EDC) and *N*-hydroxybenzotrizole (HOBT) as coupling reagents. Ring closure of **3** to β-lactam **4** was achieved using a Mitsunobu reaction [7]. Subsequently, ring opening of β-lactam **4** to key intermediate **5** was carried out with LiOH in a mixed solvent system of THF/H_2O [10]. The desired β-amino acid **7** was obtained by catalytic hydrogenation (Pd/C) and reaction with Boc_2O.

Various heterocycles **13** were synthesized via the routes depicted in Scheme 2. Compound **8** was easily accessible in two steps starting from piperazine-2-carboxylic acid through known procedure. [11] Subsequently, the conversion of **8** to primary amine **9** was completed under a standard Mitsunobu reaction condition, followed by ammonolysis in refluxing methylamine ethanol solution. The key intermediate **11** was successfully obtained in excellent yield by refluxing **10** in THF with NaH. The Buchwald–Hartwig reactions of **11** with aryl bromides proceeded in the conditions of [1,1′-bis (diphenylphosphino)ferrocene] dichloropalladium [$PdCl_2$(dppf)]/NaO-*t*-Bu/toluene. The intermolecular amidation of alkyl or benzyl halides with lactams **11** were carried out successfully in the presence



Scheme 2. Synthesis of heterocycles **13**. (a) PPh_3, DIAD, THF, 93%; (b) $MeNH_2$, EtOH, 77%; (c) NaH, THF, reflux, 90%; (d) aryl bromides, $PdCl_2$(dppf), NaO-*t*-Bu, toluene,15–98%; (e) benzylhalides, NaH, THF, reflux, 48–90%; (d) HCl/Et_2O, DCM, 71–99%.

of NaH in THF. Finally, after the removal of Boc-protection group of **12** in HCl/Et_2O solution obtained target free amines **13**.

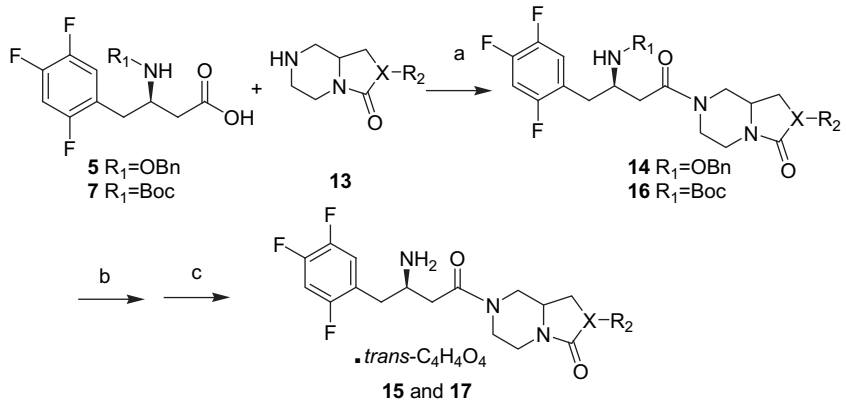
Synthetic efforts then focused on coupling β-amino acids **5** and **7** with heterocycles of type **13** to provide compounds **14** and **16** (Scheme 3). The desired compounds **15a**–**e** and **17a**–**o** (Table 1) were obtained after deprotection of the amine. Analogue **17h** was synthesized from compound **16h** by oxidation with DMSO and H_2O_2 and then deprotection of the amine (Scheme 4). For *in vitro* evaluation, the HCl salts of compounds **15** and **17** were converted to their fumaric acid salts [6].

3. Results and discussion

3.1. Inhibitory activity against DPP-IV in vitro

The derivatives **15a**–**e** and **17a**–**o** were evaluated for their inhibition of DPP-IV *in vitro* [12]. (Table 1) Soluble DPP-IV was purified from human seminal fluid as described in reference [13, 14], and sitagliptin was used as a reference compound.

As a first-in-class drug, sitagliptin (IC_{50} = 18 nM) shows excellent selectivity and oral bioavailability. To study the bioisosteres of sitagliptin, the triazolopiperazine subunit of sitagliptin was replaced by an oxazolopyrazinone or imidazopyrazinone group. Table 1 lists data for the initial series of DPP-IV inhibitors containing oxazolopyrazinone, imidazopyrazinone, as well as 2-alkyl or 2-benzyl substituted imidazopyrazinone moieties. The unsubstituted imidazopyrazinone analogue **15b** (IC_{50} = 1060 nM) showed micromolar activity and was 2-fold more active than the oxazolopyrazinone derivative **15a**. Introducing a methyl group at the 2 position of the imidazopyrazinone (**15c**) led to about a 12-fold decrease in potency. However, the 2-ethyl-imidazopyrazinone derivative **15d** (IC_{50} = 520 nM) exhibited about a 2-fold increase in potency over **15b**, while the 2-benzyl analogue **15e** (IC_{50} = 300 nM) showed significant improvement in activity (about 4-fold). Further investigations involved introducing various substitutions onto the benzyl group. Unfortunately, the 4-fluorobenzyl analogue **17a** (IC_{50} = 1236 nM) was less active than **10e** (4-fold), and the 4-methylbenzyl and 4-chlorobenzyl derivatives (**17b**–**c**) lost their potency completely. Interestingly, the 4-methoxylbenzyl derivative **17d** (IC_{50} = 360 nM) exhibited a potency similar to that of compound **15e**. To investigate the effect that the position and

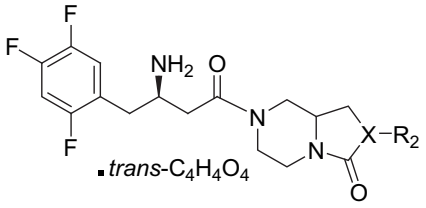


Scheme 3. Synthesis of **15** and **17**. (a) EDC, HOBT, CH_2Cl_2, rt, 30–98%; (b) 10% Pd/C, H_2, MeOH, rt; or TFA, CH_2Cl_2, rt; (c) EtOH, Et_2O, fumaric acid, rt, 45–98%.

quantity of methoxy groups had on the potency of inhibition, compounds **17e–g** were prepared. The 2-methoxybenzyl, 3-methoxybenzyl and 3,5-dimethoxybenzyl analogues displayed about a 3-fold increase *in vitro* activity compared with compound **15e**. It is interesting that the 3-$CONH_2$- analogue **17h** ($IC_{50} = 78$ nM) showed the most potent activity and is comparable to the approved drug Sitagliptin ($IC_{50} = 18$ nM).

We then turned our attention to introducing various 2-aryl functional groups onto the imidazopyrazinone. Table 1 shows the enzyme assay data for derivatives **17i–o**, but unfortunately these analogs lost *in vitro* activity.

Table 1
In vitro activity against DPP-IV.[a]



Entry	X	R_2	Percent inhibition (at 10 μM)	IC_{50} (nM)
15a	O	–	93.5	2300
15b	N	H	97.1	1060
15c	N	Me	87.0	1240
15d	N	Et	95.4	460
15e	N	Bn	96.5	300
17a	N	4-$FPhCH_2$	95.2	1236
17b	N	4-$MePhCH_2$	−0.8	NT[b]
17c	N	4-$ClPhCH_2$	0.6	NT
17d	N	4-$MeOPhCH_2$	98.7	360
17e	N	2- $MeOPhCH_2$	99.6	101
17f	N	3- $MeOPhCH_2$	98.9	124
17g	N	3,5-Di-$MeOPhCH_2$	98.9	114
17h	N	3-$CONH_2PhCH_2$	98.9	78
17i	N	Pyridin-2-yl	1.5	NT
17j	N	4-CF_3Ph	2.5	NT
17k	N	2,4,5-Tri-FPh	1.3	NT
17l	N	4-FPh	1.6	NT
17m	N	4-ClPh	−0.6	NT
17n	N	4-CNPh	−0.8	NT
17o	N	4-$MeSO_2Ph$	2.2	NT
Sitagliptin				11

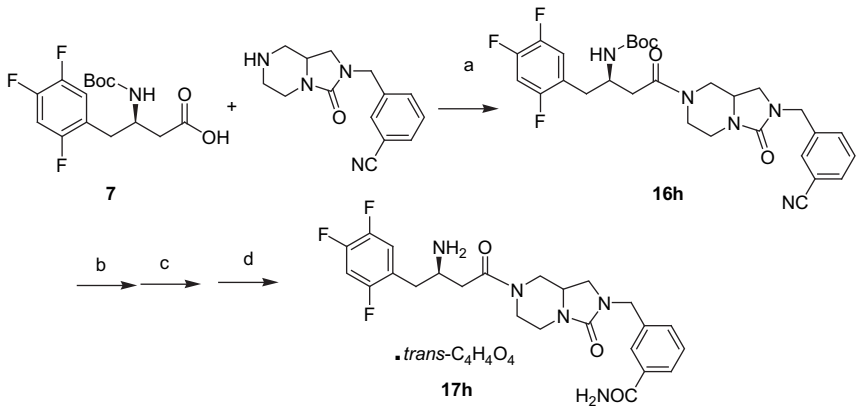
[a] The percent inhibition and IC_{50} results are an average of three independent titrations, unless otherwise noted, having calculated standard errors below 15%.
[b] NT = not tested.

3.2. Molecular docking studies

With *In vitro* activity against DPP-IV enzyme in hand, it is thought worth-while to do molecular docking studies to support the potency of inhibition. Docking studies were performed to illustrate the binding mode of these compounds. The X-ray structure of DPP-IV was downloaded from the Protein Data Bank (PDB code: 1N1M) [6, 14] and was modified by adding hydrogen atoms and removing water and cocrystallized inhibitors using sybyl 7.0 [15]. Compounds with weak activity (**15c**) and potent activity (**17h**) were built and minimized within sibyl 7.0. Then, all of the inhibitors' stereo isomers were docked into the active site pocket of DPP-IV by using GOLD version 3.2 with the ChemScore function used to evaluate the docking results [16]. All atoms within 10 Å of the ligand in the 1N1M structure were considered in the docking study. The docking results are depicted in Fig. 1.

It can be seen from Fig. 1 that all of the isomeric structures can interact with DPP-IV's binding pocket. Many interactions are evident in the binding of **15c** with DPP-IV. The 2,4,5-trifluorophenyl moiety fully occupies the S_1 hydrophobic pocket and forms a π–π interaction with the side chain of Tyr662. The (3*R*)-β-amino group forms an N-H···O hydrogen bond with the side chain of Glu205. The carbonyl group interacts with the side chain of Tyr547 via an O–H···O hydrogen bond. The imidazopyrazinone portion of the molecule could be experiencing a hydrophobic interaction with the phenyl group of Phe357, but no clear interaction could be identified in this portion of the inhibitor, so it may serve as an area of the structure that can be further modified for increased activity.

In comparison to compound **15c**, the introduction of a substituted benzyl moiety to the fused piperazine should remarkably improve the inhibitory activity, as demonstrated by compound **17h**. It is clear from the docking results that both isomeric structures of **17h** have favorable interactions with the active site of DPP-IV. The 2,4,5-trifluorophenyl moiety fully occupies the S_1 hydrophobic pocket and interacts with the side chain of Tyr662 via a π–π interaction. The (3*R*)-β-amino group can form two N–H···O hydrogen bonds with the side chains of Glu205 and Tyr662. The carbonyl group of the imidazopyrazinone moiety can interact with the side chain of Ser209 via an O–H···O hydrogen bond. A weak C–H···O hydrogen bonding interaction could also be found for both isomeric structures between the phenyl ring and the phenol group of Tyr585. In addition to the above interactions shared by both isomers, the 8*aR isomer* enjoys another O–H···O hydrogen bonding interaction to the phenol of Tyr547.



Scheme 4. Synthesis of **17h**. (a) EDC, HOBT, CH_2Cl_2, rt, 95%; (b) DMSO, 30%H_2O_2, CH_2Cl_2, 0 °C; (c) TFA, CH_2Cl_2, rt; (d) EtOH, Et_2O, fumaric acid, rt, 67%.

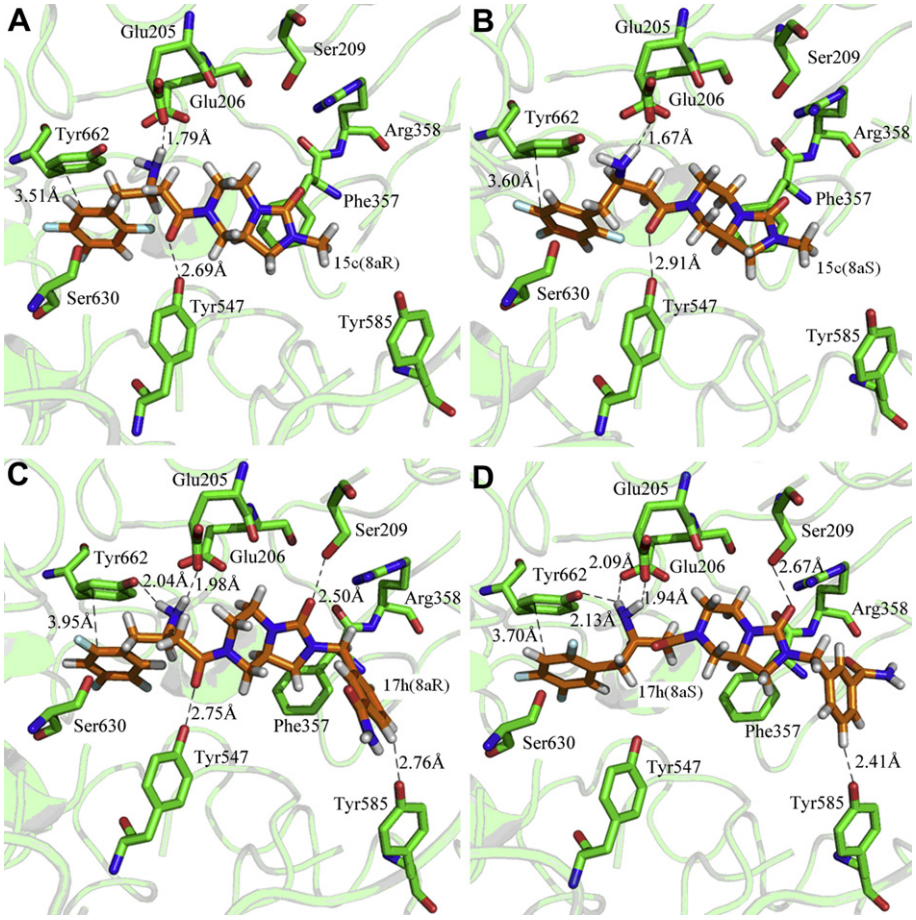


Fig. 1. Docking results obtained by combining compounds **15c** and **17h** with DPP-IV enzyme. (A) The interactions of compound **15c** (8*aR*) with the key residues of DPP-IV; (B) the interactions of compound **15c** (8*aS*) with the key residues of DPP-IV; (C) the interactions of compound **17h** (8*aR*) with the key residues of DPP-IV; (D) the interactions of compound **17h** (8*aS*) with the key residues of DPP-IV. The figures were prepared using PyMol (www.pymol.org).

4. Conclusion

In summary, we have synthesized a series of novel 2-alkyl, 2-benzyl, and substituted 2-alkyl imidazopyrazinone derivatives. Some of the target compounds, such as compounds **15e** ($IC_{50} = 300$ nM) and **17h** ($IC_{50} = 78$ nM), showed potent *in vitro* activity against DPP-IV. Molecular docking studies showed good binding of these compounds to the active site of DPP-IV. Further investigations on the optimization of these two leading compounds and individual epimers of compound **17h**, are being carried out in our laboratory.

5. Experimental

5.1. General

Tetrahydrofuran was dried over sodium. DMF was dried over calcium hydride. Other chemicals and solvents were purchased from commercial suppliers and used without further purification. Melting points were recorded on a B-540 melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400 MHz spectrometer. Chemical shifts are

given in ppm relative to tetramethylsilane (TMS) as an internal standard (0 ppm). Mass spectra were (LRMS and HRMS) recorded on a Finnigan MAT 95 mass spectrometer.

5.2. 4-(2,4,5-Trifluorophenyl)-3-oxobutanoic acid **1**

To a solution of 2-(2,4,5-trifluorophenyl)acetic acid (10.0 g, 53.0 mmol) in CH_2Cl_2 (200 mL) was added DMF (1.0 mL) and $(COCl)_2$ (8.4 mL, 106.0 mmol). After stirring for 2 h, the solution was concentrated *in vacuo*. Separately, a solution of *n*-BuLi (40.0 mL, 100 mmol) was slowly added at −60 °C under an N_2 atmosphere to a solution of bis[trimethylsilyl] malonate (25.0 g, 100 mmol) in THF (50 mL). After being stirred for 10 min, a solution of acetyl chloride in THF (30 mL) was poured into the mixture at 0 °C. The reaction mixture was quenched with 5% aqueous $NaHCO_3$, acidified to pH = 5 with 2 N HCl, and then extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered, and concentrated to give the title compound (4.9 g, 40%) as a yellow solid: mp 65–67 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.43 (s, 4H), 6.91–6.98 (m, 1H), 7.18–7.26 (m, 1H).

5.3. 4-(2,4,5-Trifluorophenyl)-3(S)-hydroxybutanoic acid **2**

Compound **1** (2.0 g, 8.6 mmol) and Et_3N (870 mg, 8.6 mmol) were dissolved in CH_2Cl_2 (20 mL) at −60 °C under an N_2 atmosphere. After being stirred for 5 min, (+)-DIP-Cl (3.3 g, 10.0 mmol) was added. The mixture was stirred at 0 °C for 2 h under an N_2 atmosphere and quenched with H_2O. The pH of the reaction mixture was adjusted to 10 with 2 N NaOH, and the aqueous phase was extracted with methyl-*tert*-butyl ether. The aqueous phase was then acidified to pH = 5 with 2 N HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered, and concentrated to give the title compound (1.3 g, 64%) in 92% ee (Chiracel AD-H, 95% hexanes, 5% IPA, 1% TFA, 1 mL/min, 35 °C, t_r = 20.5 min (S-**2**), 22.8 (R-**2**)). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.48–2.62 (m, 2H), 2.79 (d, J = 6.0 Hz, 2H), 4.24–4.29 (m, 1H), 6.91 (s, 1H), 7.18–7.30 (m, 1H).

5.4. N-benzyloxy-4-(2,4,5-trifluorophenyl)-3(S)-hydroxybutanamide **3**

To a solution of **2** (0.8 g, 3.6 mmol) in THF (20 mL) was added *O*-benzylhydroxyamine hydrochloride (1.4 g, 11.5 mmol), K_2CO_3 (2.1 g, 15.3 mmol) and HOBT (10 mg, 0.1 mmol), followed by EDC (0.8 g, 4.0 mmol) added slowly over 15 min at 20–30 °C. The resulting mixture was stirred for 3 h at 20–22 °C, quenched with H_2O, diluted with CH_2Cl_2, washed with 2 N HCl and brine, dried, filtered, concentrated and recrystallized from CH_2Cl_2 and hexane to yield the title compound (0.7 g, 60%) as a white solid: mp 99–105 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.18–2.76 (m, 5H), 4.18–4.20 (m, 1H), 4.78–4.91 (m, 2H), 6.87–6.93 (m, 1H), 7.06–7.12 (m, 1H), 7.37–7.44 (m, 5H).

5.5. N-benzyloxy-4(R)-[1-methyl-(2,4,5-trifluorobenzyl)]-2-oxoazetidine **4**

Triphenylphosphine (530 mg, 2.0 mmol) was added to a solution of compound **3** (530 mg, 1.6 mmol) in THF (30 mL) at 10 °C. DIAD (404 mg, 2.0 mmol) was then added slowly to the reaction mixture at a rate that maintained the internal reaction temperature at <10 °C. The reaction was stirred at 10 °C for additional 2 h. After the reaction was complete, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel chromatography to give the title compound (400 mg, 72%) as a white solid: mp 89–91 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.33 (m, 1H), 2.67 (m, 2H), 2.86 (m, 1H), 3.50–3.62 (m, 1H), 4.92 (m, 2H), 6.87–6.93 (m, 2H), 7.33–7.40 (m, 5H).

5.6. N-benzyloxy-4-(2,4,5-trifluorophenyl)-3(R)-aminobutanoic acid **5**

$LiOH \cdot H_2O$ (0.5 g, 12 mmol) was added to a solution of **4** (3.2 g, 10 mmol) in THF/H_2O (1:1, 20 mL) at 0 °C. After the reaction was complete, the pH was adjusted to 10 with 2 N NaOH and the aqueous phase was extracted with methyl-*tert*-butyl ether. The aqueous phase was then acidified to pH = 5 with 2 N HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered, and concentrated to give the title compound (3.1 g, 93%) as a white solid: mp 109–111 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39–2.87 (m, 4H), 4.48 (m, 1H), 4.68 (m, 2H), 4.90–4.98 (m, 1H), 6.82–7.07 (m, 2H), 7.26–7.35 (m, 5H).

5.7. 4-(2,4,5-Trifluorophenyl)-3(R)-aminobutanoic acid **6**

10% Pd/C (1.6 g) was added to a solution of **5** (2.6 g, 7.7 mmol) in MeOH (50 mL), and then the mixture was submitted to an atmosphere of hydrogen gas (40 psi H_2) for 12 h. The catalyst was filtered off and the solvent was evaporated. The crude material was recrystallized from CH_2Cl_2 and hexane to yield compound **6** (2.1 g, 92%) as a white solid: mp 169–178 °C; ^{1}H NMR (CF_3COOD, 400 MHz) δ 1.23 (dd, J_1 = 12.3 Hz, J_2 = 1.0 Hz, 2H), 2.88–2.95 (m, 2H), 3.04–3.18 (m, 2H), 4.02–4.17 (m, 1H), 6.96 (m, 1H), 7.03 (m, 1H); MS (ESI) m/z: 234 [M + 1].

5.8. N-Boc-4-(2,4,5-trifluorophenyl)-3(R)-aminobutanoic acid **7**

To a solution of **6** (2.1 g, 7.1 mmol) in 1,4-dioxane/H_2O (1:1, 40 mL) was added Et_3N (5 mL) and Boc_2O (2.0 g, 9.2 mmol). After the reaction was complete, the reaction mixture was acidified to pH = 5 with 2 N HCl, and extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered, and concentrated to give the title compound (2.4 g, 91%) as a white solid: mp 114–115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34–1.42 (m, 9H), 2.60 (m, 2H), 2.88 (d, J = 13.6 Hz, 2H), 4.10–4.18 (m, 1H), 6.86–6.94 (m, 1H), 7.01–7.10 (m, 1H).

5.9. Di-tert-butyl 2-((1,3-dioxoisoindolin-2-yl)methyl)-piperazine-1,4-dicarboxylate **9**

Compound **8** was prepared following the literature procedure. [13] To the solution of **8** (15.0 g, 47.5 mmol) in anhydrous THF (250 mL) at 10 °C was added PPh_3 (16.2 g, 61.8 mmol) and phthalimide (9.1 g, 61.8 mmol). DIAD (12.0 mL, 61.8 mmol) was then added slowly to the reaction mixture at 10 °C. The reaction was stirred at 10 °C for additional 2 h. After the reaction was completed, the reaction mixture was treated with 1 N HCl (100 mL) and then extracted with ethyl acetate. The extract was washed with 5% aqueous $NaHCO_3$ (100 mL), dried and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give the title compound (19.9 g, 93%) as a white solid. mp 95–96 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.02 (m, 9H), 1.44 (m, 9H), 2.73–2.84 (m, 1H), 2.93–3.01 (m, 1H), 3.28–3.40 (m, 1H), 3.55 (m, 1H), 3.65–4.15 (m, 4H), 4.48–4.66 (m, 1H), 7.67–7.84 (m, 4H).

5.10. Di-tert-butyl 2-(aminomethyl)piperazine-1,4-dicarboxylate (**10**)

The solution of **9** (14.1 g, 31.7 mmol) in EtOH (100 mL) was treated with methylamine alcohol solution (32%, 100 mL) and heated at reflux for 4 h, upon cooling to room temperature and concentrated. The resulting residue was dissolved in H_2O (50 mL),

acidified to pH = 3 with 2 N HCl and extracted with methyl-*tert*-butyl ether. The pH was adjusted to 10 with 2*N* NaOH, and the mixture was extracted with ethyl acetate. The extract was dried and filtered, the solvent was removed under reduced pressure to give the title compound (7.7 g, 77%) as a white solid. Mp 92–93 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 18H), 2.73–2.82 (m, 5H), 2.89–2.95 (m, 4H), 3.82–4.16 (m, 3H); MS (ESI) m/z: 316.2 $(M+H)^+$.

5.11. *tert*-Butyl-hexahydro-3-oxoimidazo[1,5-a]pyrazine-7(1H)-carboxylate (**11**)

The solution of **10** (7.7 g, 24.2 mmol) in anhydrous THF (50 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 0.7 g, 48.4 mmol) in THF (50 mL) at 0 °C. The reaction mixture was heated at reflux for 3 h. Upon cooling to room temperature, the mixture was poured into ice H_2O (50 mL), and extracted with ethyl acetate. The extracts were washed with H_2O and brine, dried, filtered, and concentrated to give the desired product (5.3 g, 90%). mp 212–213 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 2.68–2.87 (m, 3H), 3.03 (m, 1H), 3.53 (t, J = 12.0 Hz, 1H), 3.62–3.71 (m, 1H), 3.76 (d, J = 12.8 Hz, 1H), 3.98 (m, 2H), 5.18–5.36 (m, 1H); MS (ESI) m/z: 264.2 $(M+Na)^+$.

5.12. General procedure for the synthesis of compound **12**

Method A: the compound **11** (480 mg, 2.0 mmol) was dissolved in toluene (20 mL) under nitrogen. Then aryl bromides (4.0 mmol), NaO-*t*-Bu (384 mg, 4.0 mmol) and $PdCl_2$(dppf) (10 mg, 0.01 mmol) were added to the mixture and refluxing for 12–48 h. Upon cooling, the mixture was concentrated and the residue was dissolved in H_2O (30 mL), extracted with ethyl acetate. The extract was washed with brine, dried, filtered, and concentrated. The residue was purified by column chromatography to give the compounds **12**.

Method B: benzylhalide (2.0 mmol) was added to the solution of **11** (241 mg, 1.0 mmol) in anhydrous THF (10 mL). Then the mixed solution was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 4.0 mmol) in THF (20 mL) at 0 °C. The mixture was refluxing for 3–8 h. Upon cooling, the mixture was poured into ice H_2O (50 mL), extracted with ethyl acetate. The combined extracts were washed with H_2O and brine, dried, filtered, and concentrated to give the desired products **12** as yellow solids.

5.13. General procedure for the synthesis of compound **13**

To a solution of **12** (1.0 mmol) in CH_2Cl_2 (15 mL) was added HCl/Et_2O solution (1.7 mL, 4.0 N, 6.8 mmol). After being stirred overnight, the precipitated solids were filtered and washed with Et_2O to give the desired compounds.

5.13.1. Hexahydro-2-(4-trifluoromethylphenyl)-imidazo[1,5-a]pyrazin-3-one

Mp 233–235 °C; ^{1}H NMR (CF_3COOD, 400 MHz) δ 3.29–3.36 (m, 2H), 3.54–3.72 (m, 2H), 3.76 (m, 1H), 3.86 (m, 1H), 4.11–4.29 (m, 2H), 4.48 (m, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 37.3, 42.3, 45.7, 47.1, 50.3, 117.2, 126.3, 126.4, 143.9, 155.3, 160.3; HRMS (ESI): $C_{13}H_{14}N_3OF_3Na$, calc. 308.0987; found 308.0992.

5.13.2. Hexahydro-2-benzyl-imidazo[1,5-a]pyrazin-3-one

Mp 214–215 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.74 (t, J = 8.0 Hz, 1H), 2.82 (dt, J_2 = 9.6 Hz, J_2 = 4.0 Hz, 1H), 2.91 (dd, J_2 = 12.4 Hz, J_2 = 4.8 Hz, 1H), 3.16–3.30 (m, 4H), 3.78 (dd, J_1 = 14.8 Hz, J_2 = 3.8 Hz, 1H), 3.92–3.97 (m, 1H), 4.30 (t, J = 17.2 Hz, 2H), 7.22–7.27 (m, 3H), 7.33–7.37 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 37.4, 42.2, 44.9, 45.4, 47.3, 47.9, 127.7, 128.1, 129.0, 137.6, 158.8; HRMS (ESI): $C_{13}H_{17}N_3ONa$, calc. 254.1269; found 254.1277.

5.14. General acylation procedure for compounds **14a–e** and **16a–o**

To a solution of β-amino acid **5** or **7** (1.0 mmol) in CH_2Cl_2 (5 mL), fused heterocycles **13** (1.2 mmol), EDC (1.2 mmol), HOBT (0.1 mmol) and Et_3N (3.0 mmol) were added. The reaction mixture was stirred for 12 h at room temperature, quenched with H_2O, diluted with CH_2Cl_2, washed with 2*N* HCl, 2*N* NaOH and brine, and dried over with anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography to give the desire compound.

5.14.1. (3R)-3-(Benzyloxyamino)-1-[hexahydro-3-oxo-oxazolo[1,5-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one **14a**

Yield: 56%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.26–3.31 (m, 1H), 2.41 (m, 1H), 2.59–2.66 (m, 2H), 2.79–3.05 (m, 4H), 3.52–3.72 (m, 2H), 3.77–3.97 (m, 2H), 4.40 (m, 1H), 4.76–5.30 (m, 3H), 6.85–6.93 (m, 1H), 6.99–7.08 (m, 1H), 7.22–7.36 (m, 5H).

5.14.2. (3R)-3-(Benzyloxyamino)-1-[hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one **14b**

Yield: 100%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.17–2.31 (m, 1H), 2.48–2.65 (m, 2H), 2.76–3.08 (m, 5H), 3.51–3.85 (m, 5H), 4.60–4.71 (m, 4H), 6.85–6.93 (m, 1H), 7.05–7.26 (m, 1H), 7.30–7.36 (m, 5H).

5.14.3. (3R)-3-(Benzyloxyamino)-1-[2-methyl-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one **14c**

Yield: 30%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.52–2.69 (m, 3H), 2.73–2.84 (m, 3H), 2.83–2.88 (m, 3H), 2.96 (m, 2H), 3.47–3.55 (m, 4H), 3.92 (m, 2H), 4.65–4.76 (m, 2H), 6.88 (m, 1H), 7.26 (m, 1H), 7.34 (m, 5H); MS (ESI) m/z 476 [M + 1].

5.14.4. (3R)-3-(Benzyloxyamino)-1-[2-ethyl-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one **14d**

Yield: 100%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09–1.13 (m, 3H), 2.13–2.31 (m, 2H), 2.58–2.64 (m, 2H), 2.80–3.01 (m, 5H), 3.24–3.30 (m, 2H), 3.42–3.63 (m, 4H), 3.84 (m, 1H), 4.64 (m, 3H), 6.89 (m, 1H), 7.07 (m, 1H), 7.28–7.34 (m, 5H).

5.14.5. (3R)-3-(Benzyloxyamino)-1-[2-benzyl-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one **14e**

Yield: 97%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.04–2.14 (m, 2H), 2.52–2.78 (m, 2H), 2.78–2.87 (m, 4H), 2.99–3.32 (m, 1H), 3.47–3.52 (m, 1H), 3.53–3.65 (m, 1H), 3.73 (m, 1H), 3.89 (m, 1H), 4.37 (m, 2H), 4.38–4.54 (m, 1H), 4.79 (m, 2H), 6.89 (m, 1H), 7.08 (m, 1H), 7.19–7.37 (m, 10H).

5.14.6. tert-Butyl[(1R)-3-[2-(4-fluorobenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl) propyl]carbamate **16a**

Yield: 83%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (m, 9H), 2.52–2.60 (m, 3H), 2.82–3.12 (m, 5H), 3.47–3.57 (m, 2H), 3.63–3.70 (m, 1H), 3.93 (m, 1H), 4.02–4.11 (m, 1H), 4.29–4.42 (m, 2H), 5.59 (m, 1H), 6.82–6.89 (m, 1H), 7.02 (m, 3H), 7.19–7.24 (m, 2H).

5.14.7. tert-Butyl[(1R)-3-[2-(4-methylbenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate **16b**

Yield: 68%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (s, 9H), 2.34 (s, 3H), 2.53–2.60 (m, 3H), 2.80–2.96 (m, 5H), 3.08 (m, 1H), 3.27–3.32 (m, 1H), 3.52 (m, 1H), 3.68 (m, 1H), 3.91–3.97 (m, 1H), 4.03–4.10 (m, 1H), 4.35 (m, 2H), 4.58 (m, 1H), 6.87–6.92 (m, 1H), 6.99–7.07 (m, 1H), 7.14 (s, 4H).

5.14.8. tert-Butyl[(1R)-3-[2-(4-chlorobenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16c***

Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (s, 9H), 2.38–2.67 (m, 3H), 2.78–3.12 (m, 5H), 3.28–3.34 (m, 1H), 3.50–3.58 (m, 1H), 3.64–3.70 (m, 1H), 3.91–3.97 (m, 1H), 4.03–4.10 (m, 1H), 4.30–4.37 (m, 2H), 4.58–4.62 (m, 1H), 6.89 (m, 1H), 7.05 (m, J = 8.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H).

5.14.9. tert-Butyl[(1R)-3-[2-(4-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16d***

Yield: 75%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (m, 9H), 2.44–2.64 (m, 3H), 2.77–3.12 (m, 5H), 3.27–3.32 (m, 1H), 3.49–3.54 (m, 1H), 3.65–3.70 (m, 1H), 3.80 (s, 3H), 3.92–3.96 (m, 1H), 4.06–4.08 (m, 1H), 4.29–4.37 (m, 2H), 5.56–5.60 (m, 1H), 6.89 (m, 1H), 7.05 (m, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H).

5.14.10. tert-Butyl[(1R)-3-[2-(2-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16e***

Yield: 94%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 9H), 2.44–2.65 (m, 3H), 2.83–3.08 (m, 5H), 3.33–3.40 (m, 1H), 3.48–3.56 (m, 1H), 3.66 (m, 1H), 3.83 (s, 3H), 3.90–3.95 (m, 1H), 4.09 (m, 1H), 4.44 (s, 2H), 4.55–4.60 (m, 1H), 6.86–6.95 (m, 3H), 7.06 (m, 1H), 7.22–7.29 (m, 2H).

5.14.11. tert-Butyl[(1R)-3-[2-(3-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl) propyl]carbamate ***16f***

Yield: 83%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 9H), 2.40–2.68 (m, 3H), 2.82–3.14 (m, 5H), 3.29–3.35 (m, 1H), 3.51–3.57 (m, 1H), 3.60–3.65 (m, 1H), 3.69 (m, 1H), 3.80 (s, 3H), 3.94 (m, 1H), 4.05–4.11 (m, 1H), 4.36 (s, 2H), 4.59 (m, 1H), 6.78–6.84 (m, 3H), 6.88 (m, 1H), 7.06 (m, 1H), 7.22–7.27 (m, 1H).

5.14.12. tert-Butyl[(1R)-3-[2-(3,5-dimethoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16g***

Yield: 50%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 9H), 2.39–2.66 (m, 3H), 2.80–3.12 (m, 5H), 3.30–3.37 (m, 1H), 3.49–3.59 (m, 1H), 3.60–3.65 (m, 1H), 3.62–3.75 (m, 1H), 3.77 (s, 6H), 3.80–3.96 (m, 1H), 4.00–4.11 (m, 1H), 4.27–4.37 (m, 2H), 4.60 (m, 1H), 6.38 (s, 3H), 6.84–6.93 (m, 1H), 7.01–7.08 (m, 1H).

5.14.13. tert-Butyl[(1R)-3-[2-(3-cyanobenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16h***

Yield: 95%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (m, 9H), 2.52–2.71 (m, 3H), 2.81–3.27 (m, 5H), 3.39 (m, 1H), 3.65–3.79 (m, 1H), 3.89–3.98 (m, 1H), 4.02 (m, 1H), 4.06–4.17 (m, 1H), 4.37–4.50 (m, 2H), 4.54 (m, 1H), 6.89 (m, 1H), 7.05 (m, 1H), 7.45–7.62 (m, 4H).

5.14.14. tert-Butyl[(1R)-3-[2-(pyridin-2-yl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl) propyl]carbamate ***16i***

Yield: 96%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42–1.49 (m, 9H), 2.47–2.71 (m, 3H), 2.88–3.15 (m, 4H), 3.48–3.53 (m, 1H), 3.70–3.82 (m, 2H), 3.94–4.13 (m, 3H), 4.64–4.87 (m, 1H), 6.86–6.94 (m, 1H), 7.02–7.10 (m, 1H), 7.60–7.69 (m, 4H).

5.14.15. tert-Butyl[(1R)-3-[2-(4-(trifluoromethyl)phenyl)-hexahydro-3-oxoimidazo [1,5-a]pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16j***

Yield: 51%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (m, 9H), 2.53–2.67 (m, 3H), 2.92–3.15 (m, 4H), 3.52 (m, 1H), 3.76 (m, 2H), 3.96–4.11 (m, 3H), 4.64–4.85 (m, 1H), 6.90 (m, 1H), 7.05–7.10 (m, 1H), 7.59 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H).

5.14.16. tert-Butyl[(1R)-3-[2-(2,4,5-trifluorophenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16k***

Yield: 55%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42–1.49 (m, 9H), 2.55–2.65 (m, 3H), 2.90–3.15 (m, 4H), 3.48–3.59 (m, 1H), 3.72–3.78 (m, 2H), 3.94–4.00 (m, 2H), 3.09–4.10 (m, 1H), 4.62–4.83 (m, 1H), 6.90 (m, 1H), 7.05–7.10 (m, 1H), 7.32 (m, 1H), 7.52 (m, 1H).

5.14.17. tert-Butyl[(1R)-3-[2-(4-fluorophenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16l***

Yield: 34%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42–1.49 (m, 9H), 2.53–2.65 (m, 3H), 2.92–3.20 (m, 4H), 3.40–3.48 (m, 2H), 3.72–3.78 (m, 2H), 3.90–4.02 (m, 3H), 4.62–4.83 (m, 1H), 6.91 (m, 1H), 7.01–7.08 (m, 3H), 7.46–7.52 (m, 2H).

5.14.18. tert-Butyl[(1R)-3-[2-(4-chlorophenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16m***

Yield: 75%, mp 207–208 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (m, 9H), 2.22–2.70 (m, 3H), 2.88–3.14 (m, 4H), 3.43–3.49 (m, 1H), 3.70–3.78 (m, 2H), 3.90–4.13 (m, 3H), 4.65–4.84 (m, 1H), 5.40 (m, 1H), 6.90 (m, 1H), 7.05–7.10 (m, 1H), 7.28 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H).

5.14.19. tert-Butyl[(1R)-3-[2-(4-cyanophenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16n***

Yield: 97%, mp 99–100 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (m, 9H), 2.57–2.67 (m, 3H), 2.81–3.05 (m, 4H), 3.70–3.84 (m, 1H), 3.95–4.17 (m, 4H), 4.73 (m, 1H), 4.89–4.94 (m, 1H), 5.30–5.38 (m, 1H), 6.88–6.96 (m, 1H), 7.07–7.25 (m, 2H), 7.60–8.00 (m, 1H), 8.00–8.67 (m, 1H).

5.14.20. tert-Butyl[(1R)-3-[2-(4-(methylsulfonyl)phenyl)-hexahydro-3-oxoimidazo [1,5-a]pyrazin-7(8H)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate ***16o***

Yield: 49%, mp 227–230 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (m, 9H), 2.52–2.68 (m, 3H), 2.92 (m, 2H), 2.97–3.16 (m, 5H), 3.48–3.55 (m, 1H), 3.76–3.82 (m, 2H), 3.98–4.11 (m, 3H), 4.68–4.87 (m, 1H), 5.38 (m, 1H), 6.90 (m, 1H), 7.05–7.10 (m, 1H), 7.75 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.7 Hz, 1H).

5.15. General acylation procedure for compounds ***15a–e***

To a solution of **15a–e** (0.5 mmol) in MeOH was added 10% Pd/C (500 mg). The mixture was submitted to an atmosphere of hydrogen (40 psi H_2) for 12 h. The catalyst was filtered off, and the filtrate evaporated to give a crude material that was recrystallized from CH_2Cl_2 and hexane to yield compounds **15a–e**. To a solution of fumaric acid (0.2 N) in ethanol, a solution of the above-prepared compounds (0.2 N) in ethanol was added via a pipet. Anhydrous Et_2O was added in a dropwise manner to the reaction until it became cloudy. The cloudy white heterogeneous mixture was stirred vigorously at room temperature for 20 min, filtered, and washed with Et_2O to give the desired compounds as white solids.

5.15.1. (2R)-4-Oxo-4-[hexahydro-3-oxo-oxazolo[1,5-a]pyrazin-7 (8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***15a***

Yield: 55%, mp 74–75 °C. ^{1}H NMR (CF_3COOD, 300 MHz) δ 3.01–3.23 (m, 6H), 3.89–4.19 (m, 5H), 4.45–4.80 (m, 3H),

6.93–7.01 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.78, 48.51, 52.94, 56.48, 66.62, 106.28, 120.13, 121.84, 135.56, 147.49, 156.64, 169.22; HRMS (ESI) calcd for $C_{16}H_{18}F_3N_3O_3$ (357.1300 g/mol), found (MH^+) 358.1386.

5.15.2. (2R)-4-Oxo-4-[hexahydro-3-oxoimidazo[1,5-a]pyrazin-7 (8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***15b***

Yield: 50%, mp 236–238 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 3.04–3.40 (m, 9H), 3.78–4.13 (m, 5H), 6.98–7.13 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.48, 36.97, 41.41, 44.98, 48.46, 52.88, 53.32, 106.47, 111.76, 119.99, 121.29, 136.11, 147.96, 161.16, 169.04; HRMS (ESI) calcd for $C_{16}H_{19}F_3N_4O_2$ (356.1460 g/mol), found (MH^+) 357.1543.

5.15.3. (2R)-4-Oxo-4-[2-methyl-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***15c***

Yield: 71%, mp 91–92 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 3.16–3.46 (m, 10H), 3.40–3.46 (m, 1H), 3.55 (m, 2H), 3.71 (m, 1H), 4.28 (m, 3H), 7.21–7.30 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.13, 33.41, 36.27, 37.61, 43.43, 49.76, 54.14, 66.37, 106.27, 120.14, 122.41, 136.34, 150.30, 155.23, 163.57, 168.72; HRMS (ESI) calcd for $C_{17}H_{21}F_3N_4O_2$ (370.1617 g/mol), found (MH^+) 371.20.

5.15.4. (2R)-4-Oxo-4-[2-ethyl-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***15d***

Yield: 45%, mp 99–100 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 1.16 (t, $J = 3.3$ Hz, 3H), 2.95–3.33 (m, 10H), 3.40–4.20 (m, 6H), 4.20–4.40 (m, 1H), 6.89–6.99 (m, 1H), 7.03 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.81, 32.06, 36.87, 38.18, 41.35, 43.48, 46.09, 47.26, 48.37, 50.57, 106.23, 120.05, 135.72, 147.46, 149.91, 158.91, 168.76; HRMS (ESI) calcd for $C_{18}H_{23}F_3N_4O_2$ (384.1773 g/mol), found (MH^+) 385.1841.

5.15.5. (2R)-4-Oxo-4-[2-benzyl-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***15e***

Yield: 50%, mp 94–95 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 2.71–3.27 (m, 8H), 3.49–3.54 (m, 1H), 3.76–4.06 (m, 4H), 4.33–4.45 (m, 3H), 6.85–6.91 (m, 1H), 6.97–7.25 (m, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.40, 34.88, 41.36, 44.99, 46.63, 47.31, 48.18, 60.56, 106.12, 120.29, 120.89, 127.69, 128.12, 129.02, 136.30, 137.78, 147.59, 159.11, 169.91; HRMS (ESI) calcd for $C_{23}H_{25}F_3N_4O_2$ (446.1930 g/mol), found (MH^+) 447.2015.

5.16. General acylation procedure for compounds ***17a–g*** *and* ***17i–o***

To a solution of **16a–g** and **16i–o** (0.3 N) in CH_2Cl_2 was added TFA (10 equiv). The mixture was stirred for 12 h at room temperature, and evaporated to dryness. The resulting residue was dissolved in 2 N NaOH (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, concentrated and recrystallized from CH_2Cl_2 and hexane to yield desire compounds. The HCl salts of these compounds were converted to their fumaric acid salts using the procedure described in Section 5.15.

5.16.1. (2R)-4-Oxo-4-[2-(4-fluorobenzyl)-hexahydro-3-oxoimidazo [1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***17a***

Yield: 45%, mp 123–125 °C. ^{1}H NMR (CF_3COOD, 300 MHz) δ 2.83–2.86 (m, 1H), 3.00–3.42 (m, 9H), 3.60–3.65 (m, 1H), 3.80–4.17 (m, 3H), 3.50–3.58 (m, 1H), 4.46–4.62 (m, 2H), 7.02–7.40 (m, 6H); ^{13}C NMR (D_2O, 100 MHz) δ 30.82, 33.50, 40.00, 41.45, 44.84, 45.56, 46.64, 48.45, 50.60, 105.83, 114.19, 119.06, 123.46, 130.52, 134.02, 139.32, 160.34, 161.48, 163.91, 168.62, 160.48; HRMS (ESI) calcd for $C_{23}H_{24}F_4N_4O_2$ (464.1835 g/mol), found (MH^+) 465.1920.

5.16.2. (2R)-4-Oxo-4-[2-(4-methylbenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17b***

Yield: 53%, mp 118–119 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.31 (s, 2H), 2.33–2.72 (m, 2H), 2.78–3.10 (m, 5H), 3.24–3.28 (m, 2H), 3.47–3.75 (m, 3H), 3.84–3.86 (m, 2H), 4.19–4.38 (m, 2H), 4.45–4.48 (m, 1H), 6.85–6.89 (m, 1H), 7.07–7.12 (m, 4H), 7.23–7.28 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.81, 35.58, 41.34, 44.97, 45.49, 47.04, 48.27, 50.84, 56.47, 106.30, 120.25, 121.27, 128.18, 129.57, 134.66, 136.83, 146.08, 147.38, 149.97, 159.08, 168.39; HRMS (ESI) calcd for $C_{24}H_{27}F_3N_4O_2$ (460.2086 g/mol), found (MH^+) 461.2173.

5.16.3. (2R)-4-Oxo-4-[2-(4-chlorobenzyl)-hexahydro-3-oxoimidazo [1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***17c***

Yield: 60%, mp 137–138 °C.^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38–2.74 (m, 3H), 2.76–2.88 (m, 3H), 2.91–3.12 (m, 2H), 3.29–3.35 (m, 1H), 3.49–3.58 (m, 2H), 3.76–3.92 (m, 2H), 4.27–4.38 (m, 2H), 4.44–4.51 (m, 1H), 6.91 (m, 1H), 7.09 (m, 1H), 7.16 (m, 2H), 7.28 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.27, 34.54, 41.34, 44.99, 46.41, 46.63, 48.29, 50.85, 106.48, 116.11, 120.18, 128.97, 130.03, 132.31, 136.91, 146.25, 147.53, 150.14, 159.04, 168.39; HRMS (ESI) calcd for $C_{23}H_{24}ClF_3N_4O_2$ (480.1540 g/mol), found (MH^+) 481.1606.

5.16.4. (2R)-4-Oxo-4-[2-(4-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17d***

Yield: 55%, mp 123–124 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.30–2.46 (m, 3H), 2.59–2.67 (m, 2H), 2.59–2.91 (m, 3H), 3.21–3.32 (m, 1H), 3.47–3.55 (m, 2H), 4.64–3.71 (m, 1H), 3.79 (s, 3H), 3.93 (m, 1H), 4.28–4.37 (m, 2H), 4.58 (m, 1H), 6.84–6.92 (m, 3H), 7.06 (m, 1H), 7.16 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.92, 32.93, 36.30, 41.26, 44.93, 46.72, 48.57, 50.59, 55.49, 62.48, 106.43, 114.39, 120.13, 129.55, 135.73, 147.30, 158.96, 169.10; HRMS (ESI) calcd for $C_{24}H_{27}F_3N_4O_3$ (476.2035 g/mol), found (MH^+) 477.2111.

5.16.5. (2R)-4-Oxo-4-[2-(2-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17e***

Yield: 83%, mp 133–134 °C. ^{1}H NMR (D_2O, 400 MHz) δ 2.40–2.70 (m, 2H), 2.78–2.90 (m, 5H), 3.27 (m, 1H), 3.47 (m, 2H), 3.60–3.68 (m, 5H), 3.76 (m, 1H), 4.16–4.26 (m, 3H), 6.48 (s, 1H), 6.85 (m, 1H), 6.92 (m, 1H), 6.97 (m, 1H), 7.01–7.14 (m, 2H), 7.23 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.72, 36.28, 41.32, 42.22, 45.03, 46.14, 48.42, 50.94, 55.82, 56.48, 106.23, 111.26, 120.85, 125.37, 128.82, 135.55, 157.49, 159.19, 168.17; HRMS (ESI) calcd for $C_{24}H_{27}F_3N_4O_3$ (476.2035 g/mol), found (MH^+) 477.2104.

5.16.6. (2R)-4-Oxo-4-[2-(3-methoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin -7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17f***

Yield: 69%, mp 131–133 °C. ^{1}H NMR (D_2O, 400 MHz) δ 2.40–2.90 (m, 8H), 3.30 (m, 1H), 3.46–3.52 (m, 1H), 3.52–3.66 (m, 5H), 3.79 (m, 1H), 4.13–4.25 (m, 3H), 6.42 (m, 1H), 6.71–6.81 (m, 3H), 7.00 (m, 1H), 7.10 (m, 1H), 7.20 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.71, 36.66, 41.28, 44.97, 45.68, 47.28, 48.51, 50.59, 55.41, 56.48, 106.23, 113.00, 113.71, 120.19, 130.12, 135.77, 139.41, 159.11, 159.90,

168.76, 169.04; HRMS (ESI) calcd for $C_{24}H_{27}F_3N_4O_3$ (476.2035 g/mol), found (MH^+) 477.2118.

5.16.7. (2R)-4-Oxo-4-[2-(3,5-dimethoxybenzyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17g***

Yield: 60%, mp 136–137 °C. ^{1}H NMR (D_2O, 400 MHz) δ 2.40–3.13 (m, 8H), 3.51–3.73 (m, 3H), 3.79–3.87 (m, 7H), 4.00 (m, 1H), 4.30–4.45 (m, 3H), 6.58 (m, 2H), 6.60 (m, 1H), 6.63 (m, 1H), 7.23 (m, 1H), 7.31 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.97, 36.81, 41.30, 44.99, 45.73, 47.42, 48.58, 50.6, 51.08, 55.56, 99.27, 105.98, 120.14, 121.82, 135.76, 140.23, 145.17, 147.46, 155.41, 159.12, 161.60, 168.74; HRMS (ESI) calcd for $C_{25}H_{29}F_3N_4O_4$ (506.2141 g/mol), found (MH^+) 507.2209.

5.16.8. (2R)-4-Oxo-4-[2-(pyridin-2-yl)-hexahydro-3-oxoimidazo [1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine fumaric acid ***17i***

Yield: 95%, mp 115–116 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 2.94–3.40 (m, 8H), 3.90–4.02 (m, 3H), 4.17 (m, 3H), 4.29 (m, 1H), 4.41–4.46 (m, 1H), 6.97–7.04 (m, 1H), 7.09–7.18 (m, 2H), 7.32 (d, J = 8.8 Hz, 1H), 7.56 (t, J = 6.8 Hz, 1H), 8.42–8.49 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 32.99, 36.84, 41.13, 44.98, 45.64, 48.47, 49.03, 49.55, 50.00, 106.20, 112.23, 118.00, 119.99, 121.86, 135.90, 137.81, 147.93, 152.64, 155.34, 169.13; HRMS (ESI) calcd for $C_{21}H_{22}F_3N_5O_2$ (433.1726 g/mol), found (MH^+) 434.1890.

5.16.9. (2R)-4-Oxo-4-[2-(4-(trifluoromethyl)-phenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17j***

Yield: 87%, mp 183–185 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.53–3.05 (m, 9H), 3.57–3.79 (m, 4H), 3.94–4.00 (m, 1H), 7.51–7.57 (m, 2H), 7.67 (m, 2H), 7.75 (m, 2H), 7.89–7.94 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.39, 34.62, 41.14, 44.82, 46.71, 48.23, 49.72, 55.31, 106.20, 116.99, 120.19, 122.03, 122.35, 123.64, 126.34, 144.18, 145.30, 147.70, 150.29, 155.55, 168.47; HRMS (ESI) calcd for $C_{23}H_{22}F_6N_4O_2$ (500.1647 g/mol), found (MH^+) 501.1706.

5.16.10. (2R)-4-Oxo-4-[2-(2,4,5-trifluorophenyl)-hexahydro-3-oxoimidazo[1,5-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17k***

Yield: 73%, mp 120–121 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.50–2.69 (m, 3H), 2.76–3.05 (m, 5H), 3.56–3.78 (m, 4H), 3.92 (m, 1H), 4.39–4.58 (m, 1H), 7.48–7.70 (m, 2H), 7.76–7.97 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.39, 34.58, 41.18, 45.21, 48.22, 49.14, 50.67, 106.71, 113.43, 120.19, 120.24, 128.63, 145.27, 147.77, 151.02, 153.76, 155.96, 158.96, 168.54; HRMS (ESI) calcd for $C_{22}H_{20}F_6N_4O_2$ (486.1490 g/mol), found (MH^+) 487.1556.

5.16.11. (2R)-4-Oxo-4-[2-(4-fluorophenyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17l***

Yield: 85%, mp 134–135 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.58–2.79 (m, 3H), 3.01–3.21 (m, 5H), 3.43–3.45 (m, 1H), 3.72–4.02 (m, 4H), 4.77–4.86 (m, 1H), 6.95 (m, 1H), 7.01–7.06 (m, 1H), 7.14 (m, 2H), 7.47 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 30.86, 33.46, 39.91, 41.36, 44.86, 47.05, 48.45, 50.01, 106.89, 115.78, 119.10, 122.03, 134.40, 135.02, 159.14, 160.61, 169.73, 170.29; HRMS (ESI) calcd for $C_{22}H_{22}F_4N_4O_2$ (450.1679 g/mol), found (MH^+) 451.1765.

5.16.12. (2R)-4-Oxo-4-[2-(4-chlorophenyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17m***

Yield: 98%, mp 163–165 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 3.16–3.41 (m, 8H), 3.50–3.64 (m, 1H), 3.94–4.40 (m, 5H), 7.21–7.38 (m, 4H), 7.48–7.62 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.27, 34.43, 41.22, 44.75, 45.43, 46.02, 48.08, 49.53, 106.39, 119.06, 120.34, 126.35, 128.95, 135.35, 139.47, 147.97, 155.96, 168.59; HRMS (ESI) calcd for $C_{22}H_{22}ClF_3N_4O_2$ (466.1383 g/mol), found (MH^+) 467.1443.

5.16.13. (2R)-4-Oxo-4-[2-(4-cyanophenyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17n***

Yield: 98%, mp 147–148 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 2.93–3.25 (m, 8H), 3.77–3.83 (m, 1H), 4.01–4.22 (m, 4H), 7.02 (m, 1H), 7.11–7.17 (m, 2H), 7.70 (m, 1H), 7.78 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 31.16, 34.29, 40.14, 41.18, 45.85, 48.23, 48.91, 65.52, 103.68, 106.26, 117.31, 119.77, 133.39, 135.68, 144.28, 155.62, 168.86, 170.39; HRMS (ESI) calcd. for $C_{23}H_{22}F_3N_5O_2$ (457.1726 g/mol), found (MH^+) 458.1801.

5.16.14. (2R)-4-Oxo-4-[2-(4-(methylsulfonyl)phenyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17o***

Yield: 94%, mp 171–173 °C. ^{1}H NMR (CF_3COOD, 400 MHz) δ 2.93–3.46 (m, 10H), 3.76–3.81 (m, 1H), 4.02–4.28 (m, 5H), 4.58–4.82 (m, 1H), 6.99 (m, 1H), 7.10 (m, 2H), 7.15 (m, 1H), 7.80 (m, 2H), 8.02 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 29.68, 38.86, 44.82, 46.03, 48.31, 50.14, 60.58, 106.47, 117.83, 120.61, 129.41, 133.38, 134.86, 130.13, 147.65, 155.88, 167.16, 168.48; HRMS (ESI) calcd for $C_{23}H_{25}F_3N_4O_4S$ (510.1549 g/mol), found (MH^+) 511.1624.

5.17. (2R)-4-oxo-4-[2-(3-carbamoylbenzyl)-hexahydro-3-oxoimidazo[1,5-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine fumaric acid ***17h***

To a solution of **16h** (280 mg, 0.5 mmol) in CH_2Cl_2 (20 mL), DMSO (1 mL) and H_2O_2 (30%, 5 mL) were added at 0 °C. After 1 h, the mixture was poured into H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was then submitted to amine deprotection conditions and converted to its fumaric acid salt using the procedure in Section 5.9, which gave compound **17h** (202 mg, 67%), mp 221–223 °C. ^{1}H NMR (D_2O, 400 MHz) δ 2.60–2.89 (m, 9H), 3.23 (m, 1H), 3.55–3.63 (m, 2H), 3.72–3.80 (m, 1H), 4.14–4.20 (m, 3H), 6.46 (s, 2H), 6.95 (m, 1H), 7.06 (m, 1H), 7.25–7.31 (m, 2H), 7.45 (s, 1H), 7.52 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 30.84, 33.72, 40.30, 41.43, 44.91, 45.55, 46.84, 48.42, 50.90, 64.20, 105.79, 119.05, 126.64, 129.20, 131.69, 134.93, 137.22, 160.56, 169.64, 172.67; HRMS (ESI) calcd for $C_{24}H_{26}F_3N_5O_3$ (489.1988 g/mol), found (MH^+) 490.2053.

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