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Identification of BACE-1 inhibitors through directed C(sp^3)–H activation on 5-oxo-pyrrolidine-3-carboxylic acid derivatives†

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Convenient synthesis of stereochemically dense 5-oxo-pyrrolidines was obtained from succinic anhydride and imines by combining the Castagnoli–Cushman reaction with directed Pd-catalyzed C(sp^3)–H functionalization, taking advantage of the developing carboxylic group properly derivatized with 8-aminoquinoline as a directing group. These fully substituted 5-oxopyrrolidines were found to be able to inhibit BACE-1 enzyme with sub-micromolar activity, thanks to the interaction of the key aryl appendage introduced by C(sp^3)–H activation within BACE-1 S2′ subsite.

Introduction

A convenient synthetic approach for fragment elaboration and decoration of a scaffold in fragment-based approaches[1–4] is the site-selective formation of bonds at all growing points of a fragment[5] taking advantage of C–H functionalization strategies.[6] The site-selective C(sp^3)–H functionalisation to form stereocenters is of high value for adding functionalized chemical moieties,[7] and in particular late stage aromatic C(sp^3)–H functionalization[8] can be used to introduce elements that can assist the molecules in passing the BBB.[9–12] Among the many different C(sp^3)–H arylation methods, Pd-catalyzed C–H activation reactions employing a Directing Group (DG)[13] are often used for their high site selectivity, being able to discriminate a particular C–H bond, using distance as the distinctive parameter.[14] This strategy represents an optimal tool for the straightforward elaboration of molecular scaffolds and it has been applied to several different *N*-containing heterocycles.[15–18]

Among the many different *N*-containing heterocycles, 5-oxopyrrolidines is a privileged structure in medicinal chemistry.[19,20] In particular, fully substituted 5-oxopyrrolidines bearing a carbonyl moiety in position 3 are found in many bioactive molecules and natural products involved in the treatment of CNS diseases. Just to give some examples, different Endothelin Receptor Antagonists[21] and Glutaminyl-Peptide CycloTransferase-Like (QPCTL) inhibitors,[22] used for the treatment of Alzheimer's Disease, contain a fully substituted 5-oxopyrrolidines. Also, this scaffold is present in heliotropamide A and bisavenanthramide B-6 natural products used in traditional Chinese medicine,[23] for their acetylcholinesterase inhibitory activity and their neuroprotective effects (Fig. 1).[24]

Several methods have been developed for the preparation of densely functionalized 5-oxopyrrolidines, including cycliza-

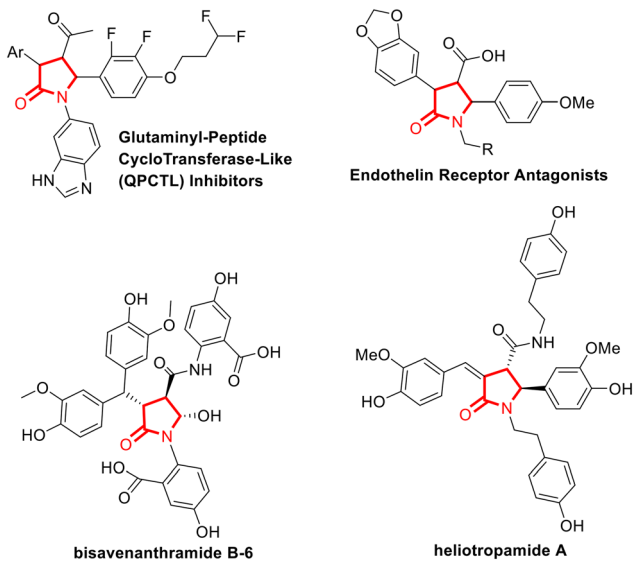

Fig. 1 Representative examples of bioactive compounds (top) and natural products (bootom) used in the treatment of CNS diseases, that contains fully substituted 5-oxopyrrolidines bearing a carbonyl moiety in position 3.

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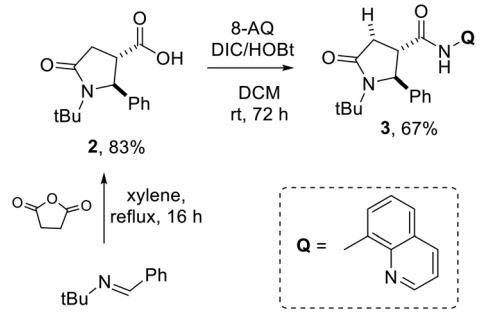
†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for compounds **1–3**, **4a–4j**, **5a**, **5b**, crystallographic data for **4g**, kinetic NMR studies, inhibition curves of **4g** and **5b** on BACE-1 enzyme, CNS MPO calculations for compounds **2–3**, **4a–4j**, **5a**, **5b**, coordinates of top-ranked pose of **4g**. CCDC 2165903. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d3ob02117c

tion, [3 + 2] or [4 + 1] cycloaddition,[25] cascade reactions, or related transformations.[20,26] However, to the best of our knowledge, short and convenient synthesis of fully substituted 5-oxopyrrolidines, containing appendages in each available positions and bearing a carbonyl moiety in position 3 were not reported in the literature. In our previous synthetic efforts in the field of CNS drug discovery,[27–29] we reported the exploitation of the Castagnoli–Cushman reaction (CCR) to generate *C*-2 substituted morpholinone scaffolds as BACE-1 inhibitors.[28] The CCR is a multicomponent reaction that is capable to give functionalized N-heterocycles depending on the building blocks employed.[30–32] In this work, we reasoned to expand the chemical complexity of 5-oxo-pyrrolidine-3-carboxylic acid derivatives resulting from the CCR reaction with a directed C(sp^3)–H functionalization to obtain fully substituted 5-oxopyrrolidines. The synthesized compounds were then analyzed for their drug-like properties, considering the range of optimal parameters for CNS drugs, as defined by Wager and coworkers,[33] and evaluated for their inhibition activity against different enzymes involved in CNS diseases. In particular, we performed a screening on representative enzymes involved in Alzheimer's Disease (AD), namely BACE-1, an aspartic protease involved in the amyloidogenic pathway by cleaving amyloid precursor protein (APP),[34–36] and legumain, also called δ-secretase, a cysteine protease responsible for tau cleaving in AD.[37]

Results and discussion

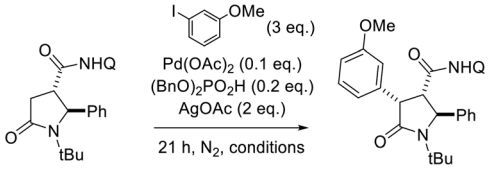
The directed C(sp^3)–H functionalization of 5-oxo-pyrrolidine-3-carboxamide was developed by taking advantage of the COOH handle resulting from the CCR reaction to install a suitable directing group for Pd-catalysis. In particular, 8-aminoquinoline (8-AQ) was chosen among various directing groups for its capability of chelating the transition metal catalyst and bringing it close to the β-C(sp^3)–H bond, thus resulting in the use of distance as chemo- and stereoselective parameters for functionalizing the desired C–H bond. To study the versatility of this novel C(sp^3)–H functionalization method, we synthesized 5-oxo-2-phenylpyrrolidine-3-carboxylic acid **2** as a representative substrate of the C–H arylation reaction. Specifically, 5-oxopyrrolidine **2** was obtained in high yield from *N*-benzylidene-2-methylpropan-2-amine (**1**) and succinic anhydride, following the reported protocols (Scheme 1).[38,39]

Then, the carboxylic acid function resulting from the Castagnoli–Cushman reaction was exploited for inserting the 8-AQ as a directing group through amide coupling employing HOBt and DIC in anhydrous CH_2Cl_2, to give compound **3** in 67% yield (Scheme 1). As no examples of 8-aminoquinoline-promoted C(sp^3)–H arylation of 5-oxopyrrolidine-3-carboxylic acid derivatives are reported in the literature, solvent, substrate concentration and reaction temperature were studied in order to find out best reaction conditions. As reported in Table 1, best conditions for the C–H bond activation step were found to be the employment of 3 equivalents of aryl iodide and 0.2 equivalents of $(BnO)_2PO_2H$, heating at 110 °C for 21 h in anhydrous 1,4-dioxane at 0.2 M concentration. The increase of the concentration to 0.9 M and the reduction of reaction temperature to 80 °C did not improve the reaction conversion.



Scheme 1 Synthesis of 8-aminoquinoline-functionalized 5-oxo-2-phenylpyrrolidine-3-carboxamide **3**.

Table 1 Method optimization for C(sp^3)–H functionalization of functionalized 5-oxo-2-phenylpyrrolidine-3-carboxamide **3**



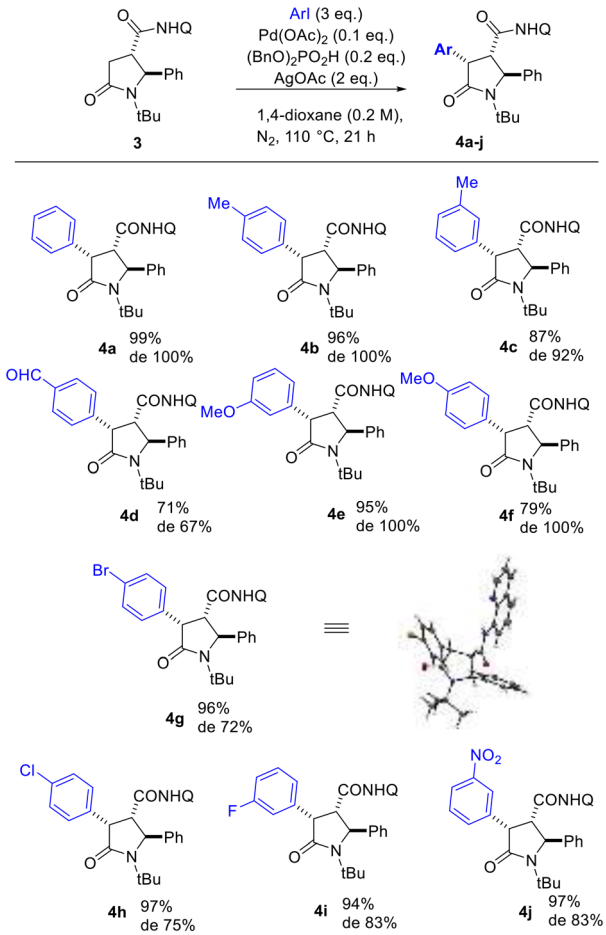
3 **4**

Entry	Solvent (conc.)	Temp., °C	Yield, %
1	Toluene (0.05 M)	110	14
2	*t*-AmylOH (0.05 M)	110	15[a]
3	DCE (1 M)	110	36[a]
4	HFIP (0.2 M)	110	68
5	TFE (0.02 M)	110	59
6	**1,4-Dioxane (0.2 M)**	**110**	**95**
7	*t*-BuOH (0.2 M)	110	29[a]
8	1,4-Dioxane (0.2 M)	80	14[a]
9	1,4-Dioxane (0.9 M)	80	10[a]
10	1,4-Dioxane (0.9 M)	110	73[a]

[a] NMR conversion.

The optimized conditions were applied for conducting a substrate scope study, producing with good to excellent yields 2,4-diaryl-5-oxo-pyrrolidines **4a–j** (Scheme 2), with the rare *trans* 2,4-diaryl substitution pattern. The reaction proved to possess a broad scope for aryl iodide with a high functional group tolerance in the presence of aldehyde or nitro groups. For compound **4g**, an X-ray crystallographic analysis was carried out to confirm the relative configuration of the substituents at the scaffold (Scheme 2), indicating the *trans* 2,3- and *cis* 3,4-configuration for the major isomer formed during the directed C(sp^3)–H bond activation step. Also, gCOSY of **4g** allowed to assign the ^{1}H NMR peaks to each proton (Fig. 2).

In particular, 4-H was found significantly deshielded in C(sp^3)–H functionalized 5-oxo-2-phenylpyrrolidines **4a–j** (4.4 ppm with respect to 3.06 ppm found for **3**), whereas 3-H



Scheme 2 Scope of directed C(sp^3)–H functionalization of 5-oxo-2-phenylpyrrolidine-3-carboxamide **3**. Diastereoselectivity ratio (de) was determined by the two sets of peaks in the ^{1}H NMR spectrum of the crude mixture attributed to the hydrogen atoms 2-H, 3-H and 4-H of the heterocyclic ring. The X-Ray crystallographic structure of **4g** is reported in the Scheme (CCDC: 2165903†).

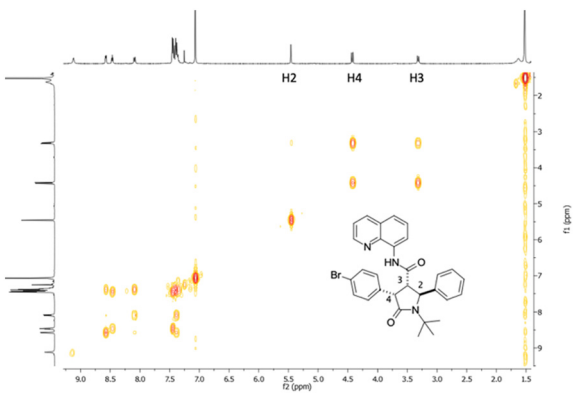


Fig. 2 gCOSY NMR plot of compound **4g**.

and 2-H protons did not shift considerably (3.06 and 5.35 ppm, respectively for compound **3**, and around 5.5 and 3.3 ppm for compounds **4a–j**).

The diastereoselectivity ratio was determined by ^{1}H NMR, specifically evaluating the 2-H signal appearing as a singlet for the major isomer and as an upfield doublet for the minor one (see Fig. 3 for **4j**), resulting in good to high de values depending on the installed aryl moiety (Scheme 2). To give an insight to the conversion of the C(sp^3)–H bond functionalization reaction and the epimerization degree at C-3 at different times of the reaction mixture, we carried out a kinetic study by NMR employing 1-iodo-3-nitrobenzene (Fig. 3) and 4-iodobenzaldehyde as aryl iodides (see ESI, Fig. S1 and S2†). The reaction temperature was kept fixed at 110 °C to allow for the reaction to proceed to completion (Table 1), and at given times (1, 2, 4, 8, 16, 21 hours) aliquots were taken from the reaction mixture and analyzed by ^{1}H NMR to assess % conversion and epimerization. The results indicated reaction completion at 16 to 21 h and epimerization starting after 4 h reacting, although not increasing significantly during the reaction time.

The proposed mechanism for the diastereoselective β-C (sp^3)–H bond activation reaction involves the coordination of the Pd by the directing group *via* ligand exchange to form the intermediate species **II**, followed by insertion to generate the palladacycle **III** (Fig. 4). The oxidative addition of the aryl species in **IV** enables the following C–H functionalization to form the arylated specie **V** and subsequent reductive elimination, resulting to the release of Pd catalyst **I** and to the diastereostereoselective attachment of the aryl group with the relative *cis* configuration with respect to the amide bond bearing the DG.

Finally, a representative directing group removal was carried out under standard H_2O_2/LiOH conditions to allow for potential additional functionalization of the carboxylic group of the 5-oxopyrrolidines. Thus, the 8-AQ directing group was removed from compounds **4e** and **4g** through a two step-procedure, which foresees the Boc protection of the nitrogen atom of the carboxamide group, followed by oxidative cleavage employing H_2O_2 and LiOH (Scheme 3). Such procedure

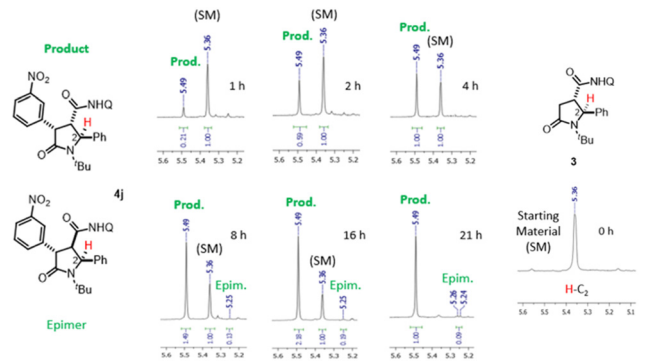


Fig. 3 Kinetic study by NMR of the C(sp^3)–H functionalization for the synthesis of **4j**. The expansions of ^{1}H NMR spectra recorded at different times (1, 2, 4, 6, 8, 16, 21 h) of the reaction mixture, employing 1-iodo-3-nitrobenzene as aryl iodide, highlight the reaction conversion and the epimerization degree at the NMR signals of 2-H proton.

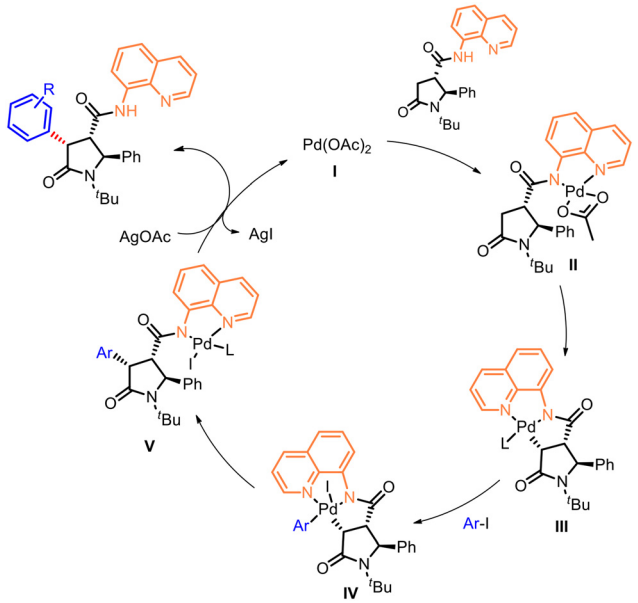
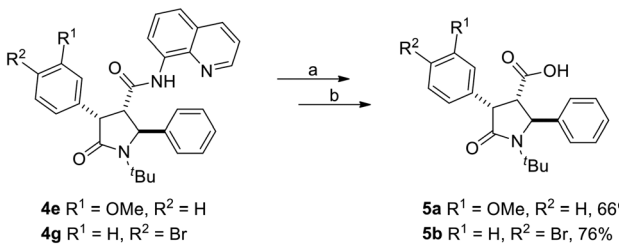


Fig. 4 Proposed mechanism for the directed C–H aryl functionalization at C-4 of 5-oxopyrrolidines.



Scheme 3 Representative two-steps removal of 8-AQ directing groups. Reagents and conditions: (a) $(Boc)_2O$, DMAP, MeCN, 25 to 50 °C, 2 h, then r.t., 18 h; (b) H_2O_2, LiOH, 0 °C, 20 min, then 25 °C, 18 h.

allowed to obtain the corresponding carboxylic acids **5a** and **5b** with an overall yield of 66 and 76%, respectively.

Compounds **4a–j** and their corresponding unsubstituted precursors **3** and **2** were assessed for their physicochemical properties in order to establish their valence as potential compounds for central nervous system drug discovery. As reported by Wager and coworkers,[33] a strict relationship between several physicochemical properties and favorable *in vitro* attributes for the CNS drugs (including high permeability, low P-gp efflux liability, low metabolic clearance, and high safety) is observed. In particular, the CNS MPO (CNS MultiParameter Optimization) method provides a single score (spanning from 0 to 6), that weights and condenses the penalty of six different physicochemical properties that are not in the desirable range. Particularly, the desirable properties for a drug to be able to cross the blood brain barrier (BBB) are: (a) calculated partition coefficient (clog *P*) less than 3; (b) distribution coefficient at pH = 7.4 (clog *D*) less than 2; (c) molecular weight (MW) less than 360 Da; (d) topological polar surface area (tPSA) between 40 and 90; (e) number of hydrogen bond donors (HBD) less than 0.5; (f) pK_a less than 8. Thus, cLog *P*, tPSA, HBD and MW values were calculated using the web-based public tool SwissADME,[40] whereas pK_a and cLog *D* values were calculated with ACD Labs Software v 6.0 [41] (Table 2). Then, the CNS MPO method was applied to measure penalty values for each physicochemical parameter (see ESI†) and the overall CNS MPO score was found. As shown in Table 2, the good scores (higher > 4) observed for the final compounds **5a–5b** confirmed that the C–H arylated method did not alter the desirable physicochemical properties required for the CNS drugs and revealed the need of removing the 8-AQ directing group, as the corresponding compounds **4e** and **4g** possess CNS scores around 3, with cLog *D*, cLog *P* and MW values outside the desirable ranges. To demonstrate the utility of these frag-

Table 2 Physicochemical properties and CNS MPO scores of compounds **2**, **3**, **4a–j**, **5a–b**

Cmpd.	cLog *P*	cLog *D*	MW	tPSA	HBD	pK_a	CNS MPO
2	1	1	1	1	0.83	1	**5.83**
3	0.98	0	0.8	1	0.83	1	**4.61**
4a	0.13	0	0.26	1	0.83	1	**3.22**
4b	0	0	0.16	1	0.83	1	**3**
4c	0	0	0.16	1	0.83	1	**3**
4d	0.4	0	0.06	1	0.83	1	**3.3**
4e	0.14	0	0.05	1	0.83	1	**3.02**
4f	0.14	0	0.05	1	0.83	1	**3.02**
4g	0	0	0	1	0.83	1	**2.83**
4h	0	0	0.01	1	0.83	1	**2.84**
4i	0.08	0	0.14	1	0.83	1	**3.05**
4j	0.21	0	0	0.4	0.83	1	**2.44**
5a	0.93	1	0.95	1	0.83	1	**5.71**
5b	0.57	1	0.6	1	0.83	1	**4**

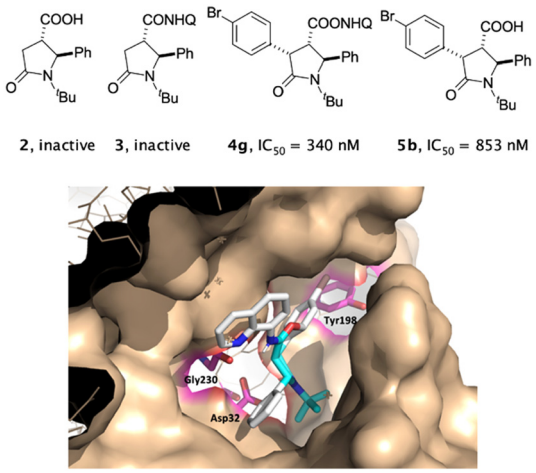


Fig. 5 Top: hit compounds identified as BACE-1 inhibitors (**4g** and **5b**) in comparison with the parent corresponding 5-oxo-pyrrolidines **2** and **3**. Bottom: (2*S*,3*S*,4*S*)-**4g** docked into the active site of BACE1 (PDB: 5CLM), highlighting protein residues (purple) that form key interactions: Gly230 *vs.* 8-AQ, Tyr198 *vs.* 4-Br-phenyl ring. Nonpolar hydrogen atoms are omitted for clarity.

ments in CNS drug discovery, we performed a screening of this library on representative enzymes involved in neurodegenerative diseases, in particular BACE-1 and legumain. While no active compounds were found towards legumain, two molecules (**4g** and **5b**) showed >50% inhibition towards BACE-1 at 10 μM concentration.

A dose–response measurement for **4g** and **5b** in the range 0.01–100 μM of inhibitor concentration was performed, confirming the inhibition profile of the compounds with an IC_{50} value of 340 nM and 853 nM, respectively (Fig. 5, top and Fig. S3 at ESI†). These compounds both contain the newly installed 4-Br-phenyl moiety, suggesting the importance of this moiety as a new vector interacting with the enzyme, as the corresponding parent compounds **2** and **3** were found inactive. This result was confirmed by molecular docking studies carried out with Autodock 4.0 and low energy ligand–protein AMMP energy minimization, where an interaction between the 4-bromophenyl moiety of most active compound **4g** and Tyr198 of hydrophobic S2′ subsite in the catalytic cleft of BACE-1 enzyme was observed (Fig. 5, bottom).

Conclusion

In conclusion, in view of developing a short synthesis for the generation and growth of sp^3-rich fragments, we set up a straightforward synthetic pathway which combines the use of a DOS-compatible reaction like CCR with the 8-AQ directed Pd-catalyzed β-C(sp^3)–H bond activation reaction. This protocol allows for the generation of densely functionalized 5-oxopyrrolidines possessing three stereocenters. The reaction conditions of the C–H arylation were optimized and the generality of the process was demonstrated for a range of aryl iodides bearing different substituents, resulting in high yields and good to high diastereoselectivity. Preliminary enzyme inhibition assays of newly synthesised compounds towards BACE-1 allowed to identify two molecules both bearing the key 4-Br-phenyl moiety required for inhibition potency at submicromolar concentration, resulting in the identification of new hit compounds useful for subsequent elaboration of such new chemical entities as BACE-1 inhibitors. Finding potent ligands for BACE-1 is still a challenging task in drug discovery, particularly for the requirement of potent inhibitors of this enzyme while passing efficiently the blood–brain barrier (BBB), so the identification of a new moiety capable of interacting strongly within the catalytic cleft of the enzyme is a powerful tool for fragment-based approaches, which have been particularly useful for BACE-1 drug discovery,[5–7] followed by optimization of such fragment hits using fragment-linking.[42,43]

Experimental

General

Analytical grade solvents and commercially available reagents were used without further purification. Glutaric acid was recrystallized from CH_2Cl_2/Et_2O. Reactions requiring an inert atmosphere were carried out under a nitrogen atmosphere. CH_2Cl_2, 1,2-DCE, and 1,4-dioxane were freshly distilled over CaH_2. THF, toluene, and xylene were dried by distillation over Na/benzophenone. ^{t}BuOH was dried over Na_2SO_4. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Mercury 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), Varian Inova (^{1}H: 400 MHz, ^{13}C: 100 MHz) or Varian Mercury 200 (^{1}H: 200 MHz, ^{13}C: 50 MHz) spectrometer. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million (ppm) and hertz (Hz), respectively. Flash column chromatography (FCC) purifications were performed manually using glass columns with Merck silica gel (40–63 μm) or automatically using the Biotage Isolera system and SNAP silica cartridges. TLC analyses were performed on Merck silica gel 60 F254 plates. Elemental analyses were performed on a Thermoscientific FlashSmart Elemental Analyzer. ESI-MS spectra were recorded on a Thermo Scientific LCQ fleet ion-trap double quadrupole mass spectrometer using electrospray (ES^+) ionization techniques. A VWR® Ultrasonic cleaner (frequency 45 kHz, power 80 W) was used for reactions conducted under ultrasound irradiation.

N-Benzylidene-2-methylpropan-2-amine (1)

Following the procedure reported by Stefani *et al.*,[38] in a dry flask, *tert*-butyl amine (116 mg, 1.59 mmol) and benzaldehyde (168 mg, 1.59 mmol) were dissolved in absolute EtOH (2.65 mL), and silica (477 mg) was added. The mixture was reacted in an inert atmosphere under ultrasound irradiation at room temperature for 2 h. Silica was filtered, and the solvent was removed under reduced pressure to provide the title compound **1**, that was used without further purification (256 mg, quantitative yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.29 (s, 1H, HC═N), 7.76 (dd, J = 6.6, 3.0 Hz, 2H, HC_{Ar}), 7.41 (m 3H, HC_{Ar}), 1.31 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.1 (C═N), 129.1 (C_{Ar}), 127.5 (2C, C_{Ar}), 126.9 (2C, C_{Ar}), 56.2 ($C(CH_3)_3$), 28.7 (3C, CH_3).

(2*S**,3*S**)-1-(*tert*-Butyl)-5-oxo-2-phenylpyrrolidine-3-carboxylic acid (2)

In a dry round bottom flask charged with a magnetic stir bar, imine **1** (1.85 g, 11.5 mmol, 1 eq.) was dissolved in 12 mL of dry xylene (1 M), succinic anhydride (1.15 g, 1 eq.) was added and the reaction mixture was refluxed overnight. The reaction mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The oily crude was dissolved in a mixture of Et_2O and a saturated aqueous solution of $NaHCO_3$, and the two phases were separated. The aqueous phase was then acidified to pH = 1 and extracted three times with CH_2Cl_2. The organic phase was collected, washed with Brine, and concentrated *in vacuo*. Pure **2** was obtained in 83% yield (2.5 g, 9.57 mmol) after flash chromatography (EtOAc/*n*-hexane (2 : 1), R_f = 0.05). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.84 (s, 1H, CO_2H), 7.40–7.25 (m, 5H, HC_{Ar}), 5.29 (s, 1H, HC_2), 2.95 (dd, J = 17.1, 9.2 Hz, 1H, HC_4), 2.78 (dd, J = 21.7, 13.4 Hz, 2H, HC_3 + H′C_4), 1.34 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2 (COOH), 174.9 (NC═O), 142.3 (C_{Ar}), 129.1 (C_{Ar}), 128.0

(C_{Ar}), 125.3 (2C, C_{Ar}), 64.7 ($C(CH_3)_3$), 55.7 (C_2), 46.0 (C_3), 33.8 (C_4), 27.9 (3C, CH_3). MS (ESI) *m*/*z* (%) = 260.10 (100, $[M - H]^-$). Anal. calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.08; H, 7.36; N, 5.33.

(2*S**,3*S**)-1-(*tert*-Butyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl) pyrrolidine-3-carboxamide (3)

Compound **2** (2.31 g, 8.86 mmol, 1 eq.), HOBt·H_2O (1.44 g, 10.6 mmol, 1.2 eq.) and 44 mL of dry CH_2Cl_2 (0.2 M) were added to a dry round bottom and stirred for 5 minutes under inert atmosphere. Then, DIC (1.34 g, 10.6 mmol, 1.2 eq.) was added, and the reaction mixture was stirred for other 5 minutes before adding 8-aminoquinoline (1.41 g, 9.75 mmol, 1.1 eq.). The mixture was left stirring at room temperature for 72 h under inert atmosphere, then it was diluted with CH_2Cl_2, and a saturated aqueous $NaHCO_3$ solution was added and extracted three times with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was chromatographed (Et_2O/*n*-hexane (2 : 1), R_f = 0.29) to provide pure **3** (2.3 g, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H, O═CNH), 8.80–8.68 (m, 2H, HC_{Ar}), 8.15 (dd, J = 8.3, 1.6 Hz, 1H, HC_{Ar}), 7.58–7.47 (m, 2H, HC_{Ar}), 7.48–7.35 (m, 3H, HC_{Ar}), 7.35–7.30 (m, 3H, HC_{Ar}), 5.35 (s, 1H, HC_2), 3.06 (m, 2H, HC_4 + HC_3), 2.75 (dd, J = 16.4, 1.5 Hz, 1H, H′C_4), 1.40 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5 (C═ONH), 171.1 (C═ON*t*Bu), 148.1 (C_{Ar}), 143.6 (C_{Ar}), 138.1 (C_{Ar}), 136.4 (C_{Ar}), 134.0 (C_{Ar}), 129.1 (C_{Ar}), 127.9 (C_{Ar}), 127.8 (C_{Ar}), 127.3 (C_{Ar}), 125.4 (2C, C_{Ar}), 121.9 (C_{Ar}), 121.6 (C_{Ar}), 116.7 (C_{Ar}), 64.7 ($C(CH_3)_3$), 55.5 (C_2), 48.4 (C_3), 41.8 (C_4), 28.0 (3C, CH_3). MS (ESI) *m*/*z* (%) = 388.16 (100, $[M + H]^+$). Anal. calcd for $C_{24}H_{25}N_3O_2$: C, 74.39; H, 6.50; N, 10.84. Found: C, 74.51; H, 6.53; N, 10.56.

General procedure for the C(sp^3)–H bond arylation reaction

A dry microwave reaction vial was charged with compound **3** (1 eq.), dibenzyl phosphate (0.2 eq.), AgOAc (2 eq.), ArI (3 eq.), Pd $(OAc)_2$ (0.1 eq.), and vacuum/N_2 cycles were performed (n.b. When aryl iodides were liquid, they were added immediately after vacuum/N_2 cycles). Dry 1,4-dioxane was added, and the reaction mixture was reacted at 110 °C in a pre-heated oil bath under rapid stirring for 21 h under inert atmosphere. After cooling to room temperature, the reaction mixture was diluted with EtOAc, filtered over Celite and concentrated *in vacuo*. The crude product was purified by FCC (Et_2O/*n*-hexane) to provide the pure arylated product.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-5-oxo-2,4-diphenyl-*N*-(quinolin-8-yl) pyrrolidine-3-carboxamide (4a)

Compound **4a** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (60 mg, 99% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.31). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, O═CNH), 8.58 (dd, J = 4.1, 1.3 Hz, 1H, HC_{Ar}), 8.46 (dd, J = 6.0, 3.0 Hz, 1H, HC_{Ar}), 8.07 (d, J = 8.1 Hz, 1H, HC_{Ar}), 7.48–7.32 (m, 8H, HC_{Ar}), 7.21 (d, J = 7.4 Hz, 2H, HC_{Ar}), 6.98 (t, J = 7.7 Hz, 2H, HC_{Ar}), 6.78 (t, J = 7.4 Hz, 1H, HC_{Ar}), 5.48 (s, 1H, HC_2), 4.49 (d, J = 9.5 Hz, 1H, HC_4), 3.37 (d, J = 9.4 Hz, 1H, HC_3), 1.55 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C═ONH), 169.4 (C═ON*t*Bu), 147.5 (C_{Ar}), 143.0 (C_{Ar}), 137.8 (C_{Ar}), 136.3 (C_{Ar}), 135.2 (C_{Ar}), 133.5 (C_{Ar}), 132.1 (C_{Ar}), 129.6 (2C, C_{Ar}), 129.2 (2C, C_{Ar}), 128.1 (2C, C_{Ar}), 127.9 (C_{Ar}), 127.9 (C_{Ar}), 127.2 (C_{Ar}), 126.9 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.6 (CAr), 121.4 (C_{Ar}), 62.2 ($C(CH_3)_3$), 56.3 (C_2), 56.0 (C_4), 50.5 (C_3), 28.1 (3C, CH_3). MS (ESI) *m*/*z* (%) = 486.27 (100, $[M + Na]^+$). Anal. calcd for $C_{30}H_{29}N_3O_2$: C, 77.73; H, 6.31; N, 9.06. Found: C, 77.95; H, 6.38; N, 8.99.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)-4-(*p*-tolyl)pyrrolidine-3-carboxamide (4b)

Compound **4b** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (59 mg, 96% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.42). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H, O═CNH), 8.58 (dd, J = 4.2, 1.4 Hz, 1H, HC_{Ar}), 8.47 (dd, J = 6.8, 2.1 Hz, 1H, HC_{Ar}), 8.08 (d, J = 8.0 Hz, 1H, HC_{Ar}), 7.50–7.31 (m, 8H, HC_{Ar}), 7.06 (d, J = 8.0 Hz, 2H, HC_{Ar}), 6.74 (d, J = 7.9 Hz, 2H, HC_{Ar}), 5.48 (s, 1H, HC_2), 4.44 (d, J = 9.4 Hz, 1H, HC_4), 3.33 (d, J = 9.4 Hz, 1H, HC_3), 1.82 (s, 3H, OCH_3), 1.54 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6 (C═ONH), 169.5 (C═ON*t*Bu), 147.3 (C_{Ar}), 143.0 (C_{Ar}), 137.1 (CAr), 136.4 (C_{Ar}), 133.6 (C_{Ar}), 132.0 (C_{Ar}), 131.1 (C_{Ar}), 129.4 (2C, C_{Ar}), 129.2 (2C, C_{Ar}), 128.8 (2C, C_{Ar}), 127.8 (C_{Ar}), 127.6 (C_{Ar}), 127.2 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.5 (C_{Ar}), 121.2 (C_{Ar}), 116.6 (C_{Ar}), 62.1 ($C(CH_3)_3$), 56.3 (C_2), 55.9 (C_4), 50.1 (C_3), 28.1 (3C, CH_3), 20.6. MS (ESI) *m*/*z* (%) = 500.27 (100, $[M + Na]^+$). Anal. calcd for $C_{31}H_{31}N_3O_2$: C, 77.96; H, 6.54; N, 8.80. Found: C, 78.14; H, 6.59; N, 8.73.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)-4-(*m*-tolyl)pyrrolidine-3-carboxamide (4c)

Compound **4c** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (53 mg, 87% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.41). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H, O═CNH), 8.56 (d, J = 3.3 Hz, 1H, HC_{Ar}), 8.48 (dd, J = 6.4, 2.3 Hz, 1H, HC_{Ar}), 8.06 (d, J = 8.1 Hz, 1H, HC_{Ar}), 7.50–7.31 (m, 8H, HC_{Ar}), 6.97 (d, J = 7.2 Hz, 2H, HC_{Ar}), 6.82 (t, J = 7.7 Hz, 1H, HC_{Ar}), 6.49 (d, J = 7.5 Hz, 1H, HC_{Ar}), 5.49 (s, 1H, HC_2), 4.45 (d, J = 9.5 Hz, 1H, HC_4), 3.33 (d, J = 9.5 Hz, 1H, HC_3), 1.91 (s, 3H, OCH_3), 1.55 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C═ONH), 169.4 (C═ON*t*Bu), 147.5 (C_{Ar}), 143.0 (C_{Ar}), 137.5 (C_{Ar}), 136.1 (C_{Ar}), 134.9 (C_{Ar}), 133.7 (C_{Ar}), 130.2 (C_{Ar}), 129.2 (2C, C_{Ar}), 128.0 (C_{Ar}), 127.8 (C_{Ar}), 127.6 (C_{Ar}), 127.5 (C_{Ar}), 127.1 (C_{Ar}), 126.5 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.4 (C_{Ar}), 121.3 (C_{Ar}), 116.1 (C_{Ar}), 110.4 (C_{Ar}), 62.0 ($C(CH_3)_3$), 56.3 (C_2), 55.9 (C_4), 50.5 (C_3), 28.1 (3C, CH_3), 20.9 (CH_3). MS (ESI) *m*/*z* (%) = 478.23 (100, $[M + H]^+$). Anal. calcd for $C_{31}H_{31}N_3O_2$: C, 77.96; H, 6.54; N, 8.80. Found: C, 78.13; H, 6.61; N, 8.73.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-4-(4-formylphenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4d)

Compound **4d** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (45 mg, 71% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.33). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H, CHO), 9.12 (s, 1H, O═CNH), 8.54 (d, J = 4.0 Hz, 1H, HC_{Ar}), 8.50–8.38 (m, 1H, HC_{Ar}), 8.04 (d, J = 8.2 Hz, 1H, HC_{Ar}), 7.46 (t,

J = 7.7 Hz, 4H, HC_{Ar}), 7.43–7.31 (m, 8H, HC_{Ar}), 5.48 (s, 1H, HC_2), 4.55 (d, J = 9.3 Hz, 1H, HC_4), 3.37 (d, J = 9.3 Hz, 1H, HC_3), 1.55 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5 (CHO), 172.4 (C═ONH), 168.8 (C═ON*t*Bu), 147.8 (C_{Ar}), 142.5 (C_{Ar}), 142.4 (C_{Ar}), 137.9 (C_{Ar}), 136.1 (C_{Ar}), 135.0 (C_{Ar}), 133.4 (C_{Ar}), 130.5 (2C, C_{Ar}), 129.5 (2C, C_{Ar}), 129.3 (2C, C_{Ar}), 128.1 (C_{Ar}), 127.6 (C_{Ar}), 127.0 (C_{Ar}), 125.6 (2C, C_{Ar}), 121.9 (C_{Ar}), 121.5 (C_{Ar}), 116.2 (C_{Ar}), 62.3 ($C(CH_3)_3$), 56.3 (C_2), 56.2 (C_4), 50.4 (C_3), 28.1 (3C, CH_3). MS (ESI) m/z (%) = 514.28 (100, $[M + Na]^+$). Anal. calcd for $C_{31}H_{29}N_3O_3$: C, 75.74; H, 5.95; N, 8.55. Found: C, 75.91; H, 6.99; N, 8.51.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-4-(3-methoxyphenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4e)

Compound **4e** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (60 mg, 95% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.36). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H, O═CNH), 8.60–8.52 (m, 1H, HC_{Ar}), 8.49 (dd, J = 6.0, 3.0 Hz, 1H, HC_{Ar}), 8.06 (dd, J = 8.2, 1.3 Hz, 1H, HC_{Ar}), 7.48–7.32 (m, 8H, HC_{Ar}), 6.90–6.67 (m, 3H, HC_{Ar}), 6.30–6.18 (m, 1H, HC_{Ar}), 5.50 (s, 1H, HC_2), 4.45 (d, J = 9.5 Hz, 1H, HC_4), 3.49 (s, 3H, OCH_3), 3.40–3.26 (m, 1H, HC_3), 1.53 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2 (C═ONH), 169.3 (C═ON*t*Bu), 159.3 (C_{Ar}), 147.6 (C_{Ar}), 142.9 (C_{Ar}), 136.5 (C_{Ar}), 133.7 (C_{Ar}), 130.7 (C_{Ar}), 129.7 (C_{Ar}), 129.2 (2C, C_{Ar}), 129.1 (C_{Ar}), 127.9 (C_{Ar}), 127.5 (C_{Ar}), 127.0 (CAr), 125.7 (2C, C_{Ar}), 122.9 (C_{Ar}), 121.8 (C_{Ar}), 121.5 (C_{Ar}), 121.3 (C_{Ar}), 114.7 (C_{Ar}), 113.0 (C_{Ar}), 62.1 ($C(CH_3)_3$), 56.2 (C_2), 56.0 (C_4), 54.8 (OCH_3), 50.5 (C_3), 28.0 (3C, CH_3). MS (ESI) m/z (%) = 516.18 (100, $[M + Na]^+$). Anal. calcd for $C_{31}H_{31}N_3O_3$: C, 75.43; H, 6.33; N, 8.51. Found: C, 75.60; H, 6.35; N, 8.47.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-4-(4-methoxyphenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4f)

Compound **4f** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (50 mg, 79% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.38). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H, O═CNH), 8.58 (d, J = 4.0 Hz, 1H, HC_{Ar}), 8.52–8.45 (m, 1H, HC_{Ar}), 8.07 (s, 1H, HC_{Ar}), 7.48–7.31 (m, 8H, HC_{Ar}), 7.10 (d, J = 8.5 Hz, 2H, HC_{Ar}), 6.50 (d, J = 8.5 Hz, 2H, HC_{Ar}), 5.47 (s, 1H, HC_2), 4.44 (d, J = 9.4 Hz, 1H, HC_4), 3.37 (s, 3H, OCH_3), 3.32 (d, J = 9.2 Hz, 1H, HC_3), 1.53 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6 (C═ONH), 169.5 (C═ON*t*Bu), 158.4 (C_{Ar}), 147.5 (C_{Ar}), 143.0 (C_{Ar}), 137.8 (C_{Ar}), 136.3 (C_{Ar}), 133.7 (C_{Ar}), 130.7 (2C, C_{Ar}), 129.2 (2C, C_{Ar}), 127.9 (C_{Ar}), 127.6 (C_{Ar}), 127.1 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.6 (C_{Ar}), 121.3 (C_{Ar}), 116.4 (C_{Ar}), 113.7 (2C, C_{Ar}), 108.5 (C_{Ar}), 62.1 ($C(CH_3)_3$), 56.3 (C_2), 55.9 (C_4), 54.8 (OCH_3), 49.7 (C_3), 28.1 (3C, CH_3). MS (ESI) m/z (%) = 516.30 (100, $[M + Na]^+$). Anal. calcd for $C_{31}H_{31}N_3O_3$: C, 75.43; H, 6.33; N, 8.51. Found: C, 75.61; H, 6.36; N, 8.47.

(2*S**,3*S**,4*S**)-4-(4-Bromophenyl)-1-(*tert*-butyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4g)

Compound **4g** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (67 mg, 96% yield, EtOAc/*n*-hexane (1 : 3), R_f = 0.38). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, O═CNH), 8.58 (dd, J = 4.2, 1.5 Hz, 1H, HCAr), 8.47 (t, J = 4.5 Hz, 1H, HCAr), 8.10 (d, J = 8.1 Hz, 1H, HCAr), 7.48–7.30 (m, 8H, HCAr), 7.07 (s, 4H, HC_{Ar}) 5.46 (s, 1H, HC_2), 4.43 (d, J = 9.3 Hz, 1H, HC_4), 3.33 (d, J = 9.2 Hz, 1H, HC_3), 1.53 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8 (C═ONH), 169.1 (C═ON*t*Bu), 147.8 (C_{Ar}), 142.7 (C_{Ar}), 136.2 (C_{Ar}), 134.2 (C_{Ar}), 131.4 (2C, C_{Ar}), 131.2 (2C, C_{Ar}), 129.3 (2C, C_{Ar}), 128.0 (C_{Ar}), 127.7 (C_{Ar}), 127.1 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.9 (C_{Ar}), 121.6 (C_{Ar}), 121.2 (C_{Ar}), 110.5 (C_{Ar}), 110.0 (C_{Ar}), 108.5 (C_{Ar}), 62.2 ($C(CH_3)_3$), 56.2 (C_2), 56.1 (C_4), 49.8 (C_3), 28.1 (3C, CH_3). MS (ESI) m/z (%) = 564.21 (100, $[M + Na]^+$). Anal. calcd for $C_{30}H_{28}BrN_3O_2$: C, 66.42; H, 5.20; N, 7.75. Found: C, 66.59; H, 5.30; N, 7.68.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-4-(4-chlorophenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4h)

Compound **4h** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (63 mg, 97% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.31). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H, O═CNH), 8.57 (dd, J = 4.2, 1.5 Hz, 1H, HC_{Ar}), 8.48 (t, J = 4.5 Hz, 1H, HC_{Ar}), 8.08 (dd, J = 8.3, 1.5 Hz, 1H, HC_{Ar}), 7.48–7.32 (m, 8H, HC_{Ar}), 7.14 (d, J = 8.4 Hz, 2H, HC_{Ar}), 6.93 (d, J = 8.4 Hz, 2H, HC_{Ar}), 5.47 (s, 1H, HC_2), 4.45 (d, J = 9.3 Hz, 1H, HC_4), 3.33 (d, J = 9.3 Hz, 1H, HC_3), 1.54 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9 (C═ONH), 169.1 (C═ON*t*Bu), 147.8 (C_{Ar}), 142.6 (C_{Ar}), 137.9 (C_{Ar}), 136.1 (C_{Ar}), 133.6 (C_{Ar}), 133.5 (C_{Ar}), 132.9 (C_{Ar}), 131.0 (2C, C_{Ar}), 129.2 (2C, C_{Ar}), 128.2 (2C, C_{Ar}), 128.0 (C_{Ar}), 127.6 (C_{Ar}), 127.0 (C_{Ar}), 125.6 (2C, C_{Ar}), 121.8 (C_{Ar}), 121.5 (C_{Ar}), 116.3 (C_{Ar}), 62.2 ($C(CH_3)_3$), 56.2 (C_2), 56.0 (C_4), 49.7 (C_3), 28.0 (3C, CH_3). MS (ESI) m/z (%) = 520.28 (100, $[M + Na]^+$). Anal. calcd for $C_{30}H_{28}ClN_3O_2$: C, 72.35; H, 5.67; N, 8.44. Found: C, 72.54; H, 5.69; N, 8.38.

(2*S**,3*S**,4*S**)-1-(*tert*-Butyl)-4-(3-fluorophenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4i)

Compound **4i** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (68 mg, 94% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.40). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H, O═CNH), 8.60 (dd, J = 4.2, 1.5 Hz, 1H, HC_{Ar}), 8.48 (dd, J = 5.6, 3.3 Hz, 1H, HC_{Ar}), 8.07 (d, J = 8.2 Hz, 1H, HC_{Ar}), 7.49–7.31 (m, 8H, HCAr), 6.99 (dd, J = 7.6, 4.7 Hz, 2H, HC_{Ar}), 6.90 (td, J = 8.1, 6.1 Hz, 1H, HC_{Ar}), 6.48–6.40 (m, 1H, HC_{Ar}), 5.48 (s, 1H, HC_2), 4.48 (d, J = 9.4 Hz, 1H, HC_4), 3.35 (d, J = 9.4 Hz, 1H, HC_3), 1.54 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7 (C═ONH), 169.0 (C═ON*t*Bu), 163.7 (C_{Ar}), 161.2 (C_{Ar}), 147.7 (C_{Ar}), 142.7 (C_{Ar}), 137.6 (d, J = 7.7 Hz, 1C, C_{Ar}), 136.2 (C_{Ar}), 133.5 (C_{Ar}), 129.5 (d, J = 8.4 Hz, 1C, C_{Ar}), 129.2 (2C, C_{Ar}), 128.0 (C_{Ar}), 127.6 (C_{Ar}), 127.1 (C_{Ar}), 125.6 (2C, C_{Ar}), 125.2 (d, J = 2.8 Hz, 1C, C_{Ar}), 121.7 (C_{Ar}), 121.4 (C_{Ar}), 116.8 (d, J = 22.2 Hz, 1C, C_{Ar}), 116.2 (C_{Ar}), 113.8 (d, J = 21.2 Hz, C_{Ar}), 62.1 ($C(CH_3)_3$), 56.1 (C_2), 56.0 (C_4), 50.0 (C_3), 28.0 (3C, CH_3). MS (ESI) m/z (%) = 504.27 (100, $[M + Na]^+$). Anal. calcd for $C_{30}H_{28}FN_3O_2$: C, 74.82; H, 5.86; N, 8.73. Found: C, 75.21; H, 5.82; N, 8.68.

(2*S,3*S**,4*S**)-1-(*tert*-Butyl)-4-(3-nitrophenyl)-5-oxo-2-phenyl-*N*-(quinolin-8-yl)pyrrolidine-3-carboxamide (4j)**

Compound **4j** was obtained following general procedure for the C(sp^3)–H bond arylation reaction (67 mg, 97% yield, Et_2O/*n*-hexane (2 : 1), R_f = 0.27). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H, O═CNH), 8.49 (dd, J = 4.2, 1.5 Hz, 1H, HCAr), 8.43 (dd, J = 5.8, 3.1 Hz, 1H, HC_{Ar}), 8.13 (s, 1H, HC_{Ar}), 8.05 (dd, J = 8.3, 1.5 Hz, 1H, HC_{Ar}), 7.57 (dd, J = 10.7, 4.8 Hz, 2H, HC_{Ar}), 7.50–7.44 (m, 2H, HC_{Ar}), 7.44–7.37 (m, 5H, HC_{Ar}), 7.35 (dd, J = 8.3, 4.2 Hz, 1H, HC_{Ar}), 7.08 (t, J = 8.0 Hz, 1H, HC_{Ar}), 5.49 (s, 1H, HC_2), 4.60 (d, J = 9.3 Hz, 1H, HC_4), 3.40 (d, J = 9.3 Hz, 1H, HC_3), 1.55 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0 (C═ONH), 168.7 (C═ON*t*Bu), 147.9 (C_{Ar}), 147.7 (C_{Ar}), 142.4 (C_{Ar}), 137.8 (C_{Ar}), 137.5 (C_{Ar}), 136.2 (C_{Ar}), 135.7 (C_{Ar}), 133.2 (C_{Ar}), 129.4 (2C, C_{Ar}), 129.0 (C_{Ar}), 128.2 (C_{Ar}), 127.6 (C_{Ar}), 127.0 (C_{Ar}), 125.6 (2C, C_{Ar}), 125.1 (C_{Ar}), 121.9 (2C, C_{Ar}), 121.7 (C_{Ar}), 116.2 (C_{Ar}), 62.2 ($C(CH_3)_3$), 56.3 (C_2), 56.1 (C_4), 50.0 (C_3), 28.1 (3C, CH_3). MS (ESI) *m*/*z* (%) = 531.23 (100, $[M + Na]^+$). Anal. calcd for $C_{30}H_{28}N_4O_4$: C, 70.85; H, 5.55; N, 11.02. Found: C, 71.08; H, 5.62; N, 10.93.

(2*S,3*S**,4*S**)-1-(*tert*-Butyl)-4-(3-methoxyphenyl)-5-oxo-2-phenylpyrrolidine-3-carboxylic acid (5a)**

Compound **4e** (209 mg, 0.423 mmol) was solubilized in MeCN (2.2 mL), then $(Boc)_2O$ (277 mg, 1.27 mmol) and DMAP (5 mg, 0.042 mmol) were added. The reaction solution was warmed to 50 °C and stirred for 2 hours. Then, it was cooled to room temperature, stirred for additional 18 hours, and the solvent was removed *in vacuo*. The crude Boc-derivative was solubilized in THF/H_2O (3 : 1) (4.2 mL), then $LiOH{\cdot}H_2O$ (106 mg, 2.52 mmol) and 30% H_2O_2 solution (480 µL, 1.90 mmol) were successively added at 0 °C. The reaction mixture was stirred for 20 minutes at 0 °C, then warmed to room temperature and stirred for additional 18 hours. Et_2O was added to the reaction mixture, the aqueous phase was acidified to pH = 2 with 6M HCl and extracted three times with EtOAc. The collected organic phase was washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford pure carboxylic derivative **5a** (136 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, J = 7.2 Hz, 2H, HC_{Ar}), 7.34 (dd, J = 13.2, 7.2 Hz, 3H, HC_{Ar}), 7.14 (t, J = 7.8 Hz, 1H, HC_{Ar}), 6.78–6.72 (m, 3H, HC_{Ar}), 5.13 (s, 1H, HC_2), 4.23 (d, J = 9.3 Hz, 1H, HC_4), 3.71 (s, 3H, OCH_3), 3.16 (d, J = 9.3 Hz, 1H, HC_3), 1.39 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7 (COOH), 173.5 (C═ON*t*Bu), 159.2 (C_{Ar}), 142.2 (C_{Ar}), 136.6 (C_{Ar}), 129.2 (2C, C_{Ar}), 128.1 (C_{Ar}), 125.7 (2C, C_{Ar}), 121.7 (C_{Ar}), 114.7 (C_{Ar}), 113.3 (C_{Ar}), 108.5 (C_{Ar}), 62.4 ($C(CH_3)_3$), 56.0 (C_2), 55.0 (C_4), 53.9 (OCH_3), 49.4 (C_3), 27.9 (3C, CH_3). MS (ESI) *m*/*z* (%) = 733.00 (100, $[2M - H]^-$), 366.17 (55, $[M - H]^-$). Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 72.12; H, 6.90; N, 3.77.

(2*S,3*S**,4*S**)-1-(*tert*-Butyl)-4-(4-bromo)-5-oxo-2-phenylpyrrolidine-3-carboxylic acid (5b)**

Compound **5b** was obtained in 76% yield as described for **5a** starting from **4g** (209 mg, 0.423 mmol) as a 2 : 1 mixture of unseparable epimers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.28 (m, 7H, HC_{Ar}), 7.14 (d, J = 8.5 Hz, 2H minor, HC_{Ar}), 7.08 (d, J = 8.4 Hz, 2H major, HC_{Ar}), 5.13 (s, 1H major + 1H minor, HC_2), 4.26 (d, J = 9.2 Hz, 1H major, HC_4), 3.96 (d, J = 7.1 Hz, 1H minor, HC_4), 3.20 (d, J = 9.3 Hz, 1H major, HC_3), 3.06–2.99 (m, 1H minor, HC_3), 1.42 (s, 9H major, CH_3), 1.34 (s, 9H minor, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8 (major, COOH), 173.3 (minor, COOH), 172.7 (2C, major + minor, C═ON^{t}Bu), 144.2 (minor, C_{Ar}), 141.9 (major, C_{Ar}), 136.7 (minor, C_{Ar}), 134.2 (minor, C_{Ar}), 131.8 (2C, minor, C_{Ar}), 131.4 (2C, major, C_{Ar}), 131.3 (2C, major, C_{Ar}), 129.8 (2C, minor, C_{Ar}), 129.3 (2C, major, C_{Ar}), 129.1 (2C, minor, C_{Ar}), 128.3 (major, C_{Ar}), 128.1 (minor, C_{Ar}), 125.8 (2C, minor, C_{Ar}), 125.6 (2C, major, C_{Ar}), 121.6 (major, C_{Ar}), 121.4 (minor, C_{Ar}), 63.1 (minor, $\underline{C}(CH_3)_3$), 62.3 (major, $\underline{C}(CH_3)_3$), 56.7 (minor, C_2), 56.1 (major, C_2), 53.7 (major, C_4), 53.5 (minor, C_4), 51.2 (minor, C_3), 48.6 (major, C_3), 28.2 (3C, minor, CH_3), 28.0 (3C, major, CH_3). MS (ESI) *m*/*z* (%) = 438.10 (100, $[M + Na]^+$). Anal. calcd for $C_{21}H_{22}BrNO_3$: C, 60.59; H, 5.33; N, 3.36. Found: C, 60.98; H, 5.40; N, 3.28.

X-Ray data collection

Single crystals were mounted in a loop and intensity data collected at 100° K with a Bruker Apex-II CCD diffractometer, using a Cu–Kα (λ = 1.54184 Å) radiation. Data were collected with the Bruker APEX2 program, integrated and reduced with the Bruker SAINT software. The integrated intensities, measured using the φ and ω scan mode, were corrected for Lorentz and polarization effects. The substantial redundancy in data allows empirical absorption corrections to be applied using SADABS-2016/2 (Bruker AXS area detector scaling and absorption correction).40 Structures were solved by direct methods of SIR201941 and refined using the full-matrix least squares on F^2 provided by SHELXL-2014/6.42 The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms as isotropic.

Crystallographic data for 4g

Asymmetric unit contains two independent molecules. This is probably due to a slightly different orientation of the two condensed rings in molecule A compared to B. Molecular formula 2 × ($C_{30}H_{28}N_3O_2Br$), M = 2 × 542.46, triclinic, space group $P\bar{1}$, a = 9.6503(2), b = 12.2687(2), c = 22.1064(4) Å, α = 81.658(1) β = 84.899(1) γ = 77.132(1) V = 2520.2(1) Å^3, Z = 2 D_c = 1.430, μ = 2.483 mm^{-1}, F(000) = 1120. 43 937 reflections were collected with a 2.023 < θ < 68.556 range with a completeness to theta 98.7%; 9157 were independent, the parameters were 658 and the final R index was 0.365 for reflections having $I > 2\sigma I$. Hydrogen atoms were assigned in calculated positions except for H on N2 (A + B) that were found in FD map. No relevant hydrogen bonds are detected.

Enzymatic BACE-1 assay

Primary BACE-1 enzymatic activity was assessed using a fluorometric assay kit (SensoLyte® 520 β-Secretase Assay Kit, Anaspec, USA) employing the fluorescent substrate HiLyte Fluor™ 488-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys (QXL™ 520)-

OH. All the measurements were performed in 96-well plates with a BMG labtech OptimaStar microplate reader. The inhibitors (10 μM) were pre-incubated with the BACE-1 enzyme (1 μg mL^{-1}) for 10 minutes at 25 °C before starting the reaction by substrate addition. The fluorescence was monitored over 30 minutes (λ_{ex} = 490 nm, λ_{em} = 520 nm) at 25 °C. The percentages of inhibition for the test compounds were determined through the eqn $(1 - V_s/V_o) \times 100$, where V_s is the initial velocity in the presence of the inhibitor and V_o is the initial velocity of the unhibited reaction. The IC_{50} value of **4g** and **5b** were obtained by dose–response measurements using 0.01–100 μM as the range of inhibitor concentrations. All the experiments were performed in triplicate and the collected data were analyzed using Graphpad 4.0 software package (Graphpad Prism, San Diego, CA).

Docking calculations

Automated docking studies were carried out using the Lamarckian Genetic Algorithm (LGA) as a search engine implemented in the Autodock 4.0.1 program.[44] The AutoDockTools 1.4.5 (ADT) graphical interface[45] was used to prepare BACE1 and inhibitor PDBQT files. Coordinates of **4g** were generated using Spartan (version 5.147), and then energy-minimized through the AM1 semi-empirical method to calculate the equilibrium geometry. The coordinates of BACE1 protease were retrieved from the Protein Data Bank (PDB code: 5CLM), and enzyme-inhibitor complex was unmerged for achieving the free enzyme structure. Water molecules were removed. Hydrogen atoms were added to the enzyme and the ligand, Gasteiger charges were added and non-polar hydrogens were merged. Three-dimensional energy scoring grids of 0.375 Å resolution and 40 Å × 40 Å × 40 Å dimensions were computed. The center of the grid was set using the coordinates of one of the oxygen atoms of D228. A total of 50 runs with a maximum of 2 500 000 energy evaluations were carried out for **4g**, using the default parameters for LGA. Low energy ligand–protein complexes were subjected to AMMP energy minimization using VegaZZ,[46] then Cluster analysis was performed on docked results using a root-mean-square (rms) tolerance of 1.5 Å. The analysis of the binding mode of the docked conformations was carried out using the Autodock plugin within PyMol software v0.99.[47]

Conflicts of interest

There are no conflicts to declare.

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