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New phenylaniline derivatives as modulators of amyloid protein precursor metabolism

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ABSTRACT

The chloroquinoline scaffold is characteristic of anti-malarial drugs such as chloroquine (CQ) or amodiaquine (AQ). These drugs are also described for their potential effectiveness against prion disease, HCV, EBV, Ebola virus, cancer, Parkinson or Alzheimer diseases. Amyloid precursor protein (APP) metabolism is deregulated in Alzheimer's disease. Indeed, CQ modifies amyloid precursor protein (APP) metabolism by precluding the release of amyloid-beta peptides (Aβ), which accumulate in the brain of Alzheimer patients to form the so-called amyloid plaques. We showed that AQ and analogs have similar effects although having a higher cytotoxicity. Herein, two new series of compounds were synthesized by replacing 7-chloroquinolin-4-amine moiety of AQ by 2-aminomethylaniline and 2-aminomethylphenyle moieties. Their structure activity relationship was based on their ability to modulate APP metabolism, Aβ release, and their cytotoxicity similarly to CQ. Two compounds **15a**, **16a** showed interesting and potent effect on the redirection of APP metabolism toward a decrease of Aβ peptide release (in the same range compared to AQ), and a 3–10-fold increased stability of APP carboxy terminal fragments (CTFα and AICD) without obvious cellular toxicity at 100 μM.

1. Introduction

4-Aminoquinoline moiety is present in many biologically active compounds. Compounds bearing this sub-structure are described with anti-tuberculosis,[1] anti-leishmania[2] or anti-inflammatory,[3] or antiviral (Ebola[4]) activity or against Parkinson's disease[5] although an anti-malarial therapeutic indication is most well-known. The most used compounds of this family are chloroquine (CQ) and amodiaquine (AQ) (Fig. 1). For decades, CQ has been one of the two most widely used antimalarial drugs with moderate acute toxicity. Following a repositioning strategy, CQ and CQ-derived compounds have already been evaluated in several medical indications such as prion disease,[6–9] HCV[10,11] and even cancer.[12,13] CQ-derived compounds such as hydroxychloroquine are administered, for instance, for the treatment of systemic lupus erythematosus[14] or rheumatoid polyarthritis.[15] AQ proved to be effective against CQ-resistant malarial strains,[16] and was also described with antiviral (Ebola,[4] dengue,[17]) and antibacterial (anthrax[18]) activity or even cancer.[19,20] The AQ major drawback is its weak metabolic stability leading to hepatotoxic derivatives with cases of agranulocytosis, neutropenia and hepatitis. The 4-hydroxyanilino moiety of AQ explained its toxicity.[21–23] Thus, a great number of AQ analogs without this 4-hydroxyaniline sub-structure have been synthesized to circumvent this drawback. Others and we succeeded in designing compounds with nanomolar activities on CQ-resistant strains of *P. falciparum*, the parasite responsible for malaria.[24–33] Isoquine derivatives are the most advanced compounds.

Considering Alzheimer's disease (AD), we previously reported indirect modulatory effect of CQ on APP metabolism.[34] The metabolism of this type transmembrane protein is central to AD pathophysiology. This complex metabolism leads to the production and release of amyloid-beta peptides (Aβ) of 35–43 amino acids among which $A\beta_{42}$ form neurotoxic oligomers and abnormally accumulates as parenchymal amyloid deposits in Alzheimer patient's brains. As illustrated in Fig. 2, $A\beta_{42}$ is an intermediate proteolytic product arising from the carboxy-peptidase activity of γ-secretase.[35] The ratio of $A\beta_{42}/A\beta_{40}$ is increased in AD suggesting that the carboxy-peptidase activity is defective. Part of the Aβ peptides are produced in acidic cellular compartments in which beta- and

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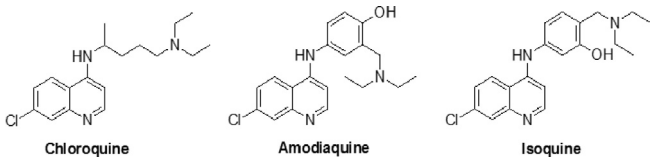


Fig. 1. Structures of anti-malarial 4-aminoquinolines, Chloroquine, Amodiaquine, and Isoquine.

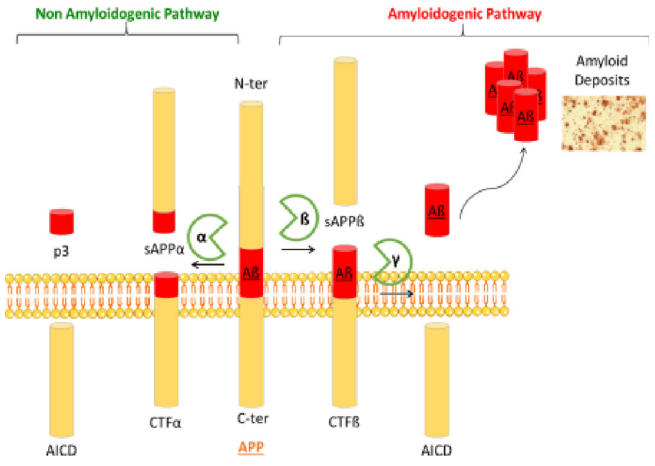


Fig. 2. Amyloid protein precursor (APP) metabolism.

gamma-secretase have both optimal activity.[36–42] CQ is known to repress Aβ peptides production through its lysomotropic activity. Considering the lack of curative therapeutic option, current strategies propose repositioning compounds or multifunctional molecules. For example, numerous works described the synthesis of multi-target compounds, exhibited significant ability to inhibit β-amyloid (Aβ) aggregation but also displayed antioxidant activity, biometal chelating ability[43] or cholinesterase inhibition.[44–46]

Our laboratory described *N,N′*-disubstituted piperazine derivatives of CQ and their ability to reduce the release of Aβ species and increase the amount of APP metabolites (carboxy terminal fragments CTFα and AICD).[47–50] We also demonstrated that it was possible to replace 7-chloroquinoline by other heterocycles or benzyl group. AQ and related anti-malarial analogs have also been evaluated and showed an improved ability to inhibit the release of Aβ species although both CTFα and AICD levels were reduced for AQ when compared to CQ effect (respectively 0.5 and 0.4) (Table 1).[50] Compounds **1**, **3**–**4** and **8** showed similar activities when compared to CQ or AQ for the inhibition of the secretion of Aβ species reaching 50% at 3.1–10 μM and 7.1–13.8 μM respectively for $A\beta_{1-40}$ and $A\beta_{1-42}$. Derivatives **2**, **5**–**7** and **9** showed better activities than CQ and AQ with 1.5–2.5 μM and 1.9–4.9 μM respectively. Increase amounts of CTFα were also achieved for compounds **2**, **5**–**7** and **9**, with a range of 1.2–2.1 when compared to CQ at 3 μM. Consequently, these compounds **2**, **5**–**7** and **9** also presented upsurge of AICD with a range of 0.9–3.4 when compared to CQ at 3 μM. These five compounds had better profile toward the reduction of both $A\beta_{40}$ and $A\beta_{42}$ production and increase of CTFα, AICD when compared to CQ. Especially, derivative **6** displayed an excellent profile for the APP metabolism with high CTFα, AICD versus to CQ, respectively 2.1 and 3.4.

Structure-activity relationships of CQ, AQ, and compounds **1**–**9** have shown (Table 1) that: 1) AQ seems more efficient than CQ to decrease Aβ secretion, 2) phenol function is not necessary (**1**), 3) addition of an amino side chain could improve of activity (**2** for instance, 4) modulation of diethylamino group to morpholine or 1-dimethylamine-2-methylaminoethyl is possible (**4**, **5**, **6** and **7**), 5) very small modifications could improve cytotoxicity (**6** and **7**) and 6) symmetric substitution with amino side chain is possible (**8** and **9**). Nevertheless, these compounds, though interesting hits, provided relatively high cytotoxicity in comparison with their activity.

Considering the challenge to provide new drugs for the treatment of neurodegenerative diseases, such as AD, we designed two original series of compounds (**16a**–**d**, **22a**–**d**) starting from compounds **6** and **9** with general structure I (Fig. 3). We aimed to evaluate the replacement of the 7-chloroquinolin-4-amine scaffold by 2-aminomethylaniline moiety for the first series of compounds **16a**–**d** and by 2-aminomethylphenyle for the other series of compounds **22a**–**d**. Previous results underlined the potential interest of amide compounds (**2**, **6** and **7**). Thus the dicarboxamide intermediate derivatives **15a**–**d**, **21a**–**d** were also considered and allowed us to determine the importance of the dicarboxamide groups (piperidinoethyl carboxamide moieties) when compared to their analogs **16a**–**d**, **22a**–**d**, with diamine groups (piperidinoethyl aminomethyl moieties). The amino groups (CH_2NR_2) introduced were piperidine, morpholine, piperazine and dimethylamine. Compounds **16a**–**d**, **22a**–**d** and their dicarboxamide analogs **15a**–**d**, **21a**–**d** were tested toward the modulation of APP metabolism and their cytotoxicity was also evaluated.

2. Chemistry

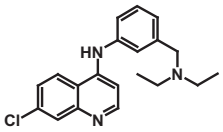
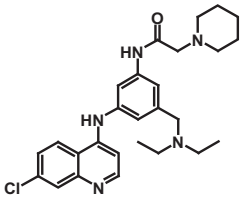
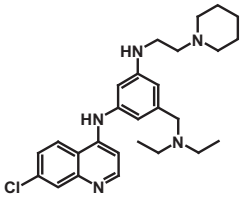
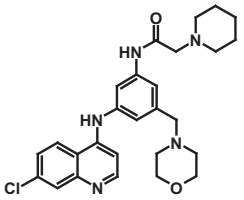
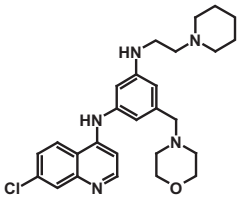
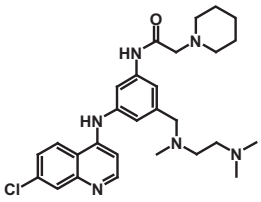
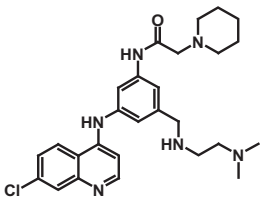
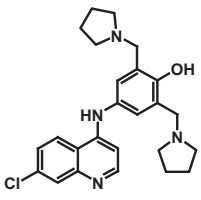
For the synthesis of compounds **16a**–**d**, aminomethylaniline intermediate **12a**–**d** were prepared in two steps (Scheme 1). For the first reaction, various attempts were engaged with piperidine as reagent. With $NaBH(OAc)_3$,[29,51] no reaction was observed and starting material was recovered. With $NaBH_4$ or $NaBH_3CN$ a mixture of derivatives was observed as compounds **11a** (27–47%), 2-nitrophenylmethanol (34–46%), resulting from the reduction of the aldehyde function, and with or without starting material (0–20%). Then, reduction with $NaBH_3CN$ and $ZnCl_2$ gave only compound **11a** with 85% yields.[52,53] The other derivatives were synthesized in the same conditions with 77–83%. The nitro group of compounds **11a**–**d** was reduced in ethanol with Pd/C and ammonium formate,[54] the resulting unstable aniline derivatives **12a**–**d** were then immediately introduced in the Buchwald reaction.

For the synthesis of compounds **16a-d** two ways were attempted in 3 or 4 steps (Scheme 2).

The first way was realized starting from 5-bromoisophthalic acid (**13**) with 1-piperidineethanamine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), hydroxybenzotriazole (HOBt) in DMF afforded dicarboxamide derivative **14** with a good yield of 89%.[51,55] A Buchwald reaction with the previously and freshly prepared aniline derivatives **12a**–**d** gave compounds **15a**–**d**. Best yields (30–88%) were in dioxane with Cs_2CO_3, Xantphos, and Pd_2dba_3. All attempts of reduction of the amide group in derivatives **15a**–**d** in THF with $LiAlH_4$ gave degradation of the reaction media. Then a second way was attempted in 4 steps. Starting from 5-bromoisophthalic acid (**13**), the Weinreb amide derivative **17** was prepared in a solution of DCM/ACN with EDC, hydroxyl benzotriazole (HOBt), *N,O*-dimethylhydroxylamine, *N*-methylmorpholine with good yield (89%). A Buchwald reaction, in the same optimized conditions, allowed to furnish derivatives **18a**–**d** (yields 26–92%), which were reduced in THF with $LiAlH_4$ and afforded derivatives **19a**–**d**. The last reaction was a reductive amination in DCE with the corresponding amine and $NaBH(OAc)_3$, to furnish compounds **16a**–**d**.[51,55]

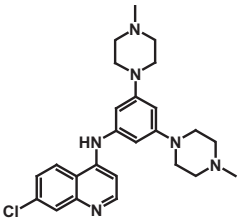
For the synthesis of compounds **22a**–**d**, two ways were also evaluated in 4 or 5 steps (Scheme 3). Same intermediates **14** and **17** were used.

Table 1
In vitro evaluation of anti-malarial drugs CQ, AQ and AQ analogs (**1**–**9**) on APP metabolism (SY5Y cells).

Cpd[a]	Structure	Cytotoxicity CC_{50} (μM)[b]	$A\beta_{1-40}$ IC_{50} (μM)[c,d]	$A\beta_{1-42}$ IC_{50} (μM)[c,d]	CTFα vs CQ (3μM)[d,e]	AICD vs CQ (3μM)[d,f]
CQ	–	30	7.0	12.8	1	1
AQ	–	10	4.0	5.4	0.5	0.4
1		12	4.5	10.4	0.6	0.5
2		4	1.5	2.8	1.6	0.9
3		15	10	13.8	0.5	0.4
4		10	3.1	7.1	0.7	0.5
5		10	2.5	4.9	1.2	1.2
6		4	1.7	2.9	2.1	3.4
7		15	2.0	2.1	1.7	2.3
8		30	5.1	11.2	0.5	0.6

(*continued on next page*)

Table 1 (*continued*)

Cpd[a]	Structure	Cytotoxicity CC_{50} (μM)[b]	$A\beta_{1-40}$ IC_{50} (μM)[c,d]	$A\beta_{1-42}$ IC_{50} (μM)[c,d]	CTFα vs CQ (3μM)[d,e]	AICD vs CQ (3μM)[d,f]
9		>15	2.3	1.9	1.4	1.1

[a] Compounds are described in Refs. 29–33.
[b] Compound concentration causing 50% of SY5Y cell death after 24 h treatment, done in duplicate.
[c] Compound concentration inhibiting 50% of Aβ peptide secretion.
[d] Mean values calculated on the basis of at least three independent experiments with less than 10% deviation.
[e] CTFα increase compared to chloroquine ([CTF]compound/[CTF]CQ) at 3 μM.
[f] AICD increase compared to chloroquine ([AICD]compound/ [AICD]CQ) at 3 μM.

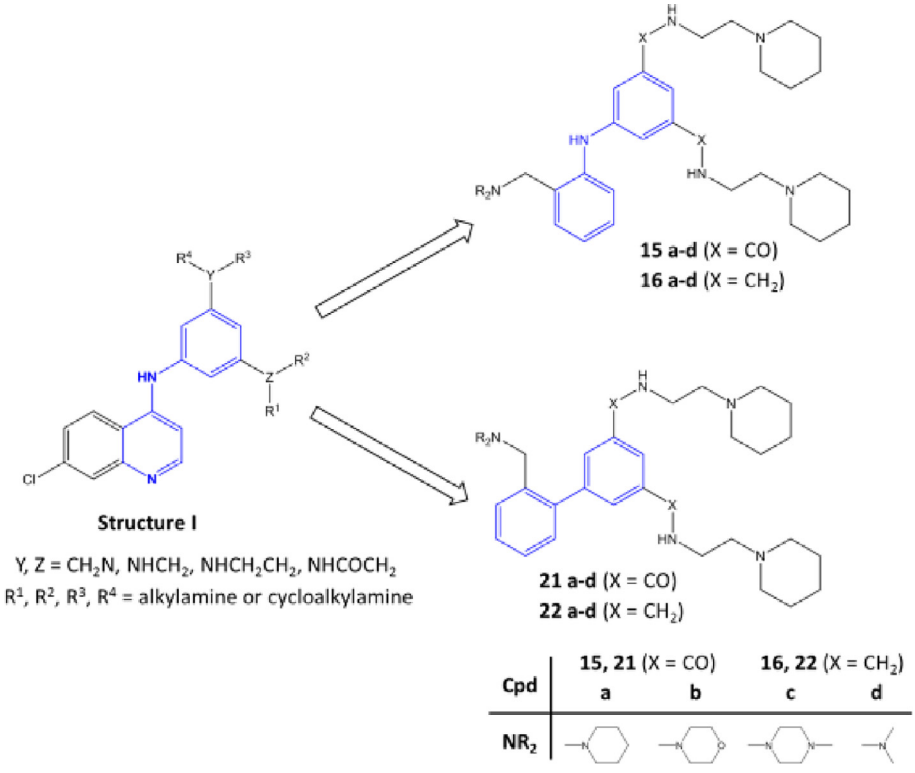
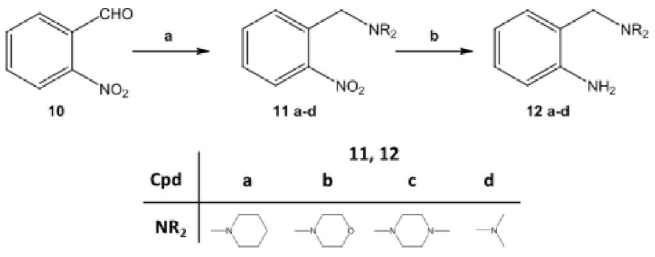
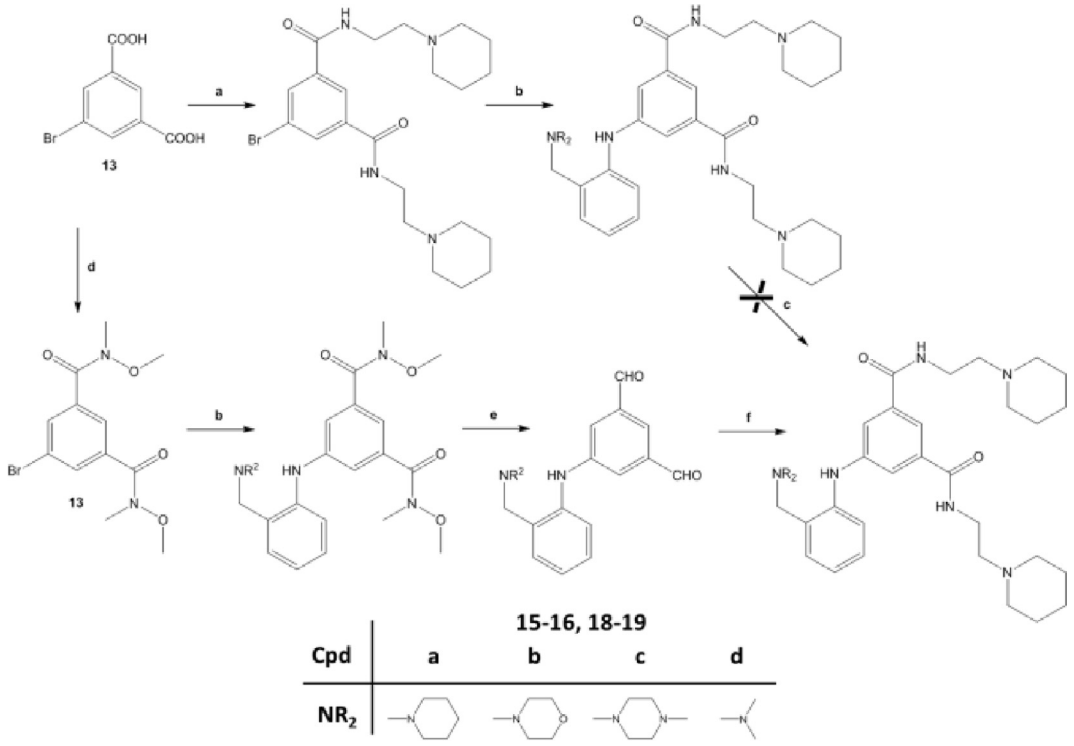


Fig. 3. Structure of general structure I and target compounds **16a–d** and **22a–d**.

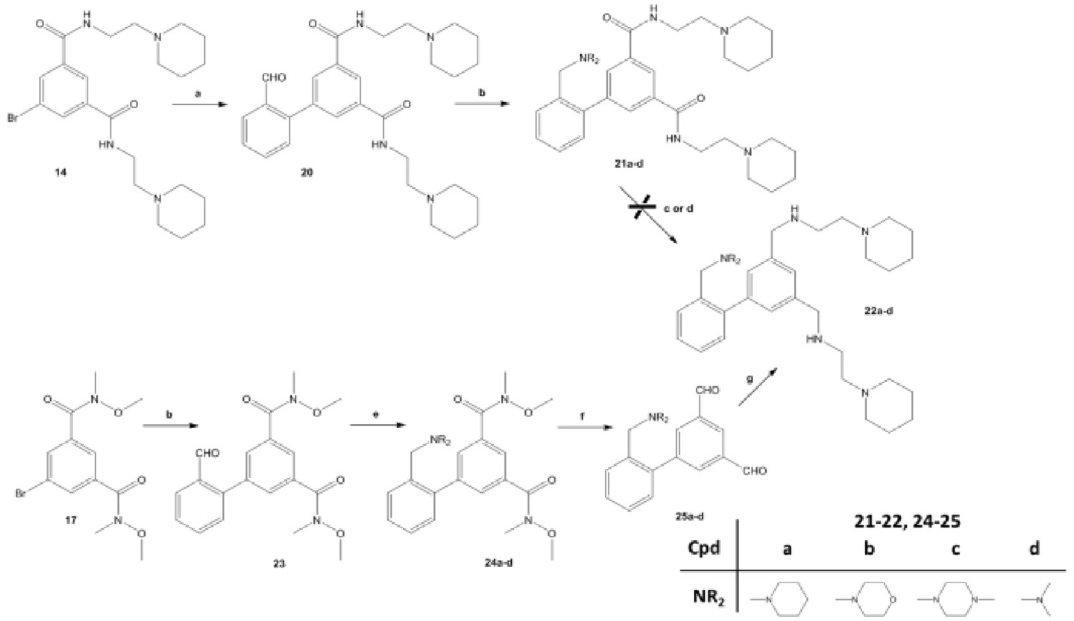


Scheme 1. Synthesis of intermediate compounds **12a–d**. Reagents and conditions: (a) (i): Secondary amine, $ZnCl_2$, DCE, rt, 3 h (ii): $NaBH_3CN$, rt, 18 h, 77–84% (b) Pd/C 10%, ammonium formate, EtOH, rt, 30–60 min.

By the first route, various attempts were considered for the Suzuki coupling reaction by varying catalyst with $Pd(PPh_3)_4$, $Pd(OAc)_2$, $Pd(dba)_3$ and a ligand PPh_3 or P(*o*-tol)$_3$ when necessary, to afford the corresponding compound **20** with 43–44% yield. The best conditions were found with $Pd(OAc)_2$, and P(*o*-tol)$_3$ with 62% yield. We observed degradation of compound **20** during the purification step on silica gel. Thus reductive amination was engaged with crude aldehyde **20** in DCE with the corresponding commercial amine and $NaBH(OAc)_3$, to furnish compounds **21a–d**.[56] The last step was the reduction of the amide group, two reactions were attempted.[57,58] No conversion could be observed in a first reaction in THF with BH_3-THF as reductive agent. A second reaction in THF with lithium aluminium hydride afforded degradation of the reaction. The second route was engaged starting from derivative **17** in a Suzuki coupling reaction with $Pd(OAc)_2$, and P(*o*-tol)$_3$ in DMF and afforded derivative **21** (87% yield). During this reaction we observed the formation of the debrominated compound as *N*1,*N*3-dimethoxy-*N*1,*N*3-dimethylbenzene-1,3-dicarboxamide. The next reaction was a



Scheme 2. Synthesis of phenylaniline target compounds **16a**–**d**. Reagents and conditions: (a) 1-piperidineethanamine, EDC·HCl, HOBt-H_2O, DMF, rt, 16 h, 89%; (b) 2-(amine-1-ylmethyl)aniline **12a**–**d**, Cs_2CO_3, Xantphos, Pd_2dba_3, dioxane, reflux, 16 h, 26–92%; (c) $LiAlH_4$, THF; (d) *N*,*O*-dimethylhydroxylamine, EDC·HCl, HOBt-H_2O, *N*-methylmorpholine, ACN, DCM, rt, 16 h, 89%; (e) $LiAlH_4$, THF, 0 °C, 1 h, (f) (i): 1-piperidineethanamine, toluene, reflux, 1 h, (ii): $NaBH(OAc)_3$, AcOH, DCE, rt, 15 h, 8–26%.



Scheme 3. Synthesis of aminomethyldiphenyl compounds **22a**–**d**. *Reagents and conditions:* (a) 2-formylphenylboronic acid, K_2CO_3, P(o-tol)$_3$, $Pd(OAc)_2$, toluene, EtOH, reflux, 18 h; (b) (i): Commercial amine (R_1H), DCE, rt, 5 h (ii): $NaBH(OAc)_3$, AcOH, rt, 24 h, 26–67%; (c) BH_3-THF, THF, reflux, 2 h; (d) $LiAlH_4$, THF, rt, 4 h; (e) (i): Commercially amine (R_1H), toluene, reflux, 1 h (ii): $NaBH(OAc)_3$, AcOH, DCE, rt, 15 h, 42–67%; (f) $LiAlH_4$, dry THF, 0 °C, 1 h, 50–92%.; (g) (i): 1-piperidineethanamine, toluene, reflux, 1 h (ii): $NaBH(OAc)_3$, AcOH, DCE, rt, 15 h, 4–54%.

reductive amination, in the same conditions as previously described for the first route, with the corresponding commercially amine and $NaBH(OAc)_3$, to obtain compounds **24a**–**d**. The amide Weinreb derivatives **24a**–**d** were reduced in dry THF with lithium aluminium hydride to give compounds **25a**–**d** with 50% yields. The derivative **25c** was directly engaged in the next step, without further purification, indeed we observed degradation during the purification on silica gel. The last step, to obtain compounds **22a**–**d**, was a reductive amination in toluene with 1-piperidine ethanamine to give the imine intermediate derivatives and then $NaBH(OAc)_3$ in DCE to afford the desired compounds **22a**–**d** with yields not higher than 54%.

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3. Results and discussion

The compounds were tested for modulating APP metabolism in SY5Y human neuroblastoma cell line stably expressing the neuronal isoform of human wild-type APP695 (SY5Y-APP^{wt}). This cell line is a well-established cellular model for the study of APP metabolism.

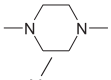
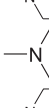
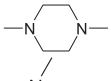
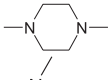
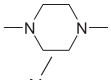
Cytotoxicity, APP carboxy-terminal fragments (CTFα and AICD) and Aβ levels ($Aβ_{1-40}$ and $Aβ_{1-42}$) were defined as outcome parameters and compared to CQ and AQ. We have also evaluated the capability of the synthesized compounds to cross the blood brain barrier (LogD at pH = 7.4), and the polar surface area. Compounds **15**,**16a–d** and **21**,**22a–d** were charged at physiological pH, so it was important to calculate the LogD at pH = 7.4 instead of LogP.

Cytotoxicity is expressed as the compound concentration causing 50% of cell death (CC_{50}). IC_{50} indicates the concentration of a compound effective for inhibiting the yield of Aβ secretion by 50%. Aβ peptides of 1–40 or 1–42 amino acids were considered. Only compounds that inhibit Aβ secretion with an IC_{50} lower than 10 μM were evaluated on APP metabolism through a western blot analysis of APP-CTFs. In Table 2, the effect of the compounds on the metabolism of APP is expressed as the intensity of the western-blot band corresponding to the APP-CTFs resulting from the secretase cleavages. The effect of the compounds on APP metabolism were measured at various concentrations (1, 3, 10 μM, Fig. 4) and compared to those of CQ at 3 μM (Table 2).

Considering Aβ release, the IC_{50} of compounds **15b–d**, **16c–d**, **21b–d** and **22a–d** were high compared to CQ or AQ. Best results were obtained for derivatives **15a**, **15d**, **16a–b**, and **21a** with range from 4.1 to 6.6 μM and 4.7–9.4 μM respectively for $Aβ_{40}$ and $Aβ_{42}$. Among these compounds, **15a** and **16a** have IC_{50} similar to those of AQ, whereas the others are closer to CQ. It is important to emphasize that the quantity of CTFα and AICD were higher for **15a** and **16a** with 3.2–10 more than AQ. These compounds **15a**, **15d**, **16a–b**, and **21a** showed suitable LogD from −0.12 to 1.34. Their polar surface area were all inferior to 90. They could cross the blood brain barrier as expected.

The effect of the target compounds on APP metabolism was studied toward the production of CTFα (Fig. 4A) and AICD (Fig. 4B) by Western blot analysis. Only five derivatives (**15a**, **15d**, **16a**, **16b**, **21a**), with significant results toward $Aβ_{40}$ and $Aβ_{42}$, were evaluated at three concentrations 1, 3 and 10 μM and compared to

Table 2
In vitro evaluation of compounds **15**,**16a–d** and **21**,**22a–d** on APP metabolism (SY5Y cells).

Cpd	R	Cytotoxicity CC_{50} (μM)[a]	$Aβ_{40}$ IC_{50} (μM)[b,c]	$Aβ_{42}$ IC_{50} (μM)[b,c]	CTFα vs CQ (3μM)[c,d]	AICD vs CQ (3μM)[c,e]	LogD[f] pH = 7.4	PSA[f] Å^2
CQ		30	7.0	12.8	1	1	–	
AQ		10	4.0	5.4	0.5	0.4	–	
15a		>100	4.1	4.7	2.8	2.3	0.43	80
15b		>100	≫10	≫10	–	–	0.72	89
15c		>100	≫10	≫10	–	–	0.47	83
15d		>100	8.2	≫10	1.0	3.0	−0.03	80
16a		>100	4.6	6.0	2.6	4.0	−0.12	46
16b		77	6.6	7.9	0.9	1.3	0.21	55
16c		>100	≫10	≫10	–	–	−0.07	49
16d		>100	≫10	≫10	–	–	−0.56	46
21a		>100	6.2	9.4	1.4	1.9	1.34	68
21b		>100	≫10	≫10	–	–	1.10	77
21c		>100	≫10	≫10	–	–	0.86	71
21d		>100	≫10	≫10	–	–	0.88	68
22a		>100	≫10	≫10	–	–	1.04	34
22b		>100	≫10	≫10	–	–	0.78	43
22c		>100	≫10	≫10	–	–	0.38	37
22d		>100	≫10	≫10	–	–	0.41	34

[a] Compound concentration causing 50% of SY5Y cell death after 24 h treatment, done in duplicate.
[b] Compound concentration inhibiting 50% of Aβ peptide secretion.
[c] Mean values calculated on the basis of at least three independent experiments with less than 10% deviation.
[d] CTFα increase compared to chloroquine ([CTF]compound/[CTF]CQ) at 3 μM.
[e] AICD increase compared to chloroquine ([AICD]compound/[AICD]CQ) at 3 μM.
[f] LogD and polar surface area PSA were calculated using ACD/ADME Suite 4.95 soft.

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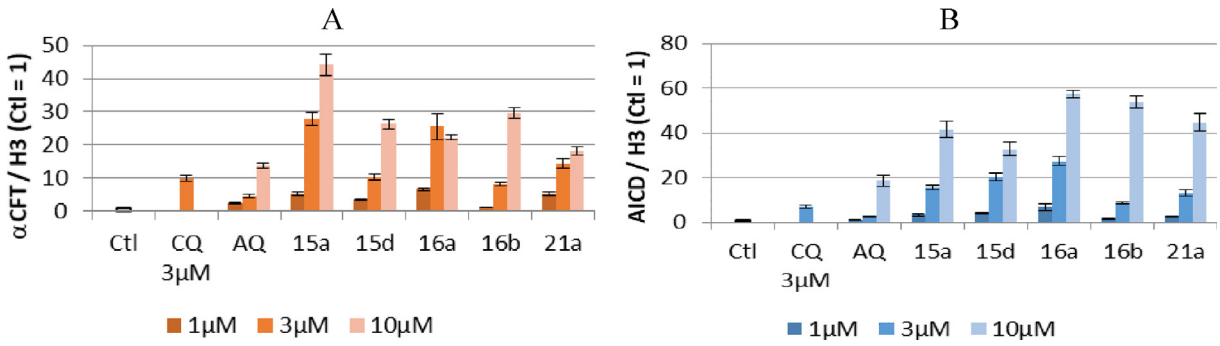

Fig. 4. Effect of target compounds on APP metabolism (A: APP CTFα, B: AICD).

control CQ and AQ. Dose-dependent profiles on the quantification of CTFα and AICD were obtained for all compounds and all showed higher efficiency than AQ (Fig. 4).

In Fig. 4A, compound **15a** with the best IC_{50} for $A\beta_{40}$ and $A\beta_{42}$ release had similar effect at 3 μM than all derivatives **15d**, **16a**, **16b**, **21a** at 3 or 10 μM concentrations. At 10 μM, the release of CTFα for compound **15a** was 1.5 times better than all compounds evaluated and 3 times better than CQ and AQ. At 3 μM, derivatives **15a**, **16a** and **21a** gave better release of CTFα compared to AQ at 10 μM.

In Fig. 4B, the best result was obtained for compound **16a** and had similar effect at 10 μM compared to derivative **16b** and a little bit higher than **15a** and **21a**. At the concentration of 3 μM, AICD was 3-fold and 10-fold times higher than with CQ and AQ respectively. At 3 μM, compounds **15d** and **16a** gave much more AICD releasing compared to AQ evaluated at 3 or 10 μM concentrations.

All together, aminomethylaniline series seemed more efficient than aminomethylphenyle series. Considering the substituent R, the piperidine induced the best profile: lower Aβ release IC_{50} and higher increase in APP-CTFs (APP-CTFα and AICD). On the contrary, in all cases, *N*-methylpiperazine substitution leads to non-efficient compounds (Aβ release $IC_{50} \gg 10$ μM).

All the compounds, except compound **16b** with a CC_{50} of 77 μM, showed no cytotoxicity at the concentration of 100 μM, underlining the interest of aminomethylaniline and aminomethylphenyle series compared to reference compounds CQ, AQ and **1**–**9**.

The two compounds **15a**, **16a** showed the best profiles as there were able to inhibit both $A\beta_{40}$ and $A\beta_{42}$ at the micromolar range (4.1–6.0 μM), at the same range of AQ and CQ. They also increased the quantity of neurotrophic APP-CTFs: CTFα (2.8 and 2.6-times more) and AICD (2.3 and 4.0-times more) versus CQ but also versus AQ with respectively 5.6–5.75 more for CTFα and 3.2–10 more for AICD.

4. Conclusion

Two series of compounds **15a**–**d**, **16a**–**d** and **21a**–**d**, **22a**–**d** bearing aminomethylaniline and aminomethylphenyle scaffold, in replacement of the 7-chloroquinolin-4-amine found in CQ, AQ and derivatives **1**–**9** previously tested in our laboratory, were designed and synthesized. These compounds were evaluated for their inhibition of the secretion of Aβ peptides (50%) by determining their IC_{50} and their activity on APP metabolism in SY5Y-APP695 human neuroblastoma cell line. Two compounds **15a**, **16a** showed interesting profiles as they were able to inhibit both $A\beta_{40}$ and $A\beta_{42}$ at the micromolar range (4.1–6.0 μM) and increase the quantity APP-CTF: CTFα and AICD versus AQ (range: 3.2–10). Furthermore, no cytotoxicity could be observed for concentrations up to 100 μM. Their structures were engaged in further chemistry modulations considering their moderate ability to modulate the APP metabolism.

In a longer term, these non-toxic compounds could also be tested on other models of pathologies where CQ and AQ proved efficient, especially towards antimalarial properties.

5. Experimental section

5.1. Chemistry

Chemicals and solvents were purchased from various suppliers (Sigma-Aldrich, Alfa Aesar, Fisher, VWR) and used without purification. The reaction monitoring was performed by thin layer chromatography (TLC) on Macherey-Nagel Alugram® Sil 60/UV_{254} (thickness 0.2 mm). TLC were revealed by UV (λ = 254 nm) and/or the appropriate stain. Purification of the compounds was carried out by column chromatography (flash or manual). Manual chromatography was performed using Macherey-Nagel silica gel (0.04–0.063 mm of particle size), while flash chromatography was performed on a Reveleris® Flash Chromatography System using Macherey-Nagel Chromabond flash RS columns. NMR spectra were recorded on a Bruker DRX 300 spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS) or to residual proton signal in deuterated solvents. Chemical shifts are reported as position (δ in ppm), multiplicity (s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad and M = massif), coupling constant (*J* in Hz), relative integral and assignment. The attributions of protons and carbons were achieved by analysis of 1D and 2D experiments (^{1}H, ^{13}C, COSY, HQC and HMBC). LC–MS were performed on a Varian triple quadrupole 1200 W mass spectrometer equipped with a non-polar C_{18} TSK-gel Super ODS (4.6 × 50 mm) column, using electrospray ionisation and a UV detector (diode array). Elution was performed at a flow rate of 2 mL/min with water-formic acid (pH = 3.8) as eluent A and ACN-formic acid (pH = 3.8) as eluent B, employing a 0.25 min plateau with 0% B and a linear gradient from 0% B to 98% B in 3.25 min, followed by a 0.5 min plateau with 98% B. Then, column re-equilibration was performed for 1 min. The injection duty cycle was 5 min, taking into account the column equilibration time. HRMS were recorded on a Hight Resolution Mass Spectrometer (HRMS) Thermo Scientific™ Exactive™. Analysed compounds were dissolved in methanol and directly introduced in the ionisation source ESI, in positive or negative mode according to the analysed compound, and recorded for 1 min. The Xcalibur software was used to determine the elementar composition of main pics of the spectrum. The purity of final compounds was determined by high pressure liquid chromatography (HPLC) using to columns: C_{18} Interchrom UPTISPHERE and C_4 Interchrom UPTISPHERE. The HPLC analysis was carried out on a Shimadzu LC-2010AHT system equipped with a UV detector set at

254 and 215 nm. The compounds were dissolved in 100 μL of buffer B and 900 μL of buffer A. The eluent system used was: buffer A (H_2O/TFA, 100:0.1) and buffer B (ACN/H_2O/TFA, 80:20:0.1). Retention times (t_r) were obtained at a flow rate of 0.2 mL/min for 37 min using a gradient form 100% of buffer A over 1 min, to 100% buffer B over the next 30 min, to 100% of buffer A over 1 min and 100% of buffer A over 1 min. The melting point analyses were performed on Barnstead Electrothermal Melting Point Series IA9200. All final compounds were transformed into their hydrochloride salts following this procedure: the compound was dissolved in MeOH and HCl_{aq} 2 M was added dropwise until pH1. The solvent was evaporated and the compound was freeze-dried.

5.1.1. General procedure for the synthesis of compounds ***11a–d***

2-Nitrobenzaldehyde **10** (1.00 g, 6.62 mmol) was dissolved in DCE (95 mL). The appropriate commercially amine (9.93 mmol), and $ZnCl_2$ (0.90 g, 6.62 mmol) were added. After 3 h of stirring at room temperature, $NaBH_3CN$ (624 mg, 9.93 mmol) was added. After 18 h of stirring at room temperature, a saturated $NaHCO_3$ solution (40 mL) was added. The reaction mixture was stirred for 1 h at room temperature. DCM (100 mL) was added and the layers were separated. The organic layer was washed twice with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column or flash chromatography.

5.1.1.1. 1-[(2-Nitrophenyl)methyl]piperidine ***11a***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 7:3 (*v*/*v*)). The compound **11a** was obtained as a yellow oil (yield: 84%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.77 (dd, J = 8.0 Hz, J = 1.2 Hz, 1H); 7.62 (dd, J = 7.7 Hz, J = 0.4 Hz, 1H); 7.50 (td, J = 7.4 Hz, J = 1.1 Hz, 1H); 7.34 (ddd, J = 7.9 Hz, J = 7.5 Hz, J = 1.5 Hz, 1H); 3.71 (s, 2H); 2.34 (m, 4H); 1.52 (m, 4H); 1.40 (m, 2H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 149.8; 134.6; 132.3; 130.8; 127.6; 124.2; 59.7; 54.6; 26.0; 24.2. LC–MS (ESI) *m*/*z* Calculated: 221.1, Found: 221.0 $[M+H]^+$.

5.1.1.2. 4-[(2-Nitrophenyl)methyl]morpholine ***11b***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 7:3 (*v*/*v*)). The compound **11b** was obtained as an orange oil (yield: 83%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.81 (dd, J = 8.0 Hz, J = 1.2 Hz, 1H); 7.59 (dd, J = 5.9 Hz, J = 1.7 Hz, 1H); 7.54 (ddd, J = 7.7 Hz, J = 7.2 Hz, J = 1.3 Hz, 1H); 7.40 (td, J = 8.0 Hz, J = 1.8 Hz, 1H); 3.79 (s, 2H); 3.66 (m, 4H); 2.44 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 149.9; 133.4; 132.3; 131.0; 128.1; 124.4; 67.0; 59.5; 53.6. LC–MS (ESI) *m*/*z* Calculated: 223.1, Found: 223.0 $[M+H]^+$.

5.1.1.3. 1-Methyl-4-[(2-nitrophenyl)methyl] piperazine ***11c***. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.8:0.2 (*v*/*v*)). The compound **11c** was obtained as a yellow oil (yield: 77%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.81 (d, J = 8.0 Hz, 1H); 7.56–7.41 (M, 3H); 3.89 (s, 2H); 3.14 (m, 2H); 2.80 (m, 4H); 2.72 (s, 3H); 2.56 (m, 2H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 149.7; 132.6; 132.3; 131.0; 128.7; 124.6; 58.4; 58.2; 48.8; 47.0. LC–MS (ESI) *m*/*z* Calculated: 236.1, Found: 236.0 $[M+H]^+$.

5.1.1.4. Dimethyl[(2-nitrophenyl)methyl]amine ***11d***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 7:3 (*v*/*v*)). The compound **11d** was obtained as a yellow oil (yield: 79%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.82 (dd, J = 8.1 Hz, J = 1.3 Hz, 1H); 7.63 (dd, J = 7.6 Hz, J = 1.0 Hz, 1H); 7.55 (ddd, J = 7.7 Hz, J = 7.4 Hz, J = 1.3 Hz, 1H); 7.40 (td, J = 8.0 Hz, J = 1.6 Hz, 1H); 3.72 (s, 2H); 2.24 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 149.7; 134.4; 132.5; 131.0; 127.8; 124.3; 60.3; 45.6. LC–MS (ESI) *m*/*z* Calculated: 181.1, Found: 181.0 $[M+H]^+$, 135.8 $[M-N(CH_3)_2+H]^+$.

5.1.1.5. 5-Bromo-N1,N3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide ***14***. To a solution of 5-bromoisophthalic acid **1** (200 mg, 0.82 mmol) and 1-piperidineethanamine (240 μL, 1.17 mmol) in DMF (2.5 mL) was added EDC·HCl (330 mg, 1.73 mmol) and HOBt-H_2O (25.2 mg, 0.165 mmol). The reaction mixture was stirred for 16 h at room temperature. 10 mL of saturated $NaHCO_3$ solution was added. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.7:0.3 (*v*/*v*)). The title compound **14** (340 mg, 0.73 mmol, 89%) was obtained as a white solid. Mp: 127.3 °C. ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.23 (t, J = 1.5 Hz, 1H); 8.04 (d, J = 1.5 Hz, 2H); 7.46 (t, J = 4.7 Hz, 2H); 3.48 (td, J = 5.9 Hz, J = 5.5 Hz, 4H); 2.50 (t, J = 6.1 Hz, 4H); 2.38 (m, 8H); 1.54 (m, 8H); 1.40 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 165.3; 136.3; 133.2; 123.7; 123.0; 57.4; 54.3; 36.6; 25.7; 24.2. LC–MS (ESI) *m*/*z* Calculated: 465.2–467.2, Found: 465.0–466.9, 233.1–234.0 $[(M+2H)/2]^+$.

5.1.1.6. 5-Bromo-N1,N3-dimethoxy-N1,N3-dimethyl benzene-1,3-dicarboxamide ***17***. To a stirred solution of 5-bromoisophthalic acid (4.00 g, 16.3 mmol) in ACN (32 mL) and DCM (32 mL) was added EDC·HCl (8.14 g, 42.40 mmol), HOBt-H_2O (5.74 g, 42.40 mmol), *N*-méthylmorpholine (23.3 mL, 212.00 mmol) and *N*,*O*-dimethylhydroxylamine·HCl (6.69 g, 68.60 mmol). The mixture was stirred at room temperature for 16 h. The mixture was washed with a saturated solution of $NaHCO_3$ (2 × 50 mL), a 1 M solution of HCl (2 × 50 mL) and 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered and evaporated. The compound was used without further purification in the next step. The title compound (4.80 g, 14.49 mmol, 89%) was obtained as a yellow oil. ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.94 (t, J = 1.4 Hz, 1H); 7.91 (d, J = 1.4 Hz, 2H); 3.54 (s, 6H); 3.35 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 167.4; 135.5; 133.3; 126.8; 121.7; 61.3; 33.5. LC–MS (ESI) *m*/*z* Calculated: 331.0–333.0, Found: 331.0–333.0 $[M+H]^+$.

5.1.1.7. General procedure for the synthesis of compounds ***15a–d***, ***18a–d***. To a solution of 4-[(2-nitrophenyl)methyl]amine **11a–d** (2.25 mmol) in EtOH (45 mL) was added ammonium formate (0.99 g, 15.75 mmol) and Pd/C 10% (177 mg, 0.17 mmol). The mixture was stirred for 1 h at room temperature. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was dissolved in DCM (30 mL) and washed with water (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. Because of the high instability of the compounds **12a–d**, they were used directly without further purification in the next step.

5-Bromo-*N*1,*N*3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide **14** (303 mg, 0.65 mmol) or 5-Bromo-*N*1,*N*3-dimethoxy-*N*1,*N*3-dimethylbenzene-1,3-dicarboxamide **17** (215 mg, 0.65 mmol) was dissolved in dioxane (2 mL) and Cs_2CO_3 (297 mg, 0.91 mmol) were added. The reaction was stirred for 30 min and deoxygenated by passing a stream of N_2 through it. Xantphos (57 mg, 0.10 mmol), Pd_2dba_3 (30 mg, 0.03 mmol) and the previously prepared amine **12a–d** dissolved in dioxane (1 mL) were added. The reaction mixture was stirred for 15 h at reflux. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. The residues were purified by column or flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.5:0.5 (*v*/*v*)) to give compounds **15a–d**.

5.1.1.8. N1,N3-bis[2-(piperidin-1-yl)ethyl]-5-([2-(piperidin-1-ylmethyl)phenyl]amino) benzene-1,3-dicarboxamide ***15a***. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.5:0.5 (*v*/*v*)) to give compound **15a** as a colourless oil (yield: 88%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 9.25 (s, 1H);

7.68 (t, *J* = 1.5 Hz, 1H); 7.62 (d, *J* = 1.4 Hz, 2H); 7.40 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H); 7.20 (ddd, *J* = 8.0 Hz, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H); 7.11 (dd, *J* = 7.5 Hz, *J* = 1.4 Hz, 1H); 7.05 (br t, *J* = 5.0 Hz, 2H); 6.84 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 3.57–3.51 (M, 6H); 2.56 (t, *J* = 6.2 Hz, 4H); 2.46–2.37 (M, 12H); 1.67–1.55 (M, 12H); 1.49–1.41 (M, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.9; 144.3; 142.6; 136.2; 130.8; 128.1; 126.0; 120.3; 118.0; 116.1; 115.5; 62.9; 57.1; 54.3; 54.0; 36.5; 26.3; 25.9; 24.4; 24.3. LC–MS (ESI) *m*/*z* Calculated: 575.4, Found: 575.4 $[M+H]^+$, 288.2 $[(M+2H)/2]^+$. HR-MS: *m*/*z* Calculated: 575.40680, Found: 575.40367 $[M+H]^+ = C_{34}H_{51}N_6O_2$. Purity: C_4 column: t_r = 17.5 min, purity = 94%; C_{18} column: t_r = 19.4 min, purity = 95%.

5.1.1.9. 5-([2-(Morpholin-4-ylmethyl)phenyl]amino)-N1,N3-bis[2-(piperidin-1-yl)ethyl] benzene-1,3-dicarboxamide ***15b***. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.5:0.5 (*v*/*v*)) to give compound **15b** as a colourless oil (yield: 48%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.69 (s, 1H); 7.77 (s, 1H); 7.67 (s, 2H); 7.40 (d, *J* = 8.0 Hz, 1H); 7.25–7.13 (M, 4H); 6.88 (td, *J* = 7.4 Hz, *J* = 1.0 Hz, 1H); 3.77 (t, *J* = 4.3 Hz, 4H); 3.61–3.57 (M, 6H); 2.63 (t, *J* = 5.7 Hz, 4H), 2.51–2.45 (M, 12H); 1.64 (m, 8H); 1.50 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.9; 144.1; 142.4; 136.0; 131.1; 128.6; 125.1; 120.7; 118.3; 116.3; 116.1; 67.2; 62.4; 57.2; 54.3; 53.1; 36.4; 25.7; 24.2. LC–MS (ESI) *m*/*z* Calculated: 577.4, Found: 577.3 $[M+H]^+$, 289.2 $[(M+2H)/2]^+$. HR-MS: *m*/*z* Calculated: 577.38607, Found: 577.38232 $[M+H]^+ = C_{33}H_{49}N_6O_3$. Purity: C_4 column: t_r = 16.7 min, purity = 90%; C_{18} column: t_r = 18.7 min, purity = 92%.

5.1.1.10. 5-((2-[(4-Methylpiperazin-1-yl)methyl] phenyl)amino)-N1, N3-bis[2-(piperidin-1-yl)ethyl] benzene-1,3-dicarboxamide ***15c***. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.5:0.5 (*v*/*v*)) to give compound **15c** as a colourless oil (yield: 35%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.83 (s, 1H); 7.74 (t, *J* = 1.4 Hz, 1H); 7.67 (d, *J* = 1.4 Hz, 2H); 7.40 (dd, *J* = 8.0 Hz, *J* = 0.8 Hz, 1H); 7.23 (td, *J* = 7.8 Hz, *J* = 1.6 Hz, 1H); 7.16 (dd, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H); 7.10 (br t, *J* = 5.1 Hz, 2H); 6.89 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 3.60–3.54 (M, 6H); 2.61–2.43 (M, 20H); 2.34 (s, 3H); 1.62 (m, 8H); 1.49 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.9; 144.3; 142.4; 136.1; 131.0; 128.4; 125.8; 120.6; 118.0; 116.1; 116.1; 62.0; 57.1; 55.4; 54.3; 52.7; 46.0; 36.5; 25.9; 24.3. LC–MS (ESI) *m*/*z* Calculated: 590.4, Found: 590.4 $[M+H]^+$, 295.7 $[(M+2H)/2]^+$. HR-MS: *m*/*z* Calculated: 590.41770, Found: 590.41328 $[M+H]^+ = C_{34}H_{52}N_7O_2$. Purity: C_4 column: t_r = 16.1 min, purity = 95%; C_{18} column: t_r = 18.7 min, purity = 97%.

5.1.1.11. 5-((2-[(Dimethylamino)methyl]phenyl) amino)-N1,N3-bis[2-(piperidin-yl)ethyl] benzene-1,3-dicarboxamide ***15d***. The residue was purified by flash chromatography (DCM/MeOH(NH_3), 10:0 to 9.5:0.5 (*v*/*v*)) to give compound **15d** as a red oil (yield: 30%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.92 (s, 1H); 7.69 (t, *J* = 1.4 Hz, 1H); 7.65 (d, *J* = 1.4 Hz, 2H); 7.39 (d, *J* = 7.9 Hz, 1H); 7.20 (td, *J* = 8.1 Hz, *J* = 1.4 Hz, 1H); 7.11 (dd, *J* = 7.7 Hz, *J* = 1.1 Hz, 1H); 7.06 (br t, *J* = 4.2 Hz, 2H); 6.85 (td, *J* = 7.5 Hz, *J* = 0.9 Hz, 1H); 3.54 (q, *J* = 5.5 Hz, 4H); 3.46 (s, 2H); 2.55 (t, *J* = 6.0 Hz, 4H); 2.43 (br s, 8H); 2.24 (s, 6H); 1.59 (m, 8H); 1.46 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.9; 144.2; 142.6; 136.1; 130.6; 128.3; 126.5; 120.4; 118.2; 116.0; 115.6; 63.9; 57.1; 54.3; 44.9; 36.5; 25.9; 24.3. LC–MS (ESI) *m*/*z* Calculated: 535.4, Found: 535.3 $[M+H]^+$. HR-MS: *m*/*z* Calculated: 535.37550, Found: 535.37152 $[M+H]^+ = C_{31}H_{47}N_6O_2$. Purity: C_4 column: t_r = 16.6 min, purity = 90%; C_{18} column: t_r = 17.8 min, purity = 92%.

5.1.1.12. N1,N3-Dimethoxy-N1,N3-dimethyl-5-([2-(piperidin-1-ylmethyl)phenyl]amino) benzene-1,3-dicarboxamide ***18a***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 1:9 (*v*/*v*)) to give compound **18a** as a yellow oil (yield: 75%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 9.25 (br s, 1H); 7.43 (d, *J* = 1.4 Hz, 2H); 7.41 (t, *J* = 1.3 Hz, 1H); 7.36 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H); 7.18 (td, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H); 7.08 (dd, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H); 6.82 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 3.61 (s, 6H); 3.51 (s, 2H); 3.35 (s, 6H); 2.39 (br s, 4H); 1.60 (m, 4H); 1.50 (m, 2H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.3; 141.3; 142.8; 135.0; 130.7; 128.1; 125.6; 120.0; 118.8; 118.6; 115.0; 62.9; 61.2; 53.9; 33.8; 26.3; 24.4. LC–MS (ESI) *m*/*z* Calculated: 441.3, Found: 441.2 $[M+H]^+$, 356.2 $[M-\text{piperidine}+H]^+$.

5.1.1.13. N1,N3-Dimethoxy-N1,N3-dimethyl-5-([2-(morpholin-4-ylmethyl)phenyl]amino) benzene-1,3-dicarboxamide ***18b***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 0:10 (*v*/*v*)) to give compound **18b** as a yellow oil (yield: 65%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.72 (br s, 1H); 7.44 (s, 3H); 7.36 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H); 7.21 (td, *J* = 7.4 Hz, *J* = 1.6 Hz, 1H); 7.12 (dd, *J* = 7.5 Hz, *J* = 1.4 Hz, 1H); 6.85 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 3.74 (t, *J* = 4.5 Hz, 4H); 3.61 (s, 6H); 3.56 (s, 2H); 3.36 (s, 6H); 2.46 (t, *J* = 4.1 Hz, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.2; 143.1; 142.5; 135.0; 131.1; 128.5; 124.6; 120.4; 119.2; 118.8; 115.4; 67.2; 62.4; 61.2; 53.0; 33.8. LC–MS (ESI) *m*/*z* Calculated: 443.2, Found: 443.2 $[M+H]^+$, 441.1 $[M-H]^-$.

5.1.1.14. N1,N3-Dimethoxy-N1,N3-dimethyl-5-((2-[(4-methylpiperazin-1-yl)methyl]phenyl)amino) benzene-1,3-dicarboxamide ***18c***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 9.8:0.2 (*v*/*v*)) to give compound **18c** as a yellow oil (yield: 92%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.84 (br s, 1H); 7.45 (s, 3H); 7.37 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H); 7.21 (td, *J* = 7.6 Hz, *J* = 1.6 Hz, 1H); 7.13 (dd, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H); 6.86 (td, *J* = 7.4 Hz, *J* = 1.2 Hz, 1H); 3.63 (s, 6H); 3.58 (s, 2H); 3.38 (s, 6H); 2.60–2.43 (M, 8H); 2.34 (s, 3H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.3; 143.2; 142.6; 135.0; 130.9; 128.3; 125.3; 120.3; 119.0; 118.7; 115.5; 62.0; 61.2; 55.4; 52.5; 46.0; 33.9. LC–MS (ESI) *m*/*z* Calculated: 456.3, Found: 456.2 $[M+H]^+$, 454.1 $[M-H]^-$.

5.1.1.15. 5-((2-[(Dimethylamino)methyl]phenyl) amino)-N1,N3-dimethoxy-N1,N3-dimethylbenzene-1,3-dicarboxamide ***18d***. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 9.8:0.2 (*v*/*v*)) to give compound **18d** as a yellow oil (yield: 26%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.41 (s, 2H); 7.37 (s, 1H); 7.33 (dd, *J* = 8.1 Hz, *J* = 1.1 Hz, 1H); 7.15 (td, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H); 7.06 (dd, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H); 6.80 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 3.57 (s, 6H); 3.43 (s, 2H); 3.31 (s, 6H); 2.20 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.3; 143.3; 142.6; 134.9; 130.6; 128.3; 126.3; 120.3; 118.8; 118.7; 115.5; 63.4; 61.2; 44.8; 33.9. LC–MS (ESI) *m*/*z* Calculated: 401.2, Found: 401.1 $[M+H]^+$.

5.1.1.16. General procedure for the synthesis of compounds ***16a–d***. To a solution of the appropriate Weinreb amide derivatives **18a–d** (0.70 mmol) in THF (5 mL) was added $LiAlH_4$ in THF (1 M, 1.3 mL, 1.33 mmol) dropwise, under N_2 atmosphere at 0 °C. The mixture was stirred at 0 °C for 1 h. Saturated $KHSO_4$ solution (3 mL) was added dropwise. After evaporation of the THF, the residue was dissolved in DCM (30 mL) and washed twice with saturated $NaHCO_3$ solution (20 mL), twice with HCl (1 M, 10 mL) and once with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The unstable products **19a–d** were used without further purification in the next step.

The desired dicarbaldehyde derivative **19a–d** (0.23 mmol) was dissolved in toluene (5 mL). 1-Piperidineethanamine (94 µL, 0.65 mmol) was added. After 1–2 h of reflux with a Dean Stark apparatus, the solvent was evaporated and the residue was dissolved in DCE (3 mL). Acetic acid (37 µL, 0.65 mmol) and $NaBH(OAc)_3$ (138 mg, 0.65 mmol) were added. After 15 h of stirring at room

temperature, a volume of saturated $NaHCO_3$ solution (20 mL) was added. The reaction mixture was stirred for 1 h at room temperature. DCM (20 mL) was added and the layers were separated. The organic layer was washed twice with saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified flash chromatography to afford compounds **16a–d**.

*5.1.1.17. 3,5-Bis(([2-(piperidin-1-yl)ethyl]amino) methyl)-N-[2-(piperidin-1-ylmethyl) phenyl]aniline **16a**.* The residue was purified by column chromatography (DCM/MeOH(NH_3) 9.5:0.5 to 9.3:0.7 (*v*/*v*)) to give compound **16a** as a colourless oil (yield: 26%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.89 (br s, 1H); 7.35 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H); 7.17 (td, *J* = 7.6 Hz, *J* = 1.6 Hz, 1H); 7.09 (dd, *J* = 7.4 Hz, *J* = 1.4 Hz, 1H); 6.94 (d, *J* = 1.2 Hz, 2H); 6.83–6.76 (M, 2H); 3.78 (s, 4H); 3.51 (s, 2H); 2.76 (t, *J* = 6.4 Hz, 4H); 2.52–2.35 (M, 16H); 1.66–1.43 (M, 18H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 143.8; 143.5; 141.4; 130.6; 128.0; 125.2; 119.9; 119.0; 116.2; 114.8; 63.0; 58.3; 54.6; 53.9; 45.7; 26.3; 25.9; 24.5; 24.4. HR-MS: *m*/*z* Calculated: 547.44827, Found: 547.44425 $[M+H]^+ = C_{34}H_{55}N_6$. Purity: C_4 column: t_r = 14.7 min, purity = 97%; C_{18} column: t_r = 17.3 min, purity = 96%.

*5.1.1.18. N-[2-(Morpholin-4-ylmethyl)phenyl]-3,5-bis(([2-(piperidin-1-yl)ethyl]amino)methyl) aniline **16b**.* The residue was purified by column chromatography (DCM/MeOH(NH_3) 10:0 to 9:1 (*v*/*v*)) to give compound **16b** as a colourless oil (yield: 14%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.38 (br s, 1H); 7.35 (d, *J* = 7.6 Hz, 1H); 7.20 (td, *J* = 6.7 Hz, *J* = 1.4 Hz, 1H); 7.11 (dd, *J* = 7.4 Hz, *J* = 1.1 Hz, 1H); 6.94 (d, *J* = 0.9 Hz, 2H); 6.85–6.78 (M, 2H); 3.80–3.74 (M, 8H); 3.57 (s, 2H); 2.75 (t, *J* = 6.4 Hz, 4H); 2.52–2.45 (M, 8H); 2.38 (br s, 8H); 2.21 (br s, 2H); 1.57 (m, 8H); 1.44 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 143.7; 143.2; 141.8; 131.0; 128.4; 124.1; 120.2; 119.3; 116.3; 115.1; 67.2; 62.5; 58.5; 54.7; 54.0; 53.1; 46.0; 26.0; 24.4. HR-MS: *m*/*z* Calculated: 549.42754, Found: 549.42489 $[M+H]^+ = C_{33}H_{53}N_6O$. Purity: C_4 column: t_r = 14.4 min, purity = 96%; C_{18} column: t_r = 16.7 min, purity = 97%.

*5.1.1.19. N-(2-[(4-Methylpiperazin-1-yl)methyl]phenyl)-3,5-bis(([2-(piperidin-1-yl)ethyl] amino)methyl)aniline **16c**.* The residue was purified by column chromatography (DCM/MeOH(NH_3) 10:0 to 9:1 (*v*/*v*)) to give compound **16c** as a colourless oil (yield: 10%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.56 (br s, 1H); 7.34 (dd, *J* = 7.9 Hz, *J* = 0.8 Hz, 1H); 7.19 (td, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H); 7.11 (dd, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H); 6.97 (d, *J* = 1.2 Hz, 2H); 6.93 (t, *J* = 1.1 Hz, 1H); 6.82 (td, *J* = 7.3 Hz, *J* = 1.0 Hz, 1H); 3.82 (s, 4H); 3.68 (br s, 2H); 3.56 (s, 2H); 2.87 (t, *J* = 6.4 Hz, 4H); 2.68–2.48 (M, 20H); 2.40 (s, 3H); 1.65 (m, 8H); 1.47 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 143.6; 143.3; 140.3; 130.9; 128.3; 124.8; 120.1; 119.6; 116.4; 115.2; 62.0; 57.4; 55.3; 54.4; 53.4; 52.3; 45.8; 44.9; 25.2; 23.9. HR-MS: *m*/*z* Calculated: 562.45917, Found: 562.45673 $[M+H]^+ = C_{34}H_{56}N_7$. Purity: C_4 column: t_r = 14.4 min, purity = 94%; C_{18} column: t_r = 16.9 min, purity = 95%.

*5.1.1.20. N-(2-[(Dimethylamino)methyl]phenyl)-3,5-bis(([2-(piperidin-1-yl)ethyl]amino) methyl) aniline **16d**.* The residue was purified by column chromatography (DCM/MeOH(NH_3) 10:0 to 9:1 (*v*/*v*)) to give compound **16d** as a colourless oil (yield: 8%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.49 (br s, 1H); 7.35 (d, *J* = 8.0 Hz, 1H); 7.18 (td, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H); 7.11 (dd, *J* = 7.4 Hz, *J* = 1.3 Hz, 1H); 6.97 (d, *J* = 1.1 Hz, 2H); 6.85–6.78 (M, 2H); 3.78 (s, 4H); 3.45 (s, 2H); 2.77 (t, *J* = 6.4 Hz, 4H); 2.51 (t, *J* = 6.1 Hz, 4H); 2.40 (br s, 8H); 2.30–2.25 (M, 8H); 1.58 (m, 8H); 1.44 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 143.7; 143.5; 141.4; 130.5; 128.1; 125.9; 120.0; 119.2; 116.2; 115.2; 63.6; 58.3; 54.6; 53.9; 45.7; 44.9; 25.8; 24.3. HR-MS: *m*/*z* Calculated: 507.41697, Found: 507.41539 $[M+H]^+ = C_{31}H_{51}N_6$. Purity: C_4 column: t_r = 14.3 min, purity = 91%; C_{18} column: t_r = 16.5 min, purity = 90%.

*5.1.1.21. General procedure for the synthesis of compounds **21a–d**.* 2-Formylbenzeneboronic acid (350 mg, 2.32 mmol) was dissolved in a mixture of toluene (45 mL) and EtOH (15 mL). Potassium carbonate (350 mg, 2.51 mmol) and 5-bromo-*N*1,*N*3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide **14** (900 mg, 1.93 mmol) were added and the reaction was stirred for 30 min and deoxygenated by passing a stream of N_2 through it. $Pd(OAc)_2$ (10 mg, 0.04 mmol) and P(*o*-tol)$_3$ (120 mg, 0.39 mmol) were added and the mixture was refluxed for 18 h. After cooling, the mixture was poured into water, extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$ and evaporated. The derivative **20** was used without further purification in the next step.

The obtained aldehyde derivative **20** (2.90 mmol) was dissolved in DCE and the desired commercially amine (2.90 mmol) was added. After 5 h of stirring at room temperature, $NaBH(OAc)_3$ (614 mg, 2.90 mmol), acetic acid (166 µL, 2.90 mmol) were added. After 24 h of stirring at room temperature, a volume of saturated $NaHCO_3$ solution was added (20 mL). The reaction mixture was stirred for 1 h at room temperature. DCM (30 mL) was added and the layers were separated. The organic layer was washed twice with saturated $NaHCO_3$ solution (20 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (PE/EtOAc/MeOH(NH_3), 4:5/5/0.5 (*v*/*v*/*v*)).

*5.1.1.22. N1,N3-bis[2-(Piperidin-1-yl)ethyl]-5-[2-(piperidin-1-ylmethyl)phenyl] benzene-1,3-dicarboxamide **21a**.* The compound **21a** was obtained as a colourless oil (yield: 62%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.34 (t, *J* = 1.6 Hz, 1H); 8.09 (d, *J* = 1.7 Hz, 2H); 7.50 (m, 1H); 7.39–7.29 (M, 3H); 7.21 (t, *J* = 5.1 Hz, 2H); 3.61 (td, *J* = 5.8 Hz, *J* = 5.6 Hz, 4H); 3.30 (s, 2H); 2.62 (t, *J* = 6.1 Hz, 4H); 2.49 (m, 8H); 2.25 (m, 4H); 1.63 (m, 8H); 1.54–1.36 (M, 10*H*). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.7; 142.5; 141.2; 136.3; 134.3; 131.3; 130.5; 130.1; 127.6; 126.9; 123.8; 60.9; 57.4; 54.4; 54.2; 36.5; 26.1; 25.7; 24.4; 24.2. LC–MS (ESI) *m*/*z* Calculated: 560.4, Found: 560.4 $[M+H]^+$, 558.3 $[M-H]^-$. HR-MS: *m*/*z* Calculated: 560.39590, Found: 560.39511 $[M+H]^+ = C_{34}H_{50}N_5O_2$. Purity: C_4 column: t_r = 18.8 min, purity = 98%; C_{18} column: t_r = 18.4 min, purity = 98%.

*5.1.1.23. 5-[2-(Morpholine-4-ylmethyl)phenyl]-N1,N3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide **21b**.* The compound **21b** was obtained as a colourless oil (yield: 29%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.34 (t, *J* = 1.7 Hz, 1H); 8.09 (d, *J* = 1.6 Hz, 2H); 7.48 (m, 1H); 7.37–7.29 (M, 5H); 3.65–3.58 (M, 8H); 3.35 (s, 2H); 2.61 (t, *J* = 6.0 Hz, 4H); 2.48 (m, 8H); 2.35 (m, 4H); 1.63 (m, 8H); 1.49 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.6; 142.4; 141.4; 135.2; 134.3; 131.5; 130.5; 130.3; 127.6; 127.3; 123.6; 67.1; 60.6; 57.5; 54.4; 53.2; 36.5; 25.7; 24.2. LC–MS (ESI) *m*/*z* Calculated: 562.4, Found: 562.4 $[M+H]^+$, 560.4 $[M-H]^-$. HR-MS: *m*/*z* Calculated: 562.37517, Found: 562.37540 $[M+H]^+ = C_{33}H_{48}N_5O_3$. Purity: C_4 column: t_r = 18.2 min, purity = 99%; C_{18} column: t_r = 17.9 min, purity = 99%.

*5.1.1.24. 5-(2-[(4-Methylpiperazin-1-yl)methyl] phenyl)-N1,N3-bis[2-(piperidin-1-yl)ethyl] benzene-1,3-dicarboxamide **21c**.* The compound **21c** was obtained as a colourless oil (yield: 26%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.28 (t, *J* = 1.5 Hz, 1H); 8.05 (d, *J* = 1.5 Hz, 2H); 7.45 (m, 1H); 7.36–7.24 (M, 3H); 7.18 (br t, *J* = 4.9 Hz, 2H); 3.55 (td, *J* = 5.8 Hz, *J* = 5.6 Hz, 4H); 3.33 (s, 2H); 2.56 (t, *J* = 6.0 Hz, 4H); 2.50–2.25 (M, 16H); 2.22 (s, 3H); 1.58 (m, 8H); 1.44 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.7; 142.3; 141.2; 135.6; 134.4; 131.2; 130.5; 130.1; 127.6; 127.2; 123.7; 60.1; 57.4; 55.2; 54.3; 52.7; 46.0; 36.6; 25.8; 24.2. LC–MS (ESI) *m*/*z*

Calculated: 575.4, Found: 575.4 $[M+H]^+$, 573.4 $[M-H]^-$. HR-MS: *m/z* Calculated: 575.40680, Found: 575.40717 $[M+H]^+ = C_{34}H_{51}N_6O_2$. Purity: C_4 column: t_r = 19.3 min, purity = 98%; C_{18} column: t_r = 18.0 min, purity = 98%.

5.1.1.25. 5-(2-[(Dimethylamino)methyl] phenyl)-N1,N3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide ***21d***. The compound **21d** was obtained as a colourless oil (yield: 55%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 8.33 (t, *J* = 1.6 Hz, 1H); 8.07 (d, *J* = 1.7 Hz, 2H); 7.45 (m, 1H); 7.35–7.22 (M, 5H); 3.53 (td, *J* = 5.8 Hz, *J* = 5.6 Hz, 4H); 3.24 (s, 2H); 2.52 (t, *J* = 6.1 Hz, 4H); 2.39 (m, 8H); 2.13 (s, 6H); 1.55 (m, 8H); 1.42 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 166.6; 142.1; 141.0; 136.2; 134.3; 131.3; 130.5; 130.0; 127.7; 127.1; 123.9; 61.2; 57.5; 54.3; 45.7; 36.6; 25.8; 24.2. LC–MS (ESI) *m/z* Calculated: 520.4, Found: 520.4 $[M+H]^+$. HR-MS: *m/z* Calculated: 520.36460, Found: 520.36475 $[M+H]^+ = C_{31}H_{46}N_5O_2$. Purity: C_4 column: t_r = 18.1 min, purity = 96%; C_{18} column: t_r = 17.9 min, purity = 96%.

5.1.1.26. 5-(2-Formylphenyl)-N1,N3-dimethoxy-N1,N3-dimethylbenzene-1,3-dicarboxamide ***23***. 2-Formylbenzeneboronic acid (151 mg, 1.01 mmol) was dissolved in a mixture of toluene (20 mL) and EtOH (5 mL). Potassium carbonate (150 mg, 1.10 mmol) and 5-bromo-*N*1,*N*3-bis[2-(piperidin-1-yl)ethyl]benzene-1,3-dicarboxamide 17 (280 mg, 0.84 mmol) were added and the reaction was stirred for 30 min and deoxygenated by passing a stream of N_2 through it. $Pd(OAc)_2$ (4 mg, 0.02 mmol) and P(*o*-tol)$_3$ (52 mg, 0.17 mmol) were added and the mixture was refluxed for 18 h. After cooling, the mixture was poured into water, extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (PE/EtOAc, 10:0 to 3.5:6.5 (*v*/*v*)). The compound 23 was obtained as a red oil (yield: 87%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 10.00 (s, 1H), 8.11 (t, *J* = 1.4 Hz, 1H); 8.06 (dd, *J* = 7.8 Hz, *J* = 1.3 Hz, 1H); 7.83 (d, *J* = 1.5 Hz, 2H); 7.68 (td, *J* = 7.5 Hz, *J* = 1.4 Hz, 1H); 7.56 (m, 1H); 7.47 (dd, *J* = 7.7 Hz, *J* = 1.1 Hz, 1H); 3.61 (s, 6H); 3.41 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 191.5; 168.4; 144.0; 137.7; 134.2; 133.8; 133.7; 131.8; 130.9; 128.5; 128.2; 127.9; 61.3; 33.5. LC–MS (ESI) *m/z* Calculated: 357.2, Found: 357.1 $[M+H]^+$.

5.1.1.27. General procedure for the synthesis of compounds ***22a–d***, ***24a–d***. 5-(2-Formylphenyl)-*N*1,*N*3-dimethoxy-*N*1,*N*3-dimethylbenzene-1,3-dicarboxamide **23** (400 mg, 1.12 mmol) or compound **25a–d** (1.12 mmol) was dissolved in toluene (15 mL). The appropriate commercially amine (1.68 mmol) was added. After 1 h of reflux with a Dean Stark apparatus, the solvent was evaporated and the residue was dissolved in DCE (15 mL). Acetic acid (97 µL, 1.68 mmol) and $NaBH(OAc)_3$ (357 mg, 1.68 mmol) were added. After 15 h of stirring at room temperature, saturated solution of $NaHCO_3$ (20 mL) was added. The reaction mixture was stirred for 1 h at room temperature. DCM (30 mL) was added and the layers were separated. The organic layer was washed twice with saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated. The products were purified by flash chromatography (DCM/MeOH(NH_3) 10:0 to 9.6:0.4 (*v*/*v*)).

5.1.1.28. [2-(Piperidin-1-yl)ethyl](([3-(([2-(piperidin-1-yl)ethyl] amino)methyl)-5-[2-(piperidin-1-ylmethyl) phenyl]phenyl]methyl)) amine ***22a***. The compound **22a** was obtained as a colourless oil (yield: 54%). ^{1}H NMR (300 MHz), δ (ppm, MeOD): 7.54 (dd, *J* = 6.9 Hz, *J* = 2.0 Hz, 1H); 7.37–7.21 (M, 6H); 3.83 (s, 4H); 3.43 (s, 2H); 2.74 (t, *J* = 6.6 Hz, 4H); 2.49 (t, *J* = 7.0 Hz, 4H); 2.40 (m, 8H); 2.26 (m, 4H); 1.61–1.39 (M, 18H). ^{13}C NMR (75 MHz), δ (ppm, MeOD): 142.6; 142.0; 139.3; 135.1; 129.9; 129.6; 128.3; 126.9; 126.8; 126.6; 60.1; 57.7; 54.4; 54.0; 52.9; 44.7; 25.4; 25.3; 23.8. LC–MS (ESI) *m/z* Calculated: 532.4, Found: 532.4 $[M+H]^+$, 266.8 $[(M+2H)/2]^+$. HR-MS: *m/z* Calculated: 532.43737, Found: 532.43743 $[M+H]^+ = C_{34}H_{54}N_5$. Purity: C_4 column: t_r = 15.0 min, purity = 95%; C_{18} column: t_r = 17.9 min, purity = 95%.

5.1.1.29. ((3-[2-(Morpholine-4-ylmethyl)phenyl]-5-(([2-(piperidin-1-yl)ethyl]amino)methyl)phenyl) methyl)[2-(piperidin-1-yl)ethyl]amine ***22b***. The compound **22b** was obtained as a colourless oil (yield: 23%). ^{1}H NMR (300 MHz), δ (ppm, MeOD): 7.55 (m, 1H); 7.37–7.25 (M, 6H); 3.85 (s, 4H); 3.64 (t, *J* = 4.6 Hz, 4H); 3.46 (s, 2H); 2.76 (t, *J* = 6.7 Hz, 4H); 2.52 (t, *J* = 6.1 Hz, 4H); 2.43 (m, 8H); 2.34 (m, 4H); 1.59 (m, 8H); 1.48 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, MeOD): 142.7; 141.9; 139.3; 134.7; 129.9; 129.7; 128.2; 126.9; 126.7; 66.6; 59.9; 57.7; 54.4; 53.2; 52.9; 44.8; 25.3; 23.8. LC–MS (ESI) *m/z* Calculated: 534.4, Found: 534.4 $[M+H]^+$, 267.8 $[(M+2H)/2]^+$. HR-MS: *m/z* Calculated: 534.41664, Found: 534.41658 $[M+H]^+ = C_{33}H_{52}N_5O$. Purity: C_4 column: t_r = 14.7 min, purity = 98%; C_{18} column: t_r = 17.4 min, purity = 98%.

5.1.1.30. [(3-(2-[(4-Methylpiperazin-1-yl)methyl] phenyl)-5-(([2-(piperidin-1-yl)ethyl]amino)methyl) phenyl)methyl] [2-(piperidin-1-yl)ethyl]amine ***22c***. The compound **22c** was obtained as a colourless oil (yield: 4%). ^{1}H NMR (300 MHz), δ (ppm, MeOD): 7.50 (m, 1H); 7.39–7.25 (M, 6H); 3.85 (s, 4H); 3.46 (s, 2H); 2.77 (t, *J* = 6.6 Hz, 4H); 2.53 (t, *J* = 6.9 Hz, 4H); 2.36 (m, 16H); 2.26 (s, 3H); 1.60 (m, 8H); 1.47 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, MeOD): 142.6; 141.9; 139.2; 134.9; 129.9; 129.8; 128.2; 126.9; 126.8; 59.4; 57.7; 54.6; 54.3; 52.9; 51.9; 44.8; 25.3; 23.8. LC–MS (ESI) *m/z* Calculated: 547.5, Found: 547.4 $[M+H]^+$, 274.2 $[(M+2H)/2]^+$. HR-MS: *m/z* Calculated: 547.44827, Found: 547.44699 $[M+H]^+ = C_{34}H_{55}N_6$.

5.1.1.31. [(3-(2-[(Dimethylamino)methyl]phenyl)-5-(([2-(piperidin-1-yl)ethyl]amino)methyl) phenyl) methyl] [2-(piperidin-1-yl)ethyl] amine ***22d***. The compound **22d** was obtained as a colourless oil (yield: 9%). ^{1}H NMR (300 MHz), δ (ppm, MeOD): 7.55 (m, 1H); 7.42–7.24 (M, 6H); 3.85 (s, 4H); 3.48 (s, 2H); 2.76 (t, *J* = 6.5 Hz, 4H); 2.52 (t, *J* = 7.0 Hz, 4H); 2.12 (s, 6H); 1.59 (m, 8H); 1.46 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, MeOD): 142.5; 141.8; 139.4; 135.1; 129.7; 128.8; 128.2; 127.1; 126.9; 126.7; 60.1; 57.7; 54.4; 52.8; 44.8; 44.1; 25.2; 23.8. LC–MS (ESI) *m/z* Calculated: 492.4, Found: 492.4 $[M+H]^+$, 246.7 $[(M+2H)/2]^+$. HR-MS: *m/z* Calculated: 492.40607, Found: 492.40654 $[M+H]^+ = C_{31}H_{50}N_5$. Purity: C_4 column: t_r = 14.7 min, purity = 95%; C_{18} column: t_r = 17.4 min, purity = 90%.

5.1.1.32. N1,N3-Dimethoxy-N1,N3-dimethyl-5-[2-(piperidin-1-ylmethyl)phenyl]benzene-1,3-dicarboxamide ***24a***. The compound **24a** was obtained as a red oil (yield: 61%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.96 (t, *J* = 1.6 Hz, 1H); 7.89 (d, *J* = 1.6 Hz, 2H); 7.51 (m, 1H); 7.38–7.25 (M, 3H); 3.60 (s, 6H); 3.39 (s, 6H); 3.34 (s, 2H); 2.28 (m, 4H); 1.50 (m, 4H); 1.38 (m, 2H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.2; 141.3; 141.0; 136.2; 133.6; 131.5; 130.5; 130.1; 127.6; 126.9; 126.5; 61.2; 60.8; 54.2; 33.8; 26.0; 24.4. LC–MS (ESI) *m/z* Calculated: 426.2, Found: 426.2 $[M+H]^+$.

5.1.1.33. N1,N3-Dimethoxy-N1,N3-dimethyl-5-[2-(morpholine-4-ylmethyl)phenyl]benzene-1,3-dicarboxamide ***24b***. The compound **24b** was obtained as a brown oil (yield: 67%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.98 (s, 1H); 7.93 (s, 2H); 7.47 (m, 1H); 7.37–7.33 (M, 3H); 3.65 (m, 4H); 3.60 (s, 6H); 3.39 (s, 6H); 3.38 (s, 2H); 2.38 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.1; 141.2; 135.2; 133.7; 131.5; 130.6; 130.3; 127.4; 126.5; 126.5; 67.0; 61.2; 60.6; 53.2; 33.7. LC–MS (ESI) *m/z* Calculated: 428.2, Found: 428.2 $[M+H]^+$.

5.1.1.34. N1,N3-Dimethoxy-N1,N3-dimethyl-5-(2-[(4-methylpiperazin-1-yl)methyl]phenyl) benzene-1,3-dicarboxamide ***24c***. The com-

pound **24c** was obtained as a brown oil (yield: 42%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.94 (t, J = 1.6 Hz, 1H); 7.88 (d, J = 1.6 Hz, 2H); 7.47 (m, 1H); 7.36–7.25 (M, 3H); 3.57 (s, 6H); 3.36 (s, 8H); 2.40 (br s, 8H); 2.24 (s, 3H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.1; 141.2; 141.1; 135.7; 133.6; 131.5; 130.5; 130.2; 127.6; 127.2; 126.4; 61.2; 60.0; 55.1; 52.6; 46.0; 33.7. LC–MS (ESI) m/z Calculated: 441.3, Found: 441.2 $[M+H]^+$.

*5.1.1.35. 5-(2-[(Dimethylamino)methyl]phenyl)-N1,N3-dimethoxy-N1,N3-dimethylbenzene-1,3-dicarboxamide **24d**.* The compound **24d** was obtained as a red oil (yield: 56%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 7.99 (t, J = 1.6 Hz, 1H); 7.82 (d, J = 1.6 Hz,2H); 7.56 (m, 1H); 7.42–7.26 (M, 3H); 3.60 (s, 6H); 3.39 (s, 8H); 2.16 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 169.0; 141.1; 140.8; 135.9; 133.7; 131.5; 130.3; 130.0; 127.9; 127.1; 126.7; 61.2; 60.9; 45.1; 33.7. LC–MS (ESI) m/z Calculated: 386.2, Found: 386.2 $[M+H]^+$.

*5.1.1.36. General procedure for the synthesis of compounds **25a–d**.* To a solution of the appropriate Weinreb amide derivatives **24a–d** (0.70 mmol) in THF (5 mL) was added $LiAlH_4$ in THF (1 M, 1.3 mL, 1.33 mmol) dropwise, under N_2 atmosphere at 0 °C. The mixture was stirred at 0 °C for 1 h. Saturated $KHSO_4$ solution (3 mL) was added dropwise. After evaporation of the THF, the residue was dissolved in DCM (30 mL) and washed twice with saturated $NaHCO_3$ solution (20 mL), twice with HCl (1 M, 10 mL) and once with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated.

*5.1.1.37. 5-[2-(Piperidin-1-ylmethyl)phenyl]benzene-1,3-dicarbaldehyde **25a**.* The residue was purified by flash chromatography (PE/EtOAc 10:0 to 6.5:3.5 (v/v)) to give compound **25a** as a colourless oil (yield: 50%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 10.16 (s, 2H); 8.39–8.36 (M, 3H); 7.42–7.27 (M, 4H); 3.26 (s, 2H); 2.29 (m, 4H); 1.50 (m, 4H); 1.41 (m, 2H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 191.3; 143.8; 140.2; 136.6; 136.2; 131.3; 130.1; 129.1; 128.0; 127.5; 61.3; 54.0; 26.0; 24.4. LC–MS (ESI) m/z Calculated: 308.2, Found: 308.1 $[M+H]^+$.

*5.1.1.38. 5-[2-(Morpholine-4-ylmethyl)phenyl]benzene-1,3-dicarbaldehyde **25b**.* The residue was purified by flash chromatography (PE/EtOAc 10:0 to 6.5:3.5 (v/v)) to give compound **25b** as a colourless oil (yield: 50%). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 10.18 (s, 2H); 8.40–8.37 (M, 3H); 7.42–7.27 (M, 4H); 3.65 (m, 4H); 3.32 (s, 2H); 2.37 (m, 4H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 191.1; 143.6; 140.3; 136.7; 135.0; 131.3; 130.3; 129.7; 128.1; 127.8; 67.0; 61.0; 53.1. LC–MS (ESI) m/z Calculated: 310.1, Found: 310.1 $[M+H]^+$.

*5.1.1.39. 5-(2-[(4-Methylpiperazin-1-yl)methyl] phenyl)benzene-1,3-dicarbaldehyde **25c**.* The compound **25c** was obtained as a colourless oil (yield: 92%) and used without further purification in the next step (degradation). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 10.17 (s, 2H); 8.40–8.37 (M, 3H); 7.42–7.30 (M, 4H); 3.32 (s, 2H); 2.46 (br s, 8H); 2.33 (s, 3H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 191.2; 143.7; 140.3; 136.6; 136.0; 131.4; 130.2; 129.6; 128.1; 127.8; 60.5; 55.0; 52.1; 45.8. LC–MS (ESI) m/z Calculated: 323.2, Found: 323.1 $[M+H]^+$.

*5.1.1.40. 5-(2-[(Dimethylamino)methyl]phenyl) benzene-1,3-dicarbaldehyde **25d**.* The compound **25d** was obtained as a colourless oil (yield: 50%) and used without further purification in the next step (degradation). ^{1}H NMR (300 MHz), δ (ppm, $CDCl_3$): 10.17 (s, 2H); 8.38 (m, 1H); 8.30 (m, 2H); 7.52 (m, 1H); 7.46–7.37 (M, 2H); 7.30 (m, 1H); 3.32 (s, 2H); 2.18 (s, 6H). ^{13}C NMR (75 MHz), δ (ppm, $CDCl_3$): 191.2; 143.6; 139.6; 136.8; 135.9; 131.0; 130.1; 129.2; 128.4; 127.7; 61.2; 44.8. LC–MS (ESI) m/z Calculated: 268.13, Found: 268.10 $[M+H]^+$.

5.2. In vitro testing

5.2.1. Cell culture and treatment

The human neuroblastoma cell line SKNSH-SYSY (SY5Y) was cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (PAA), 2 mM L-glutamine (Invitrogen), 1 mM non-essential amino-acids and penicillin/streptomycin (Invitrogen), in a 5% CO_2 humidified incubator at 37 °C. The human APP695 cDNA was subcloned into eukaryotic expression vector pcDNA3.1 (Invitrogen), allowing for G418 antibiotic selection of stable clones. This APP cDNA was transfected into SY5Y cells using the ethyleneimine polymer ExGen 500 (Euromedex) according to the manufacturer's instructions. SY5Y cells stably expressing the APP695 were selected with Geneticin G418 (Invitrogen) and one clone named SY5Y-APP^{wt} was used here.

For treatment, SY5Y-APP^{wt} cells were plated onto 12-well plates (Falcon) 24 h before drug exposure, and cultured in Dulbecco's modified Eagle medium (Invitrogen) supplemented with 10% fetal calf serum (PAA), 2 mM L-glutamine (Invitrogen), 1 mM non-essential amino acids (Invitrogen), 50 units/mL penicillin/streptomycin (Invitrogen), and 200 μg Geneticin G418 (Invitrogen), under 5% CO_2 at 37 °C. Cells were exposed to drugs at the indicated concentrations for 24 h. After treatment, the conditioned medium was collected, spun at 200×g to eliminate the cell debris and frozen at −80 °C for $A\beta_{1-42}$ and $A\beta_{1-40}$ quantification. Treated SY5Y-APP^{wt} cells were collected in 50 μL of Laemmli lysis buffer containing protease inhibitors (Complete Mini, Roche Molecular Biochemicals, Meylan, France), sonicated for 5 min and stored at −80 °C until use. Total protein quantification of extracted samples was performed by BCA™ Protein Assay Kit (Thermo Scientific) according to the manufacturer's protocol.

5.2.2. Western blot analysis

Samples were heated at 85 °C for 2 min with Reducing Agent (Life Technologies™) and equal quantities of total proteins (20 μg/lane) were resolved in NuPAGE® Novex® 16% Tris-Tricine precast gels (Life Technologies™). After electrophoresis, the proteins were transferred onto 0.2 μM PVDF membranes (Life Technologies™) for 1 h at 20 °C using the liquid transfer system (Life Technologies™). Membranes were blocked with 5% skimmed milk in TNT (15 mM Tris buffer pH 8.4, 140 mM NaCl, 0.05% Tween-20) for 1 h at 20 °C. After washing three times, the membrane were incubated with APPCter-C17 rabbit antiserum diluted 1:4000 in TNT overnight at 4 °C. APP-Cter-C17 was raised against the last 17 amino acids of the human APP sequence.[59] To develop the immunoreaction, the blots were incubated with peroxidase-conjugated purified mouse monoclonal anti-goat/sheep IgG (Sigma A 9452, MAb clone GT-34), 1:10,000 in TNT-M, for 1 h at 20 °C, and developed with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). Membranes were scanned with LAS-4000 Mini Image System. CTFα (12 kDa) was detected. Images were obtained with a time exposure from 10 to 320 s. Each image was opened with Adobe Photo Shop CS2 (version 9.0.2) computer program, a compose containing all WB bands was created for analysis. Bands quantification was performed by using Image J 1.37v computer program. Each band was transformed in a plot and the area under the curve was calculated. Results were expressed as arbitrary units of optical density. Membranes were then rinsed for 30 min at 20 °C and reprobed with a goat polyclonal antibody against H3 (1:1000; Santa Cruz Biotechnology).

5.2.3. Secreted $A\beta_{1-40}$, and $A\beta_{1-42}$ quantification

Conditioned medium was used to determine the secreted $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations, using the Human $A\beta_{1-40}$ Assay Kits (IBL) and the INNOTEST™ $A\beta_{1-42}$ ELISA Kit (Innogenetics). Each sample was loaded in duplicate onto a 96 well plate. Experiments were done in triplicate. Results expressed in ng/ml were compared to control conditions arbitrarily given an average value of 100%. Results are presented as IC_{50}, the concentration able to decrease to 50% the basal quantity of secreted $A\beta_{1-40}$ and $A\beta_{1-42}$.

5.2.4. Cytotoxicity assay

The human neuroblastoma cell line (SY5Y) was cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco) supplemented with 2 mM L-glutamine, 100 μg/ml streptomycin, 100 IU/mL penicillin, 1 mM non-essential amino acids and 10% (v/v) heat-inactivated fetal bovine serum (Sigma Aldrich), and grown at 37 °C in a humidified incubator with 5% CO_2.

Cells were then incubated in culture medium that contained various concentrations of test compounds (100, 50, 10, 5, 1, 0.5, 0.1, 0.05 μM), each dissolved in less than 0.1% DMSO. After 72 h of incubation, cell growth was estimated by the colorimetric MTT (thiazolyl blue tetrazolium bromide) assay.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.03.016.

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