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Multi-target 1,4-dihydropyridines showing calcium channel blockade and antioxidant capacity for Alzheimer's disease therapy

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ABSTRACT

In this work we describe the synthesis, Ca^{+2} channel blockade capacity and antioxidant power of N^3,N^5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxamides **1–9**, a number of multi-target small 1,4-dihydropyridines (DHP), designed by juxtaposition of melatonin and nimodipine. As a result, we have identified antioxidant DHP **7** (Ca^{2+} channel blockade: 55%, and 8.78 Trolox/Equivalents), the most balanced DHP analyzed here, for potential Alzheimer's disease therapy.

1. Introduction

Alzheimer's disease (AD) is the main cause of memory impairment and dementia in elderly people [1]. Biochemical hallmarks of AD include the accumulation of abnormal extracellular deposits of amyloid-beta peptide, intracellular neurofibrillary tangles, extensive neuronal death and decreased levels of neurotransmitter acetylcholine [2], as well as oxidative stress (OS) [3] and imbalances in the homeostasis of biometals such as Cu, Fe and Zn [4].

Currently, acetylcholinesterase inhibitors donepezil, rivastigmine, galantamine [5] and the *N*-methyl-D-aspartate receptor antagonist memantine [6], are the only administered drugs for AD therapy, but with very limited therapeutic success. This is possibly due to the complex and multifactorial nature of AD. Consequently, a new therapeutic strategy, the multitarget small molecule (MTSM) approach [7], based on the design of drugs able to bind simultaneously at diverse enzymatic systems or receptors involved in AD pathology, is being actively pursued and investigated in a number of laboratories around the world [8,9]. Following this paradigm, we have recently developed a number of new MTSM using multicomponent reactions [10–12] as the method of choice for introducing rapidly and efficiently chemical diversity leading to highly enriched pharmacological MTSM.

Continuing with our contributions to this area, in this preliminary communication we report the design, synthesis, calcium channel blockade and antioxidant properties of *N*3,*N*5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxamides **1–9** (Scheme 1), as MTSMs belonging the new family of 1,4-dihydropyridines (DHP) **I**, resulting from the juxtaposition of DHP nimodipine [13], a well-known calcium channel blocker and melatonin [14], a reputed antioxidant agent (Fig. 1) via Hantzsch multicomponent reaction.

1,4-DHPs are privileged scaffolds in medicinal chemistry, and the core of well known calcium channel antagonists such as nimodipine or nilvadipine, which is undergoing an European multicenter, double-blind, placebo-controlled, phase III trial targeted to mild-to-moderate AD [15]. In fact, it is well known that increased cytosolic calcium level facilitates the formation of Aβ peptides through calcium-mediated β-secretase activity [16] and also regulates the glycogen synthase kinase, protein kinase C, and other kinases that hyperphosphorylate tau and potentiate neurofibrillary tangles formation [17]. Furthermore, calcium

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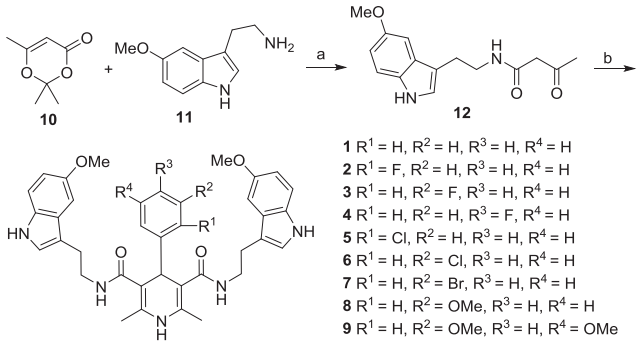


Scheme 1. Reagents and conditions: (a) Xylene, 125 °C, 30 min (69%); (b) ArCHO, $(NH_4)_2CO_3$, EtOH/H_2O, 60 °C, 16 h.

entry through voltage-gated L-type Ca^{2+} channels causes both calcium overload and mitochondrial disruption, which lead to the activation of the apoptotic cascade and cell death [18].

On the other hand, melatonin [19] protects neurons from OS [20], due to its ability to scavenge different types of radical oxygenated species in cells [21], and potent antioxidant capacity [22]. In addition, melatonin modulates the nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/ARE pathway [23], a mechanism of defense that reduces OS and inflammation by triggering the endogenous expression of detoxifying enzymes, leading to the downregulation of iNOS and COX-2 enzymes.

As shown, DHP **I** are symmetrical, non-chiral derivatives, bearing the 1,4-DHP ring as the central core and two identical melatonin motives attached to the carboxylic ester groups at C3 and C5, following our strategy "*two better than one*", that has resulted in potent antioxidant agents [11]. In order to establish structure-activity relationships (SAR) we have prepared nine DHP **I** bearing diverse substituents at the aromatic ring located at C3, in positions C2′-C5′, such as F, Cl, Br and OMe, excluding the NO_2 group due to its well known capacity to afford Pan Assay Interference Compounds (PAINS) [24] when incorporated in 1,4-DHPs. From this work we have identified 4-(3-bromophenyl)-*N*^{3},*N*^{5}-bis (2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (DHP **7**) (Scheme1) as the most balanced DHP in terms of Ca^{+2} channel antagonism and antioxidant capacity.

2. Results and discussion

2.1. Chemistry

The synthesis of DHP **1–9** has been carried out as shown in scheme 1 starting form commercially available 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**10**) and 2-(5-methoxy-1*H*-indol-3-yl)ethan-1-amine (**11**), by thermal promoted *N*-amidation to give the known *N*-(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-3-oxobutanamide (**12**) [25] in 69% yield, followed by Hantzsch 1,4-DHP synthesis using ammonium carbonate in EtOH/H_2O. The desired new compounds were obtained in yields ranging from 22 to 76% (Table 1), and their analytical and spectroscopic data are in good agreement their structures, confirming also their high purity (**Experimental Part**, and **Supporting Information**).

2.2. Calcium channel blockade

The calcium channel blockade capacity of compounds **1–9**, and nimodipine as standard, at 5 μM concentration, has been carried out following the usual methodology [26]. As shown in Table 1, the observed % values ranged from 13 (DHP **2**, R^1 = F) to 68 (DHP **5**, R^1 = Cl) and the parent compound showed no activity. The most potent DHPs corresponded, in decreasing order, to DHPs **5** (68%), **4** (66%), and **7** (55%), comparing thus very favorably with nimodipine (52%). From the point of view of the SRA some conclusions could be withdrawn. For instance, for the F-substituted DHPs, the Ca^{2+} channel blockade increases from DHP **2** to **3** and **4**, the most potent DHP bearing the fluorine atom at C4′ (66%), followed by the one bearing it at C3′ (25%) and at C2′ (13%), the contrary to what is observed for DHPs **5** and **6**, where the DHP bearing the chlorine at C2′ (68%) is more potent than the DHP bearing the Cl at C3′ (12%). In fact, DHPs **4** and **5**, bearing F at C4′ and Cl at C2′, respectively, are equipotent. Interestingly to note was also that a bromine atom at the same position, C3′ (DHP **7**), compared to the DHPs bearing F or Cl, affords the highest blockade power, while DHP **8** bearing an electron donor (OMe) substituent at C3′ is slightly less potent than DHP **7**, but greatest than the ones bearing F or Cl at the same position. However, the incorporation of a second methoxy group at C5′, as in DHP **9**, significantly decreases the Ca blockade rising it to 27%, in the same order as shown for DHP **3** (25%) bearing only one F at C3′. In any case, DHPs **4** and **5** are the most potent Ca^{2+} blockade agents detected here at 5 μM, being1.3-fold more active than nimodipine.

2.3. Antioxidant assay

The antioxidant activity of the DHPs **1–9** was determined by the ORAC-FL method. [27] Their radical scavenging properties are expressed as Trolox equivalents (TE) units, melatonin being used as positive control showing ORAC value 2.45 [10]. As shown in Table 1, all compounds presented strong antioxidant capacity, higher than the value determined for melatonin, in the same experiment. In this case the lowest ORAC value corresponded to DHP **1** (6.93) while DHP **7** (C3′ Br) showed the highest (8.78). Very interestingly, the SRA trend observed for the Ca^{2+} blockade capacity regarding DHPs **2–4** is reversed for the ORAC analysis for the same DHPs, but not for DHPs **8** and **7**, which have the same ORAC value, or exceed those shown by DHPs **4–6**, respectively.

2.4. Lipinski's rule for drug likeliness and in silico ADME prediction

In order to evaluate the potential pharmacokinetic profile of compounds DHPs **1–9** *in silico*, we have used the QikProp software [QikProp, version 3.8, Schrodinger, LLC, New York, NY, 2013]. The results are summarized in Table 1S (**Supporting Information**).

The Lipinski's rule of five [28] is the most widespread method to assess the drug like properties of molecules during the early stages of the drug discovery process. We found that DHPs **1–9** violate the Lipinski's rule due to their high MW (MW > 500) and because the partition coefficients, critical for estimation of absorption within the body, are out of the range of accepted values (QPlogPo/w < 5). The number of hydrogen bond acceptors (NHBA) and hydrogen bond donors (NHBD) were in accordance with the Lipinski's rule of five (NHBD ≤ 5, NHBA ≤ 10) for all the compounds. Nevertheless, a few violations to this rule are very common. In general, an orally active compound should not have more than 2 violations of the Lipinski's rule. DHPs in present study, except DJHP **9**, did not violate the rule more than the maximum permissible limits, showing thus drug likeness properties. Intestinal absorption or permeation is also one of the important factors to be studied regarding the absorption of the drug molecule, which was further confirmed by predicted Caco-2 cell permeability (QPPCaco), used as model for gut-blood barrier. Caco-2 cell permeability prediction for all the DHPs studied here indicates appropriate results, predicting good intestinal absorption. The most used parameter to assess the Blood Brain (BB) barrier penetration is logBB. The computed values for DHPs **1–9** lie within the indicated limits of −3 < QPlogBB < 1.2. The aqueous solubility (QPlogS) of organic compounds has a significant impact on many ADME-related properties, and only DHP **8** (QPlogS: −5.69) presents solubility value within the limits (-6.5 to 0.5). Finally, the predicted average number of possible metabolic reactions indicates that all DHPs possessed metabolic values higher than the recommended range (1–8) due to the complexity of the molecules.

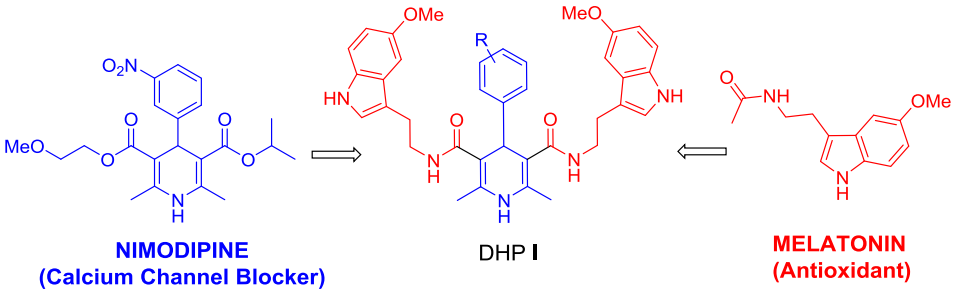
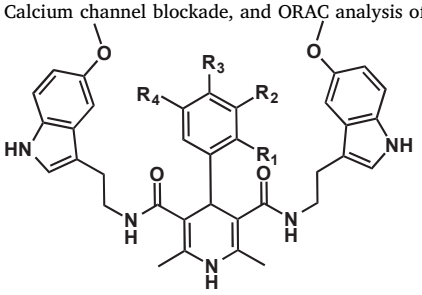


Fig. 1. Structures of melatonin and nimodipine, and the DHP **I** investigated in this work.

Table 1
Calcium channel blockade, and ORAC analysis of compounds **1–9**, nimodipine and melatonin.



DHP	R^1	R^2	R^3	R^4	Yield (%)	(%) Calcium channel bolckade[a]	ORAC[b]
1	H	H	H	H	31	Not active	6.93 ± 0.23
2	F	H	H	H	76	13	8.20 ± 0.10
3	H	F	H	H	31	25	7.70 ± 0.25
4	H	H	F	H	22	**66**	**6.99 ± 0.23**
5	Cl	H	H	H	43	**68**	**6.98 ± 0.19**
6	H	Cl	H	H	38	12	7.00 ± 0.05
7	H	Br	H	H	47	**55**	**8.78 ± 0.17**
8	H	OMe	H	H	42	**47**	**7.11 ± 0.15**
9	H	OMe	H	OMe	50	27	8.74 ± 0.09
Melatonin	–	–	–	–	–	nd[c]	2.45 ± 0.09
Nimodipine	–	–	–	–	–	**52**	nd[c]

[a] Each % value is determined at 5 μM of at least three different experiments.
[b] Data are expressed as Trolox equivalents and are the mean (n = 3) ± SEM.
[c] nd: not determined.

3. Conclusion

In this work we have reported for the synthesis of N^3,N^5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxamides **1–9**, by Hantzsch multicomponent reaction, from modest to good yields. Their calcium channel blockade power and antioxidant capacity has been investigated. From these results, we have identified 4-(3-fluorophenyl)-N^3,N^5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (DHP **4**) and 4-(2-chlorophenyl)-N^3,N^5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (DHP **5**) as the most potent Ca^{2+} channel antagonists, being 1.3-fold more active than nimodipine, and 2.9-fold more potent than melatonin as antioxidants. However, 4-(3-bromophenyl)-N^3,N^5-bis(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (DHP **7**) was not only the most potent antioxidant agent investigated here, being 3.5-fold more potent than melatonin, and 1.2-fold more potent than DHP **4** and **5**, but was equipotent to nimodipine as Ca^{2+} channel blocker (55% *vs* to 52%). These data led us to suggest that although DHP **4** and **5** are very interesting compounds, DHP **7** is possibly the most balanced MTSM investigated here, that deserves further investigation for the potential treatment of AD. Work is in progress in our laboratories and will be reported in due course.

4. Experimental part

4.1. General methods

Materials and methods. The starting materials were purchased from Sigma-Aldrich Chimie S.a.r.l (Saint-Quentin-Fallavier, France) or TCI Europe N.V. (Zwijndrecht, Belgium). Proton and Carbon JMOD NMR spectra were recorded on a Bruker AC 400 spectrometer (Bruker Biospin). The chemical shifts are expressed in parts per million (ppm) and the multiplicities are indicated by the following abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; p, pentuplet; m, multiplet, and coupling constants are expressed in Hertz (Hz). Melting points (mp) were obtained on a Köfler apparatus and were not corrected. All reactions were monitored by Thin Layer Chromatography (TLC) using Alugram Sil G/UV_{254} plates and visualized by UV light. Flash Column chromatography was carried out using silica gel 60 (70–230 mesh, Macherey-Nagel). Elemental analyses were performed on Thermo-Finnigan Flash EA 1112.

4.1.1. Synthesis of *N*-(2-(5-methoxy-1H-indol-3-yl)ethyl)-3-oxobutanamide (12) [25]

A mixture of 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**10**) (1.1 g, 7.9 mmol, 1 equiv) and 2-(5-methoxy-1*H*-indol-3-yl)ethan-1-amine (**11**) (1.5 g, 7.9 mmol, 1 equiv) in xylene (2 mL) was heated at 125 °C for 30 min. The solvent was then evaporated and the crude purified by

flash chromatography using CH_2Cl_2/MeOH/NH_3 (95:4.95:0.05), to give compound **12** (1.5 g, 69%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (br s, 1H), 7.18 (d, J = 8.8 Hz, 1H), 7.04–6.97 (m, 2H), 6.94 (d, J = 2.1 Hz, 1H), 6.81 (dd, J = 8.8, 2.4 Hz, 1H), 3.81 (s, 3H), 3.59–3.50 (m, 2H), 3.25 (s, 2H), 2.90 (t, J = 6.9 Hz, 2H), 2.12 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.45, 165.89, 153.85, 131.61, 127.63, 123.16, 112.15, 112.12, 100.47, 55.92, 49.83, 39.80, 30.72, 25.09. Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.48; H, 6.72; N, 10.15.

4.1.2. General procedure for the synthesis of the Hantzsch products 1–9

N-(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-3-oxobutanamide (**12**) (1.1 mmol, 2 equiv), the appropriate commercial benzaldehyde (0.66 mmol, 1.2 equiv) and ammonium carbonate (0.66 mmol, 1.2 equiv) were dissoved in EtOH/H_2O (1:1, 1 mL) and the mixture was heated at 60 °C overnight. Then, the mixture was cooled, diluted with water and extracted with CH_2Cl_2. The organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash chromatography using CH_2Cl_2/MeOH/NH_3 (95:4.95: 0.05).

4.1.3. N^3,N^5-Bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-4-phenyl-1,4 dihydropyridine − 3,5-dicarboxamide (1)

31%; ^{1}H NMR (400 MHz, MeOD) δ 7.21 (d, J = 8.8 Hz, 2H), 7.11–7.03 (m, 3H), 7.02–6.99 (m, 2H), 6.97–6.92 (m, 2H), 6.83 (s, 2H), 6.75 (dd, J = 8.8, 2.3 Hz, 2H), 4.58 (s, 1H), 3.81 (s, 6H), 3.42 (t, J = 7.0 Hz, 4H), 2.89–2.59 (m, 4H), 1.99 (s, 6H); ^{13}C NMR (101 MHz, MeOD) δ 172.3, 154.9, 147.2, 139.1, 133.4, 129.5, 128.9, 128.3, 127.6, 124.2, 112.9, 112.6, 106.4, 101.3, 56.3, 44.3, 41.0, 26.1, 17.5. Anal. Calcd. for $C_{37}H_{39}N_5O_4$: C, 71.94; H, 6.36; N, 11.34. Found: C, 71.77; H, 6.42; N, 11.41.

4.1.4. 4-(2-Fluorophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (2)

76%; ^{1}H NMR (400 MHz, DMSO) δ 10.59 (br s, 2H), 7.76 (s, 1H), 7.35 (t, J = 4.7 Hz, 2H), 7.26–7.18 (m, 3H), 7.17–7.11 (m, 1H), 7.10–7.03 (m, 1H), 7.03–6.94 (m, 5H), 6.71 (dd, J = 8.7, 2.2 Hz, 2H), 5.05 (s, 1H), 3.75 (s, 6H), 3.32–3.24 (m, 3H), 2.79–2.61 (m, 4H), 2.00 (s, 5H); ^{13}C NMR (101 MHz, DMSO) δ 168.2, 158.7 (d, J = 245.0 Hz), 152.9, 137.1, 133.7 (d, J = 14.6 Hz), 131.3, 130.4 (d, J = 5.2 Hz), 127.6 (d, J = 8.1 Hz), 127.4, 124.2 (d, J = 2.4 Hz), 123.1, 114.8 (d, J = 23.2 Hz), 111.9, 111.6, 110.9, 104.2, 100.1, 55.3, 35.6, 25.3, 17.2. Anal. Calcd. for $C_{37}H_{38}FN_5O_4$: C, 69.90; H, 6.03; N, 11.02. Found: C, 70.02; H, 5.97; N, 11.11.

4.1.5. 4-(3-Fluorophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (3)

31%; ^{1}H NMR (400 MHz, DMSO) δ 10.59 (d, J = 1.7 Hz, 2H), 7.78 (s, 1H), 7.42 (t, J = 5.6 Hz, 2H), 7.26–7.14 (m, 4H), 7.02–6.99 (m, 3H), 6.99–6.86 (m, 4H), 6.71 (dd, J = 8.7, 2.4 Hz, 2H), 4.85 (s, 1H), 3.76 (s, 6H), 2.80–2.61 (m, 4H), 2.03 (s, 5H); ^{13}C NMR (101 MHz, DMSO) δ 168.2, 162.2 (d, J = 242.5 Hz), 152.9, 149.9, 137.3, 131.3, 129.5 (d, J = 8.1 Hz), 127.4, 123.2, 123.1, 113.8 (d, J = 20.9 Hz), 112.4 (d, J = 21.3 Hz), 111.9, 111.6, 110.9, 104.7, 100.1, 55.3, 41.1, 25.3, 17.3. Anal. Calcd. for $C_{37}H_{38}FN_5O_4$: C, 69.90; H, 6.03; N, 11.02. Found: C, 69.81; H, 6.07; N, 11.14.

4.1.6. 4-(4-Fluorophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (4)

22%; ^{1}H NMR (400 MHz, DMSO) δ 10.61 (d, J = 1.8 Hz, 2H), 7.76 (s, 1H), 7.34 (t, J = 5.6 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H), 7.15–7.07 (m, 2H), 7.04–6.98 (m, 4H), 6.94 (t, J = 8.9 Hz, 2H), 6.71 (dd, J = 8.7, 2.4 Hz, 2H), 4.77 (s, 1H), 3.76 (s, J = 5.9 Hz, 6H), 3.32–3.24 (m, 2H), 2.81–2.60 (m, 4H), 2.03 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 168.2, 160.5 (d, J = 241.1 Hz), 152.9, 143.1, 137.2, 131.4, 128.9 (d, J = 7.8 Hz), 127.5, 123.2, 114.4 (d, J = 20.9 Hz), 111.9, 111.6, 110.9, 105.0, 100.2, 55.3, 54.9, 40.5, 40.2, 40.1, 39.9, 39.9, 39.7, 39.7, 39.5, 39.5, 39.3, 39.1, 38.8, 25.3, 17.3. Anal. Calcd. for $C_{37}H_{38}FN_5O_4$: C, 69.90; H, 6.03; N, 11.02. Found: C, 69.81; H, 6.11; N, 11.09.

4.1.7. 4-(2-Chlorophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (5)

43%; ^{1}H NMR (400 MHz, DMSO) δ 10.58 (d, J = 1.7 Hz, 2H), 7.75 (s, 1H), 7.41–7.33 (m, 1H), 7.29–7.24 (m, 2H), 7.24–7.18 (m, 4H), 7.14–7.07 (m, 1H), 7.01–6.96 (m, 4H), 6.70 (dd, J = 8.7, 2.4 Hz, 2H), 5.17 (s, 1H), 3.75 (s, 6H), 3.31–3.16 (m, 4H), 2.79–2.58 (m, 4H), 1.97 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 168.2, 152.9, 145.1, 136.5, 131.3, 131.2, 130.3, 128.6, 127.5, 127.4, 123.1, 111.9, 111.6, 110.9, 105.2, 100.1, 55.3, 25.2, 17.1. Anal. Calcd. for $C_{37}H_{38}ClN_5O_4$: C, 68.14; H, 5.87; N, 10.74. Found: C, 68.17; H, 5.81; N, 10.79.

4.1.8. 4-(3-Chlorophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (6)

38%; ^{1}H NMR (400 MHz, MeOD) δ 7.20 (d, J = 8.7 Hz, 2H), 7.12 (t, J = 1.8 Hz, 1H), 7.09–7.05 (m, 1H), 7.01 (d, J = 2.2 Hz, 2H), 7.00–6.97 (m, 1H), 6.88 (s, 2H), 6.83 (d, J = 7.6 Hz, 1H), 6.74 (dd, J = 8.8, 2.4 Hz, 2H), 4.69 (s, 1H), 3.80 (s, 6H), 3.52–3.35 (m, 4H), 2.90–2.70 (m, 4H), 1.97 (s, 6H); ^{13}C NMR (101 MHz, MeOD) δ 172.1, 154.9, 149.4, 139.1, 135.4, 133.4, 130.8, 128.9, 128.6, 127.6, 126.7, 124.1, 112.9, 112.9, 112.6, 106.0, 101.3, 56.3, 44.2, 41.1, 26.2, 17.4. Anal. Calcd. for $C_{37}H_{38}ClN_5O_4$: C, 68.14; H, 5.87; N, 10.74. Found: C, 68.29; H, 5.91; N, 10.85.

4.1.9. 4-(3-Bromophenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (7)

47%; ^{1}H NMR (400 MHz, DMSO) δ 10.60 (d, J = 1.6 Hz, 2H), 7.80 (s, 1H), 7.45 (t, J = 5.6 Hz, 2H), 7.39–7.34 (m, 1H), 7.33–7.26 (m, 1H), 7.22 (d, J = 8.7 Hz, 2H), 7.17–7.08 (m, 2H), 7.05–6.98 (m, 4H), 6.71 (dd, J = 8.7, 2.4 Hz, 2H), 5.76 (s, 1H), 4.85 (s, 1H), 3.76 (s, 6H), 3.32–3.22 (m, 2H), 2.82–2.59 (m, 4H), 2.04 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 168.1, 152.9, 149.7, 137.3, 131.3, 130.0, 130.0, 128.5, 127.5, 126.3, 123.1, 121.4, 111.9, 111.7, 110.9, 104.6, 100.1, 55.3, 41.2, 25.3, 17.4. Anal. Calcd. for $C_{37}H_{38}BrN_5O_4$: C, 63.79; H, 5.50; N, 10.05. Found: C, 63.82; H, 5.28; N, 10.11.

4.1.10. N^3,N^5-Bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-4-(3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (5)

42%; ^{1}H NMR (400 MHz, DMSO) δ 10.58 (d, J = 1.8 Hz, 2H), 7.73 (s, 1H), 7.32 (t, J = 5.6 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.08 (t, J = 7.8 Hz, 1H), 7.04–6.95 (m, 4H), 6.77–6.64 (m, 5H), 4.77 (s, 1H), 3.75 (s, 6H), 3.66 (s, 3H), 3.31–3.25 (m, 2H), 2.82–2.59 (m, 4H), 2.04 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 168.4, 159.1, 152.9, 148.6, 137.3, 131.4, 128.9, 127.5, 123.2, 119.6, 113.3, 111.9, 111.7, 110.9, 110.7, 104.9, 100.2, 55.3, 54.7, 41.2, 25.4, 17.4. Anal. Calcd. for $C_{38}H_{41}N_5O_5$: C, 70.46; H, 6.38; N, 10.81. Found: C, 70.51; H, 6.31; N, 10.85.

4.1.11. 4-(3,5-Dimethoxyphenyl)-N^3,N^5-bis(2-(5-methoxy-1H-indol-3-yl)ethyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9)

50%; ^{1}H NMR (400 MHz, DMSO) δ 10.58 (d, J = 1.9 Hz, 2H), 7.72 (s, 1H), 7.33 (t, J = 5.7 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.05–6.98 (m, 4H), 6.71 (dd, J = 8.7, 2.4 Hz, 2H), 6.36 (d, J = 2.3 Hz, 2H), 6.27 (t, J = 2.3 Hz, 1H), 4.76 (s, 1H), 3.75 (s, 6H), 3.65 (s, 6H), 2.82–2.60 (m, 4H), 2.04 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 168.4, 160.2, 152.9, 149.3, 137.2, 131.4, 127.5, 123.1, 111.9, 111.7, 110.9, 105.6, 104.8, 100.2, 97.2, 55.3, 54.8, 41.4, 25.5, 17.4. Anal. Calcd. for $C_{39}H_{43}N_5O_6$: C, 69.11; H, 6.39; N, 10.33. Found: C, 69.28; H, 6.28; N, 10.25.

4.2. Calcium channel blockade

Human neuroblastoma cell line SH-SY5Y (CRL-2266) obtained directly from American Type Culture Collection were seeded out in 96-well dark-walled plates with Dulbecco's Modified Eagle Medium/

Nutrient Mixture F-12 (DMEM/F-12) as a medium (ThermoFisher Scientific). For the assay, medium was aspirated and cells were washed two times with Hanks' Balanced Salt Solution (HBSS, ThermoFisher Scientific). Then, cells were loaded with 5 μM Fluo4-AM calcium indicator (Thermo Fisher Scientific) in HBSS for 30 min at room temperature. Subsequently, cells were washed twice with HBSS and left for another 30 min at room temperature for de-estrification of Fluo4-AM. The Fluo4-loaded cells were treated with compounds of interest (5 μM in HBSS) for 10 min. Plates were imaged with automated imaging system Pathway 855 (Becton Dickinson, USA) at excitation wavelength of 488 nm and emission bandwidth of 530–590 nm. The baseline fluorescence was imaged for 12 sec with one second interval. Then, the cells were stimulated with KCl/CaCl2 solution (dissolved in HBSS, final concentration of KCl and CaCl2 was 90 mM and 5 mM, respectively) and the cells were imaged for further 120 s with one second interval. Every cell in the microscope field of view was regarded as a separate region of interest (ROIs). Fluorescent intensities of each of the ROIs was collected at each of the timepoints. For each of analyzed compounds, the number of imaged cells and subsequent ROIs analyzed averaged at n = 1789. DMSO (0.01%) was used as a vehicle control. Nimodipine (5 μM) was used as a reference inhibitor, which exhibited ca 50% inhibition of calcium influx. Compounds were assessed at the same concentration as nimodipine in quadruplicates in two independent experiments. Fluorescent intensity values were normalized to the baseline. Raw data was collected as a normalized pixel intensity (relative fluorescence intensity) values averaged from all ROIs for each of the compounds plotted against time. Inhibition values were expressed as a maximum pixel intensity in relation to vehicle control which was established as a 100% of the signal.

4.3. Oxygen radical absorbance capacity assay

The antioxidant activity of hybrids **DHP I** was carried out by the ORAC-FL using fluorescein as a fluorescent probe. Briefly, fluorescein and antioxidant were incubated in a black 96-well microplate (Nunc) for 15 min at 37 °C. 2,2′-Azobis(amidinopropane) dihydrochloride was then added quickly using the built-in injector of Varioskan Flash plate reader (Thermo Scientific). The fluorescence was measured at 485 nm (excitation wavelength) and 535 nm (emission wavelength) each min for 1 h. All the reactions were made in triplicate and at least three different assays were performed for each sample.

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Appendix A. Supplementary material

Supporting information summary. NMR spectra of DHPs 1–9, and compound 12, and Table 1S. Predicted ADME and molecular properties for DHPs 1–9. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103205.

References

[1] H.W. Querfurth, F.M. LaFerla, Alzheimer's disease, N. Engl. J. Med. 362 (2010) 329–344, https://doi.org/10.1056/NEJMra0909142.
[2] R.T. Bartus, R.L. Dean, B. Beer, A.S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (1982) 408–414, https://doi.org/10.1126/science.7046051.
[3] M. Rosini, E. Simoni, A. Milelli, A. Minarini, C. Melchiorre, Oxidative stress in Alzheimer's disease: are we connecting the dots? J. Med. Chem. 57 (2014) 2821–2831, https://doi.org/10.1021/jm400970m.
[4] A.I. Bush, Drug development based on the metals hypothesis of Alzheimer's disease, J. Alzheimers Dis. JAD. 15 (2008) 223–240.
[5] G. Mushtaq, N.H. Greig, J.A. Khan, M.A. Kamal, Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus, CNS Neurol. Disord. Drug Targets. 13 (2014) 1432–1439.
[6] D. Wilkinson, Y. Wirth, C. Goebel, Memantine in patients with moderate to severe Alzheimer's disease: meta-analyses using realistic definitions of response, Dement. Geriatr. Cogn. Disord. 37 (2014) 71–85, https://doi.org/10.1159/000353801.
[7] M.J. Oset-Gasque, J. Marco-Contelles, Alzheimer's disease, the "one-molecule, one-target" paradigm, and the multitarget directed ligand approach, ACS Chem. Neurosci. 9 (2018) 401–403, https://doi.org/10.1021/acschemneuro.8b00069.
[8] A. Cavalli, M.L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini, C. Melchiorre, Multi-target-directed ligands to combat neurodegenerative diseases, J. Med. Chem. 51 (2008) 2326, https://doi.org/10.1021/jm800210c.
[9] R. León, A.G. Garcia, J. Marco-Contelles, Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease, Med. Res. Rev. 33 (2013) 139–189, https://doi.org/10.1002/med.20248.
[10] M. Benchekroun, L. Ismaili, M. Pudlo, V. Luzet, T. Gharbi, B. Refouvelet, J. Marco-Contelles, Donepezil–ferulic acid hybrids as anti-Alzheimer drugs, Future Med. Chem. 7 (2015) 15–21, https://doi.org/10.4155/fmc.14.148.
[11] M. Benchekroun, A. Romero, J. Egea, R. León, P. Michalska, I. Buendía, M.L. Jimeno, D. Jun, J. Janockova, V. Sepsova, O. Soukup, O.M. Bautista-Aguilera, B. Refouvelet, O. Ouari, J. Marco-Contelles, L. Ismaili, The antioxidant additive approach for Alzheimer's disease therapy: new ferulic (lipoic) acid plus melatonin modified tacrines as cholinesterases inhibitors, direct antioxidants, and nuclear factor (erythroid-derived 2)-like 2 activators, J. Med. Chem. 59 (2016) 9967–9973, https://doi.org/10.1021/acs.jmedchem.6b01178.
[12] L. Ismaili, M. do Carmo Carreiras, Multicomponent reactions for multitargeted compounds for Alzheimer's disease, Curr. Top. Med. Chem. 17 (2018) 3319–3327, https://doi.org/10.2174/1568026618666180112155424.
[13] L. Janovák, Á. Turcsányi, É. Bozó, Á. Deák, L. Mérai, D. Sebők, Á. Juhász, E. Csapó, M.M. Abdelghafour, E. Farkas, I. Dékány, F. Bari, Preparation of novel tissue acidosis-responsive chitosan drug nanoparticles: Characterization and in vitro release properties of Ca2+ channel blocker nimodipine drug molecules, Eur. J. Pharm. Sci. 123 (2018) 79–88, https://doi.org/10.1016/j.ejps.2018.07.031.
[14] E. Ramos, J. Egea, C. de Los Ríos, J. Marco-Contelles, A. Romero, Melatonin as a versatile molecule to design novel multitarget hybrids against neurodegeneration, Future Med. Chem. (2017) 765–780, https://doi.org/10.4155/fmc-2017-0014.
[15] J. Godyń, J. Jończyk, D. Panek, B. Malawska, Therapeutic strategies for Alzheimer's disease in clinical trials, Pharmacol. Rep. 68 (2016) 127–138, https://doi.org/10.1016/j.pharep.2015.07.006.
[16] C.-H. Chen, W. Zhou, S. Liu, Y. Deng, F. Cai, M. Tone, Y. Tone, Y. Tong, W. Song, Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease, Int. J. Neuropsychopharmacol. 15 (2012) 77–90, https://doi.org/10.1017/S1461145711000149.
[17] J. Avila, M. Pérez, F. Lim, A. Gómez-Ramos, F. Hernández, J.J. Lucas, Tau in neurodegenerative diseases: Tau phosphorylation and assembly, Neurotox. Res. 6 (2004) 477–482, https://doi.org/10.1007/BF03033284.
[18] S. Chakroborty, G.E. Stutzmann, Calcium channelopathies and Alzheimer's disease: insight into therapeutic success and failures, Eur. J. Pharmacol. 739 (2014) 83–95, https://doi.org/10.1016/j.ejphar.2013.11.012.
[19] J. Vriend, R.J. Reiter, Melatonin feedback on clock genes: a theory involving the proteasome, J. Pineal Res. 58 (2015) 1–11, https://doi.org/10.1111/jpi.12189.
[20] L.C. Manchester, A. Coto-Montes, J.A. Boga, L.P.H. Andersen, Z. Zhou, A. Galano, J. Vriend, D.-X. Tan, R.J. Reiter, Melatonin: an ancient molecule that makes oxygen metabolically tolerable, J. Pineal Res. 59 (2015) 403–419, https://doi.org/10.1111/jpi.12267.
[21] I.B. Zavodnik, A.V. Domanski, E.A. Lapshina, M. Bryszewska, R.J. Reiter, Melatonin directly scavenges free radicals generated in red blood cells and a cell-free system: chemiluminescence measurements and theoretical calculations, Life Sci. 79 (2006) 391–400, https://doi.org/10.1016/j.lfs.2006.01.030.
[22] H.-M. Zhang, Y. Zhang, Melatonin: a well-documented antioxidant with conditional pro-oxidant actions, J. Pineal Res. 57 (2014) 131–146, https://doi.org/10.1111/jpi.12162.
[23] J. Vriend, R.J. Reiter, The Keap1-Nrf2-antioxidant response element pathway: a review of its regulation by melatonin and the proteasome, Mol. Cell. Endocrinol. 401 (2015) 213–220, https://doi.org/10.1016/j.mce.2014.12.013.
[24] J.B. Baell, G.A. Holloway, New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays, J. Med. Chem. 53 (2010) 2719–2740, https://doi.org/10.1021/jm901137j.
[25] A.H. Jackson, P.V.R. Shannon, D.J. Wilkins, A novel two-stage route to the 2a,5a-diazacyclopenta[jk]fluorene ring system, J. Chem. Soc. Chem. Commun. (1987) 653–654, https://doi.org/10.1039/C39870000653.
[26] M. Chioua, E. Buzzi, I. Moraleda, I. Iriepa, M. Maj, A. Wnorowski, C. Giovannini, A. Tramarin, F. Portali, L. Ismaili, P. López-Alvarado, M.L. Bolognesi, K. Jóźwiak, J.C. Menéndez, J. Marco-Contelles, M. Bartolini, Tacripyrimidines, the first tacrine-dihydropyrimidine hybrids, as multi-target-directed ligands for Alzheimer's disease, Eur. J. Med. Chem. 155 (2018) 839–846, https://doi.org/10.1016/j.ejmech.2018.06.044.
[27] A. Dávalos, C. Gómez-Cordovés, B. Bartolomé, Extending applicability of the oxygen radical absorbance capacity (ORAC − Fluorescein) assay, J. Agric. Food Chem. 52 (2004) 48–54, https://doi.org/10.1021/jf0305231.
[28] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.