ChemPubSoc Europe

DOI: 10.1002/cmdc.201500383

CHEMMEDCHEM Communications

SPECIAL ISSUE

Design, Synthesis and in vitro Evaluation of Indolotacrine Analogues as Multitarget-Directed Ligands for the Treatment of Alzheimer's Disease

Ondrej Benek,[a, b, c] Ondrej Soukup,[b, c] Marketa Pasdiorova,[a, c] Lukas Hroch,[c, d] Vendula Sepsova,[a, c] Petr Jost,[a, c] Martina Hrabinova,[a, c] Daniel Jun,[a] Kamil Kuca,[c, g] Dominykas Zala,[e] Rona R. Ramsay,[e] José Marco-Contelles,*[f] and Kamil Musilek*[c, g]

Novel indolotacrine analogues were designed, synthesized, and evaluated as potential drugs for the treatment of Alzheimer's disease. By using a multitarget-directed ligand approach, compounds were designed to act simultaneously as cholinesterase (ChE) and monoamine oxidase (MAO) inhibitors. The compounds were also evaluated for antioxidant, cytotoxic, hepatotoxic, and blood–brain barrier (BBB) permeability properties. Indolotacrine **9b** (9-methoxy-2,3,4,6-tetrahydro-1*H*-indolo[2,3-*b*]quinolin-11-amine) showed the most promising results in the in vitro assessment; it is a potent inhibitor of acetylcholinesterase (AChE IC_{50}: 1.5 μM), butyrylcholinesterase (BChE IC_{50}: 2.4 μM) and MAO A (IC_{50}: 0.49 μM), and it is also a weak inhibitor of MAO B (IC_{50}: 53.9 μM). Although its cytotoxic (IC_{50}: 5.5 ± 0.4 μM) and hepatotoxic (IC_{50}: 1.22 ± 0.11 μM) profiles are not as good as those of the standard 7-methoxytacrine (IC_{50}: 63 ± 4 and 11.50 ± 0.77 μM, respectively), the overall improvement in the inhibitory activities and potential to cross the BBB make indolotacrine **9b** a promising lead compound for further development and investigation.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by progressive and irreversible cognitive impairment and memory loss.[1] Despite enormous efforts, the etiology of AD has not yet been elucidated, and the disease remains incurable.[2] According to current knowledge, β-amyloid (Aβ) aggregates,[3] τ-protein phosphorylation,[4] oxidative stress,[5] and deficits in acetylcholine (ACh)[6] are considered to play significant roles in AD pathophysiology.

The cholinergic hypothesis asserts that the decreased level of ACh in the brain leads to cognitive and memory deficits, and that sustaining or recovering cholinergic function should therefore result in amelioration of the symptoms.[7–9] Accordingly, current AD therapy is based mainly on acetylcholinesterase (AChE) inhibitors (AChEIs), which are able to increase ACh levels in cholinergic synapses. To date, the number of approved drugs is limited to three AChEIs (rivastigmine, donepezil, and galantamine) and an *N*-methyl-D-aspartic acid (NMDA) antagonist (memantine). However, these drugs cannot prevent or cure the disease, but afford only symptomatic treatment.[9,10]

The "one-target, one-compound" paradigm has been highly successful for many common diseases because their underlying molecular mechanisms were understood, allowing biologists to define the key target for a particular disease. Once the target was identified, medicinal chemists strategically designed a molecule to interact selectively with such a target, with a potential drug as the outcome. However, it is apparent that this target-based approach does not always guarantee success. Drugs directed to a single target might not always modify complex multifactorial diseases such as AD, even if they act in the way they are expected to proceed.[11] It is now widely accepted that a more effective therapy would result from the use of multipotent compounds able to intervene simultaneously in the different pathological events underlying the etiology of AD.[12,13]

Monoamine oxidase (MAO; EC 1.4.3.4) is another important target that was considered for the treatment of AD because some symptoms of AD are caused by alterations in the dopa-

[a] O. Benek, M. Pasdiorova, Dr. V. Sepsova, P. Jost, M. Hrabinova, Dr. D. Jun
Department of Toxicology and Military Pharmacy
Department of Epidemiology, Faculty of Military Health Sciences
University of Defense
Trebesska 1575, 500 01 Hradec Kralove (Czech Republic)

[b] O. Benek, Dr. O. Soukup
National Institute of Mental Health
Topolova 748, 250 67 Klecany (Czech Republic)

[c] O. Benek, Dr. O. Soukup, M. Pasdiorova, L. Hroch, Dr. V. Sepsova, P. Jost, M. Hrabinova, Prof. K. Kuca, Dr. K. Musilek
University Hospital, Sokolska 581, 500 05 Hradec Kralove (Czech Republic)

[d] L. Hroch
Department of Pharmaceutical Chemistry and Drug Control
Faculty of Pharmacy in Hradec Kralove, Charles University in Prague
Akademika Heyrovskeho 1203, 500 05 Hradec Kralove (Czech Republic)

[e] D. Zala, Dr. R. R. Ramsay
School of Biology, Biomolecular Sciences Building
University of St. Andrews, North Haugh, St. Andrews, KY16 9ST (UK)

[f] Prof. J. Marco-Contelles
Laboratory of Medicinal Chemistry (IQOG, CSIC)
Juan de la Cierva 3, 28006 Madrid (Spain)
E-mail: iqoc21@iqog.csic.es

[g] Prof. K. Kuca, Dr. K. Musilek
Department of Chemistry, Faculty of Science, University of Hradec Kralove
Rokitanskeho 62, 500 03 Hradec Kralove (Czech Republic)
E-mail: kamil.musilek@gmail.com

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201500383.

SPECIAL ISSUE This article is part of a Special Issue on Polypharmacology and Multitarget Drugs. To view the complete issue, visit: http://onlinelibrary.wiley.com/doi/10.1002/cmdc.v11.12/issuetoc.

minergic, serotoninergic, and other monoaminergic neurotransmitter systems.[14,15] Moreover, MAO-catalyzed oxidative deamination gives rise to the production of hydrogen peroxide and, consequently, reactive oxygen species (ROS) that have also been implicated in the progress of AD.[16] MAO inhibitors (MAOIs) should increase monoaminergic neurotransmission and decrease the formation of ROS; both effects are potentially valuable for the treatment of AD.[13,15] Therefore, in this context, multipotent molecules that are able to bind both ChEs and MAOs have been investigated.[17–20]

The aim of the work reported herein was to develop novel multitarget-directed ligands (MTDLs) that act primarily as MAO and cholinesterase (ChE) inhibitors (ChEIs). For this purpose we chose structural motifs contained in previously described MAO and/or ChEIs and incorporated them into the scaffold of the novel compounds. Two distinct series of molecules were designed. The first series, containing a 2-aminoindole-3-carbonitrile scaffold (referred to as the "indole" series; compounds **4a–c** and **8c**), uses an indole ring, which is a structural core feature in several MAOIs and dual-acting compounds that target both MAO and ChEs, such MBA236 (Figure 1),[21,22] as well as the β-aminonitrile motif found in some previously identified MAOIs.[23] Compounds **4b**,**c** also contain the propargylamine moiety, which is an essential part of many neuroprotective, irreversible MAOIs (Figure 1).[24] Originally, only compounds **4a**,**b** had been designed; however, during the synthesis of **4b** a side-product **4c** was isolated. Because of the low yield obtained, **4c** was tested only for its inhibitory activity against MAO. Compound **8c** was synthesized later to explore whether the *N*-allyl or *N*-propargyl substitution on the amino group at position 2 is important for MAO inhibition and also to validate the importance of the phenolic group for the antioxidant activity of other compounds in the series (discussed below).

The second series was then designed using the 2,3,4,6-tetrahydro-1*H*-indolo[2,3-*b*]quinolin-11-amine scaffold (referred to as the "indolotacrine" series; compounds **9a**,**b** and **13**) to improve the unsatisfactory anti-ChE activity of the indole series. For this purpose, the 2-aminoindole-3-carbonitrile scaffold of the indole series was fused with the structure of the potent ChEI tacrine or 7-methoxytacrine (7-MEOTA). Moreover, the resulting indolotacrines also resemble β-carboline alkaloids (e.g., harmine), which are known MAOIs (Figure 2).[25,26] Because compound **4c**, with an *N*-propargyl substituent at position 1, was found to be the most potent MAOI of the indole series, we decided to preserve this potentially favorable motif in designing compound **13** with benzyl substitution, analogous to the former *N*-propargyl moiety.

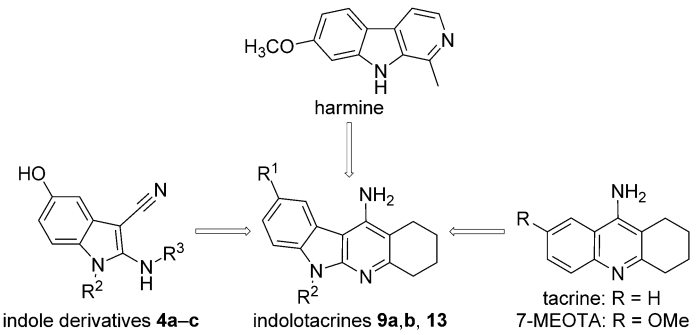
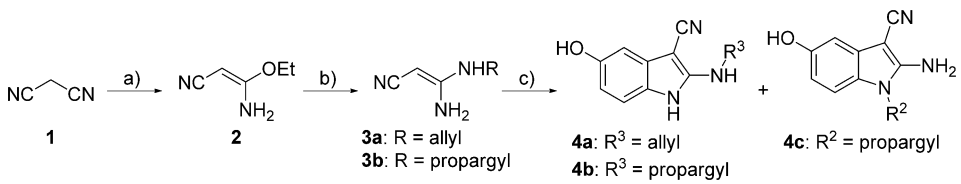


Figure 2. Design of indolotacrine series compounds **9a**,**b** and **13**.

5-Hydroxy-1*H*-indole-3-carbonitrile derivatives **4a–c** were prepared in three steps (Scheme 1). At first malononitrile (**1**) was treated with ethanol in diethyl ether saturated with gaseous hydrochloric acid to obtain 3-amino-3-ethoxyacrylonitrile (**2**). In the next step acrylonitrile **2** was treated with the corresponding alkylamine to give N-alkylated 3,3-diaminoacrylonitriles **3**. Lastly, diaminoacrylonitriles **3** were treated with *p*-benzoquinone to give 2-(alkylamino)-5-hydroxy-1*H*-indole-3-carbonitriles **4a**,**b**.[27] Moreover, a byproduct whose structure was assigned as alkylated at position 1 (compound **4c**), was also isolated from the reaction of 3-amino-3-(prop-2-yn-1-ylamino)acrylonitrile (**3b**).



Scheme 1. Synthesis of indole series **4a–4c**. *Reagents and conditions*: a) HCl, EtOH, Et_2O, 0 °C→RT, 4 h, 18%; b) alkylamine, EtOH, RT, overnight, 61–77%; c) *p*-benzoquinone, EtOH, RT, 1 h, 10–22%.

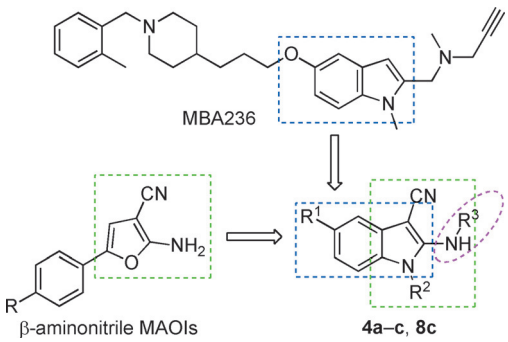
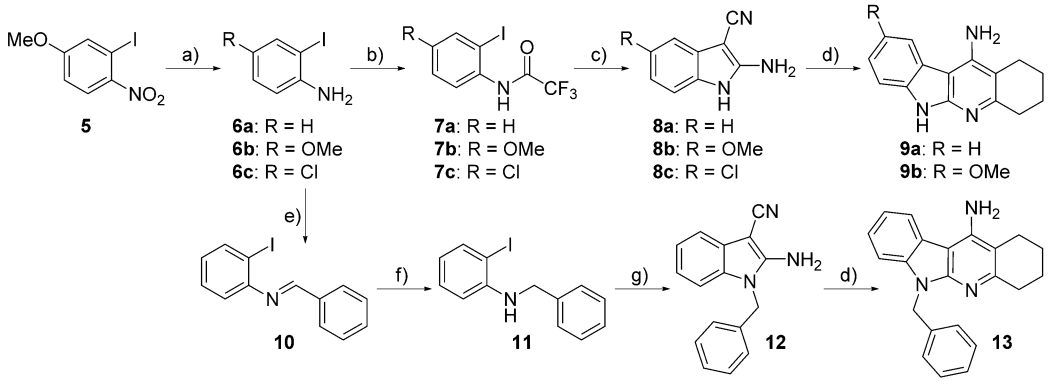


Figure 1. Design of indole series compounds **4a–c** and **8c**.

Indole **8c** and indolotacrines **9a**,**b** were prepared in two to four steps using a similar synthetic approach (Scheme 2). The synthesis of compound **9b** started from commercial 2-iodo-4-methoxy-1-nitrobenzene (**5**), which was reduced using iron powder and ammonium chloride to the corresponding aniline derivative **6b**. Intermediates **6a** and **6c** were obtained commercially. From this point, the synthesis proceeded identically



Scheme 2. Synthesis of indolotacrines **9a**, **9b**, **13** and indole **8c**. *Reagents and conditions*: a) Fe, NH_4Cl, $MeOH/H_2O$ (3:1), 50 °C, 2 h, 79%; b) trifluoroacetic anhydride, Et_3N, THF, −7 °C→RT, overnight, 97–99%; c) malononitrile, L-proline, K_2CO_3, CuI, $DMSO/H_2O$ (1:1), 60 °C, overnight, 48–90%; d) cyclohexanone, $AlCl_3$, 1,2-dichloroethane, microwave, 95 °C, 2 h, 16–54%; e) benzaldehyde, MeOH, RT, overnight, 97%; f) $NaBH_3CN$, AcOH/MeOH, 0 °C→RT, overnight, 75%; g) malononitrile, picolinic acid, K_2CO_3, CuI, DMSO, microwave, 90 °C, 12 h, 26%.

for all three compounds. The 2-iodoaniline derivatives **6a**–**6c** were treated with trifluoroacetic anhydride to give the trifluoracetamides **7a**–**7c**, which were then used for the copper iodide catalyzed cyclization with malononitrile to obtain the corresponding indole derivatives **8a**–**8c**.[28] Finally, indolotacrines **9a** and **9b** were prepared by microwave-assisted Friedländer reaction[29] of the corresponding indoles **8a** and **8b** with cyclohexanone.

Indolotacrine **13** was prepared by a slightly different synthetic procedure involving four steps (Scheme 2). Firstly, 2-iodoaniline **6** was treated with benzaldehyde to give imine **10**, which was then reduced to the corresponding amine **11** using sodium cyanoborohydride. In next step cyclization of amine **11** with malononitrile gave indole **12**.[30] In the final step, Friedländer reaction[29] of **12** with cyclohexanone gave indolotacrine **13**.

Table 1. Inhibition of MAO A and MAO B.

Compd	IC_{50} [μM][a]		SI[b]	30′ IC_{50} [μM][c]	
	MAO A	MAO B		MAO A	MAO B
4a	2.32 ± 0.26	2.02 ± 0.56	0.9	1.78 ± 0.33	10.86 ± 0.78
4b	1.32 ± 0.12	1.70 ± 0.40	1.3	1.80 ± 0.56	2.48 ± 0.32
4c	0.68 ± 0.08	1.62 ± 0.35	2.4	0.45 ± 0.03	0.87 ± 0.10
8c	2.80 ± 0.40	3.89 ± 0.02	1.4	–	–
9a	11.40 ± 1.10	> 100	8.8	30.0 ± 1.9	–
9b	0.49 ± 0.05	53.90 ± 10.70	110.0	–	–
13	> 100	> 100	–	–	–
tacrine	14.07 ± 1.47	317.2 ± 201.0	22.5	–	–
7-MEOTA	7.10 ± 0.03	98.61 ± 14.63	13.9	–	–

[a] Values are the mean ± SD of three independent measurements. [b] Selectivity index: (IC_{50} MAO B)/(IC_{50} MAO A). [c] Determined after 30 min pre-incubation of enzyme with inhibitor; values are the mean ± SEM of three independent measurements.

For biological evaluations, all final products (Figure 3) were transformed into better water-soluble hydrochlorides by stirring them in diethyl ether saturated with gaseous hydrochloric acid. Both series were assayed in vitro for their inhibitory activity against membrane-bound MAO A and MAO B (Table 1). All indoles were found to be potent and unselective MAOIs, with **4c** being the best inhibitor of both isozymes in the series. Indoles **4a**–**c** were evaluated for irreversible inhibition and, unexpectedly, none of compounds showed significantly lower IC_{50} values after 30 min pre-incubation with enzyme, despite compounds **4b**,**c** bearing the *N*-propargylamine moiety, which is present in many known irreversible MAOIs (e.g., deprenyl, clorgyline, and rasagiline). This could be due to the change in electron density on the triple bond of the *N*-propargyl motif, as its connecting nitrogen atom is part of the aromatic system in contrast to the known irreversible inhibitors in which the *N*-propargylamine moiety is separated from the aromatic system, usually by an alkyl linker. Alternatively, steric hindrance from the carbonitrile substituent could prevent the generation of the reactive intermediate or its modification of the enzyme. Based on this finding, we decided to investigate whether the *N*-allyl or *N*-propargyl substitution is necessary for MAO inhibition, and so we synthesized compound **8c**. Evaluation revealed that indole **8c**, devoid of any *N*-alkyl substituent on the amino group at position 2, retains the inhibitory activity at level similar to that of other indoles, showing that the propargyl moiety does not contribute to binding.

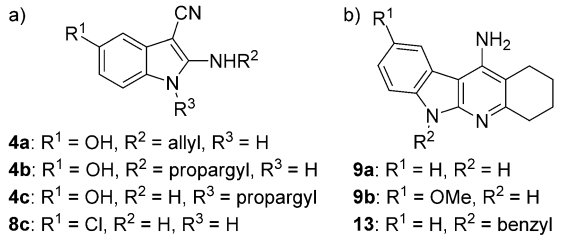


Figure 3. a) Indole and b) indolotacrine analogues prepared in this study.

Indolotacrine **9b** retained the inhibitory activity for both MAO isozymes; however, **9a** inhibited only MAO A, and **13**, with an extra *N*-benzyl substituent, showed no inhibition of either MAO isozyme. It could be assumed that the extended steric bulk of **13** would prevent entry into the active site of

MAO enzymes.[31] In addition, compound **9a** was tested for inactivation of MAO A, but it showed the expected reversible mode of inhibition. Unlike the unselective indole analogues, indolotacrines **9a**,**b** both exerted some selectivity toward MAO A inhibition, with **9b** being the most potent MAO A inhibitor among all the compounds tested (IC_{50}: 0.49 μM). Standards tacrine and 7-MEOTA showed only moderate activity, being poorer inhibitors of both MAO isozymes than the indolotacrine **9b**.

All final compounds, with the exception of **4c** (which was a byproduct of synthesis and, due to low yield, was tested only for MAO inhibition) and **8c** (prepared subsequently to enhance SAR information on MAO inhibition and antioxidant activity), were assayed in vitro for their inhibitory activity against human recombinant acetylcholinesterase (AChE) and human plasma butyrylcholinesterase (BChE) (Table 2).

Table 2. Inhibition of AChE and BChE.

Compd	IC_{50} [μM][a]		SI[b]
	AChE	BChE	
4a	319.2 ± 15.9	> 1000	3.1
4b	101.9 ± 5.4	> 1000	9.8
9a	11.6 ± 0.6	4.7 ± 0.1	0.4
9b	1.5 ± 0.1	2.4 ± 0.1	1.6
13	> 1000	1.09 ± 0.07	0.001
tacrine	0.32 ± 0.01	0.088 ± 0.001	0.3
7-MEOTA	10.0 ± 1.0	17.6 ± 0.8	1.8

[a] Values are the mean ± SEM of three independent measurements. [b] Selectivity index: (IC_{50} BChE)/(IC_{50} AChE).

No significant inhibitory activity against AChE or BChE was detected for indoles **4a**,**b**. Both compounds exerted only poor inhibition of AChE in the high micromolar range and were found to be inactive against BChE at the highest concentration tested (50 mM). A possible explanation for this observation is that compounds **4a**,**b** lack the structural complexity of other indoles or indanes, which are capable of ChE inhibition (e.g., the extra *N*-benzylpiperidine moiety present in donepezil, ASS234, and MBA236 or the carbamate moiety of ladostigil).[22] Conversely, indolotacrines **9a**,**b** were found to be potent unselective inhibitors of both ChE enzymes, with IC_{50} values in low micromolar range, and compound **13** was found to be a selective BChEI. None of the compounds were better than tacrine, but compound **9b** was a better inhibitor of both ChEs than 7-MEOTA. IC_{50} values obtained for standard inhibitors tacrine and 7-MEOTA were in good agreement with previously published results.[32]

Additionally, as ROS are likely to play a part in the development and progression of AD,[33] the compounds were evaluated for their antioxidant activity using a DPPH assay (Table 3). Indoles **4a**,**b** showed promising antioxidant properties, similar to that of the standard *N*-acetylcysteine and only slightly weaker than that of trolox. We hypothesized that this could be due to the presence of phenolic group, which is a key structural motif common of many antioxidants.[34] To prove this assumption we synthesized compound **8c**, in which the phenolic group is replaced with chlorine. Evaluation supported our hypothesis, in that indole **8c** exerts more than 20-fold weaker antioxidant activity than phenolic compounds **4a** and **4b**. Neither the indolotacrines, tacrine, nor 7-MEOTA showed any significant antioxidant activity, which is not surprising, as they all lack the phenolic group responsible for this activity, as demonstrated for the indoles. Introduction of the phenolic moiety therefore presents a possible improvement of the indolotacrine compounds for future studies.

Table 3. Antioxidant activity (EC_{50}) and cytotoxicity (IC_{50}) of prepared compounds.

Compd	EC_{50} [μM][a]	IC_{50} [μM][a]
4a	37.86 ± 5.01	> 1000
4b	25.82 ± 1.35	> 1000
8c	731.70 ± 27.17	113 ± 29
9a	> 5000	13.0 ± 1.4
9b	> 5000	5.5 ± 0.4
13	3827.0 ± 227.1	7.0 ± 0.7
tacrine	> 5000	248 ± 11
7-MEOTA	> 5000	63 ± 4
N-acetylcysteine	27.91 ± 1.82	–
trolox	16.20 ± 0.42	–

[a] Values are the mean ± SEM of three independent measurements.

The cytotoxicity of the compounds was next evaluated by using an MTT assay on the CHO-K1 cell line (Table 3). The indoles were found to possess very low toxicity, with IC_{50} values above the measurable range (> 1000 μM) in the case of **4a**,**b** and in the high micromolar range for **8c**. All indolotacrines exerted similar levels of cytotoxicity, with IC_{50} values ~10 μM. Standards 7-MEOTA and tacrine were both found to be less toxic, with tacrine being the least toxic compound among the series in vitro. This could be considered quite a surprising result, as it is known that in vivo tacrine is more toxic than 7-MEOTA.[35]

Assuming that the principal target of tacrine toxicity in vivo is the liver, we decided to evaluate tacrine and 7-MEOTA together with the most promising indolotacrine **9b** for their hepatotoxicity on the HepG2 cell line by using the MTT assay (Table 4).[36] Compound **9b** was found to be more hepatotoxic than 7-MEOTA and tacrine. As with the cytotoxicity evaluation, tacrine showed lower in vitro hepatotoxicity than 7-MEOTA, which is at odds with the in vivo results.[35] A possible explanation for this discrepancy is that the hepatotoxicity is not caused by tacrine itself, but by its metabolites, products of cytochrome P450 oxidation.[37] Therefore, it is difficult to draw a conclusion regarding the compounds' toxicity in vivo (e.g., **9b**) based on the results of in vitro testing; these cytotoxicity and hepatotoxicity assessments have, in this case, only generally informative character.

Penetration across the blood–brain barrier (BBB) is an essential property for compounds targeting the central nervous system (CNS) and should always be considered during drug development. To predict passive BBB penetration, modification of the parallel artificial membrane permeation assay (PAMPA)

Table 4. Hepatotoxicity evaluation.

Compd	IC_{50} [μM][a]
9b	1.22 ± 0.11
tacrine	17.28 ± 0.76
7-MEOTA	11.50 ± 0.77

[a] Values are the mean ± SEM of three independent measurements.

was used based on a published protocol.[38] As summarized in Table 5, it is clear that compound **9b** has high potential for availability in the CNS. Data obtained for the new compound

Table 5. Prediction of blood–brain barrier penetration of **9b** and reference compounds.

Compd	P_e [10^{-6} cm s^{-1}][a]	CNS (+/−)[b]
9b	6.6 ± 0.65	(+)
donepezil	7.3 ± 0.9	(+)
rivastigmine	6.6 ± 0.5	(+)
tacrine	5.3 ± 0.19	(+)
testosterone	11.3 ± 1.6	(+)
chlorpromazine	5.6 ± 0.6	(+)
hydrocortisone	2.85 ± 0.1	(+/−)
piroxicam	2.2 ± 0.15	(+/−)
theophylline	1.07 ± 0.18	(−)
atenolol	1.02 ± 0.37	(−)

[a] Values are the mean ± SEM of four independent measurements. [b] (+): high predicted BBB permeation, P_e: > 4.0 10^{-6} cm s^{-1}; (−): low predicted BBB permeation, P_e: < 2.0 10^{-6} cm s^{-1}; (+/−): BBB permeation uncertain, P_e: 2.0–4.0 10^{-6} cm s^{-1}.

were correlated with standard drugs for which CNS availability is known and also reported by PAMPA assay.[38] Our data show high resemblance with previously reported penetrations as well as with a general knowledge about the availability in the CNS of such standard drugs.

In summary, we report design, synthesis, and in vitro evaluation of a series of indoles and indolotacrine hybrid analogues as potential drugs for the treatment of AD. The new compounds were designed as MTDLs that target primarily ChEs and MAOs. In addition to ChE and MAO inhibition, biological evaluations also involved the determination of antioxidant, cytotoxic and hepatotoxic properties, and BBB permeability predictions. The most promising compound, indolotacrine **9b**, was found to be a potent inhibitor of AChE (IC_{50}: 1.5 μM), BChE (IC_{50}: 2.4 μM), and MAO A (IC_{50}: 0.49 μM), as well as a weak inhibitor of MAO B (IC_{50}: 53.9 μM). The inhibitory activity of **9b** against ChEs and MAOs seems quite well balanced, and thus has potential for the desired simultaneous multitarget-directed action in vivo, yet the optimal balance of inhibitory ability against each target in AD remains unknown.[39] The cytotoxic and hepatotoxic profiles of **9b** are slightly inferior to those of the standard compounds tacrine and 7-MEOTA, but the overall improvement in the enzyme inhibitory activities and potential to cross the BBB make indolotacrine **9b** a promising lead compound for further development and investigation.

Acknowledgements

This study was supported by the Czech National Institute of Mental Health (NIMH–CZ) project no. ED2.1.00/03.0078, the Ministry of Education, Youth and Sports of the Czech Republic (LD14009), MH CZ–DRO (UHHK, 00179906), the University of Defense of the Czech Republic (long-term development plan), MINECO (Government of Spain, grant SAF2012-33304), the School of Biology at the University of St. Andrews, and by COST Action CM1103.

Keywords: Alzheimer's disease · cholinesterases · cytotoxicity · inhibitors · monoamine oxidase · multitarget-directed ligands

[1] M. Goedert, M. G. Spillantini, *Science* **2006**, *314*, 777 – 781.
[2] C. Ballard, S. Gauthier, A. Corbett, C. Brayne, D. Aarsland, E. Jones, *Lancet* **2011**, *377*, 1019 – 1031.
[3] J. Hardy, *J. Neurochem.* **2009**, *110*, 1129 – 1134.
[4] B. Bulic, M. Pickhardt, B. Schmidt, E.-M. Mandelkow, H. Waldmann, E. Mandelkow, *Angew. Chem. Int. Ed.* **2009**, *48*, 1740 – 1752; *Angew. Chem.* **2009**, *121*, 1772 – 1785.
[5] A. Gella, N. Durany, *Cell Adh. Migr.* **2009**, *3*, 88 – 93.
[6] V. N. Talesa, *Mech. Ageing Dev.* **2001**, *122*, 1961 – 1969.
[7] E. K. Perry, B. E. Tomlinson, G. Blessed, K. Bergmann, P. H. Gibson, R. H. Perry, *Br. Med. J.* **1978**, *2*, 1457 – 1459.
[8] R. T. Bartus, R. L. Dean, B. Beer, A. S. Lippa, *Science* **1982**, *217*, 408 – 414.
[9] A. V. Terry, J. J. Buccafusco, *J. Pharmacol. Exp. Ther.* **2003**, *306*, 821 – 827.
[10] A. Takeda, E. Loveman, A. Clegg, J. Kirby, J. Picot, E. Payne, C. Green, *Int. J. Geriatr. Psychiatry* **2006**, *21*, 17 – 28.
[11] R. León, A. G. Garcia, J. Marco-Contelles, *Med. Res. Rev.* **2013**, *33*, 139 – 189.
[12] A. Cavalli, M. L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini, C. Melchiorre, *J. Med. Chem.* **2008**, *51*, 347 – 372.
[13] O. M. Bautista-Aguilera, G. Esteban, I. Bolea, K. Nikolic, D. Agbaba, I. Moraleda, I. Iriepa, A. Samadi, E. Soriano, M. Unzeta, J. Marco-Contelles, *Eur. J. Med. Chem.* **2014**, *75*, 82 – 95.
[14] P. Riederer, W. Danielczyk, E. Grünblatt, *Neurotoxicology* **2004**, *25*, 271 – 277.
[15] M. B. H. Youdim, D. Edmondson, K. F. Tipton, *Nat. Rev. Neurosci.* **2006**, *7*, 295 – 309.
[16] N. Pizzinat, N. Copin, C. Vindis, A. Parini, C. Cambon, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 428 – 431.
[17] J. Sterling, Y. Herzig, T. Goren, N. Finkelstein, D. Lerner, W. Goldenberg, I. Miskolczi, S. Molnar, F. Rantal, T. Tamas, G. Toth, A. Zagyva, A. Zekany, G. Lavian, A. Gross, R. Friedman, M. Razin, W. Huang, B. Krais, M. Chorev, M. B. Youdim, M. Weinstock, *J. Med. Chem.* **2002**, *45*, 5260 – 5279.
[18] I. Bolea, J. Juárez-Jiménez, C. de Los Ríos, M. Chioua, R. Pouplana, F. J. Luque, M. Unzeta, J. Marco-Contelles, A. Samadi, *J. Med. Chem.* **2011**, *54*, 8251 – 8270.
[19] I. Bolea, A. Gella, L. Monjas, C. Pérez, M. I. Rodríguez-Franco, J. Marco-Contelles, A. Samadi, M. Unzeta, *Curr. Alzheimer Res.* **2013**, *10*, 797 – 808.
[20] L. Wang, G. Esteban, M. Ojima, O. M. Bautista-Aguilera, T. Inokuchi, I. Moraleda, I. Iriepa, A. Samadi, M. B. H. Youdim, A. Romero, E. Soriano, R. Herrero, A. P. Fernández Fernández, R. Martínez-Murillo, J. Marco-Contelles, M. Unzeta, *Eur. J. Med. Chem.* **2014**, *80*, 543 – 561.
[21] G. Prat, V. Pérez, A. Rubi, M. Casas, M. Unzeta, *J. Neural Transm.* **2000**, *107*, 409 – 417.
[22] O. M. Bautista-Aguilera, A. Samadi, M. Chioua, K. Nikolic, S. Filipic, D. Agbaba, E. Soriano, L. de Andrés, M. I. Rodríguez-Franco, S. Alcaro, R. R. Ramsay, F. Ortuso, M. Yañez, J. Marco-Contelles, *J. Med. Chem.* **2014**, *57*, 10455 – 10463.
[23] J. Juárez-Jiménez, E. Mendes, C. Galdeano, C. Martins, D. B. Silva, J. Marco-Contelles, M. do C. Carreiras, F. J. Luque, R. R. Ramsay, *Biochim. Biophys. Acta Proteins Proteomics* **2014**, *1844*, 389 – 397.
[24] M.-S. Song, D. Matveychuk, E. M. MacKenzie, M. Duchcherer, D. D. Mousseau, G. B. Baker, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2013**, *44*, 118 – 124.

[25] H. Kim, S. O. Sablin, R. R. Ramsay, *Arch. Biochem. Biophys.* **1997**, *337*, 137 – 142.
[26] R. Cao, W. Peng, Z. Wang, A. Xu, *Curr. Med. Chem.* **2007**, *14*, 479 – 500.
[27] J. Landwehr, R. Troschütz, *Synthesis* **2005**, 2414 – 2420.
[28] X. Yang, H. Fu, R. Qiao, Y. Jiang, Y. Zhao, *Adv. Synth. Catal.* **2010**, *352*, 1033 – 1038.
[29] J. Marco-Contelles, E. Pérez-Mayoral, A. Samadi, M. do C. Carreiras, E. Soriano, *Chem. Rev.* **2009**, *109*, 2652 – 2671.
[30] M. Jiang, J. Li, F. Wang, Y. Zhao, F. Zhao, X. Dong, W. Zhao, *Org. Lett.* **2012**, *14*, 1420 – 1423.
[31] A. E. Medvedev, R. R. Ramsay, A. S. Ivanov, A. V. Veselovsky, V. I. Shvedov, O. V. Tikhonova, A. P. Barradas, C. K. Davidson, T. A. Moskvitina, O. A. Fedotova, L. N. Axenova, *Neurobiology* **1999**, *7*, 151 – 158.
[32] J. Korabecny, R. Dolezal, P. Cabelova, A. Horova, E. Hruba, J. Ricny, L. Sedlacek, E. Nepovimova, K. Spilovska, M. Andrs, K. Musilek, V. Opletalova, V. Sepsova, D. Ripova, K. Kuca, *Eur. J. Med. Chem.* **2014**, *82*, 426 – 438.
[33] X. Wang, W. Wang, L. Li, G. Perry, H. Lee, X. Zhu, *Biochim. Biophys. Acta Mol. Basis Dis.* **2014**, *1842*, 1240 – 1247.
[34] A. M. Pisoschi, A. Pop, *Eur. J. Med. Chem.* **2015**, *97*, 55 – 74.
[35] O. Soukup, D. Jun, J. Zdarova-Karasova, J. Patocka, K. Musilek, J. Korabecny, J. Krusek, M. Kaniakova, V. Sepsova, J. Mandikova, F. Trejtnar, M. Pohanka, L. Drtinova, M. Pavlik, G. Tobin, K. Kuca, *Curr. Alzheimer Res.* **2013**, *10*, 893 – 906.
[36] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55 – 63.
[37] J. Patocka, D. Jun, K. Kuca, *Curr. Drug Metab.* **2008**, *9*, 332 – 335.
[38] L. Di, E. H. Kerns, K. Fan, O. J. McConnell, G. T. Carter, *Eur. J. Med. Chem.* **2003**, *38*, 223 – 232.
[39] F. Prati, E. Uliassi, M. L. Bolognesi, *MedChemComm* **2014**, *5*, 853 – 861.

Received: August 28, 2015
Published online on October 2, 2015