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Novel synthesis of physovenine and physostigmine analogs

C. H. Wang [a], S. Alluri [a], G. Nikogosyan [a], C. DeCarlo [a], C. Monteiro [a], G. Mabagos [a], H. H. Feng [a], A. R. White [a], M. Bartolini [b], V. Andrisano [c], L. K. Zhang [d], A. K. Ganguly [a,*]

[a] Department of Biomedical Engineering, Chemistry and Biological Sciences Stevens Institute of Technology, Hoboken, NJ 07030, USA
[b] Department of Pharmacy and Biotechnology, University of Bologna, via Belmeloro 6, 40126 Bologna, Italy
[c] Department for Life Quality Studies, University of Bologna, Corso d'Augusto, 237, 47921 Rimini, Italy
[d] Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033-1300, USA

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ABSTRACT

This Letter describes a versatile synthetic approach to prepare physovenine and physostigmine analogs. A series of analogs were synthesized and evaluated for cholinesterase inhibition activities, including human acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) from human serum.

Introduction

(−)-Physovenine and (−)-physostigmine (Fig. 1) are known to be acetylcholinesterase (AChE) inhibitors.[1a–c] These alkaloids have been used in the past for the treatment of *myasthenia gravis* and glaucoma and more recently AChE inhibitors such as Donepezil[2] and Rivastigmine[3] have found use for the treatment of Alzheimer's patients.

The main challenge for the synthesis of physovenine and physostigmine analogs is the construction of quaternary carbon center. There are reported syntheses in the literature[4a–e] toward achieving this goal. Our approach for the synthesis of physovenine and physostigmine analogs is different and novel. The present synthesis allows us to make structurally diverse compounds for establishing structure activity relationship. The key step in our synthesis is the formation of the spiro-oxindiole ring using radical cyclization process followed by further elaboration yielding the crucial alcohol intermediate (**A**).

In a previous publication[5] we have reported the synthesis of a novel class of progesterone receptor antagonists using the intermediate (**A**) and in this publication we have used the same intermediate (**A**) to synthesize novel physostigmine and physovenine analogs represented by structure (**D**, wherein X = N and X = O respectively). These novel analogs were synthesized through the intermediacy of aryl radicals (**B**) and (**C**).

Present study

Substituted γ-butyrolactones required for the synthesis of physostigmine and physovenine analogs were prepared as follows. Treatment[6] of γ-butyrolactone **1a** with sodium methoxide and aromatic aldehydes gave substituted butyrolactones **2a–d** (Scheme 1). However, the reaction of **1a** with acetaldehyde yielded an unexpected product **2e** involving the consumption of two equivalents of acetaldehyde. For the synthesis[7] of **2f** and **2g** we treated the corresponding ketone and aldehyde with NaH and diethyl(2-oxotetrahydrofuran-3-yl)phosphonate **1c**, which in turn was derived from α-bromo-γ-butyrolactone **1b**. Compound **1a** when treated with benzyl bromide yielded **2i**. It should be noted that the reaction of **1a** with 2-bromopropane yielded **2h**, as a result of self-condensation of γ-butyrolactone **1a**.

Treatment of compounds **2a–h** with 2-bromo-4-methoxyaniline and trimethylaluminum in toluene gave amides **3a–3h** (Scheme 2), respectively. These derivatives were acetylated using acetyl chloride and pyridine in DCM to protect the hydroxyl group and then *N*-alkylated using Cs_2CO_3 and alkyl halides in DMF yielding the desired precursors **5a–5h** for reductive radical cyclization. Following the above procedure radical precursors **8a–c** used for oxidative cyclization were prepared from **2i–j**. Compounds **5a–5g** upon treatment with AIBN and TBTH in toluene solution yielded both the *exo* and *endo* cyclization derived products through the intermediacy of **9** yielding compounds **10a–g** and **11a–e**, respectively (Scheme 3). Surprisingly, two atropisomers (**10h** and **10i**) were isolated from the radical reaction of **5h**. High resolution mass

* Corresponding author. Tel.: +1 617 4843806.
E-mail address: akganguly1@aol.com (A.K. Ganguly).

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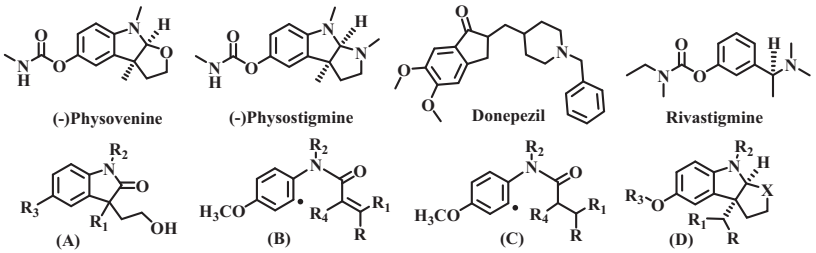
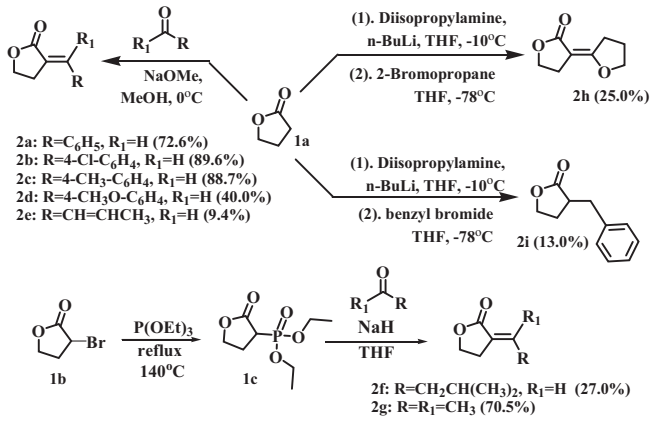


Figure 1. Examples of AChE inhibitors.



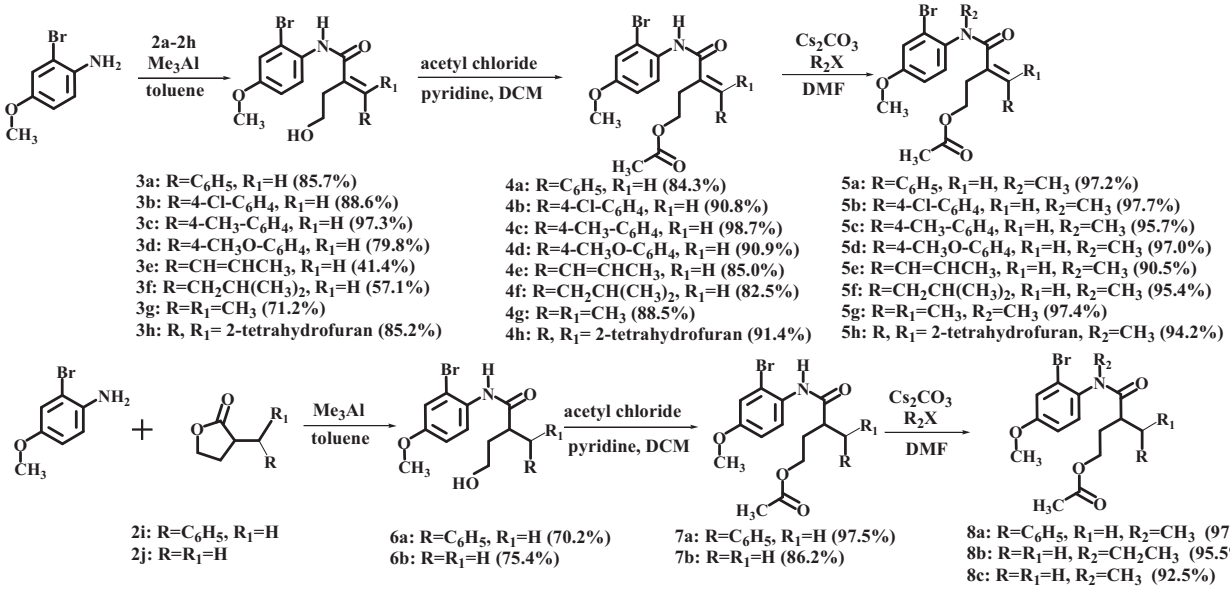
Scheme 1. Synthesis of substituted γ-butyrolactones.

spectroscopy established identical molecular compositions of both the above compounds. NMR spectra of **10h** and **10i** indicated that H_{10} in these compounds appear at δ 4.14 and δ 4.03 respectively. The quaternary carbon C_9 in **10h** appeared at δ 54.55, whereas C_9 in **10i** appeared at δ 54.22. Both spiro-oxindoles show identical correlations in COSY and HMBC. For example in HMBC, C_9 shows correlations with H_7, H_{15}, and H_{11}, whereas C_1 with H_{10}, H_{14}, and H_{17}. In addition, **10h** showed long range NOE between H_7 and H_{10}, whereas **10i** showed the correlations of H_7 with both H_{10} and H_{11} (Fig. 2). Treatment of **8a–c** with TBTH and AIBN produced the desired spirooxindoles (**10a**, **10j–k**), involving the rearrangement of **12** to the more stable radical **13**.

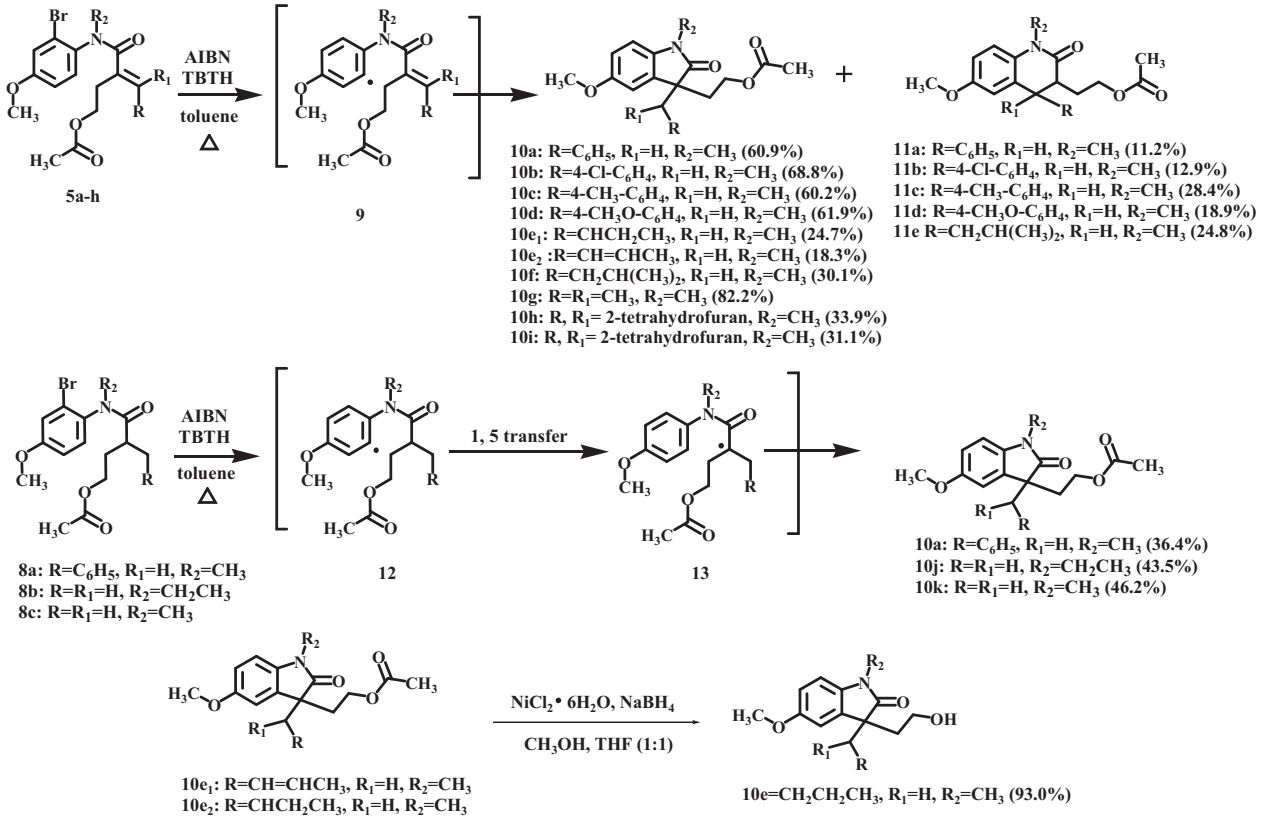
Hydrolysis of **10a–b** with 2 N NaOH in methanol yielded the corresponding alcohols **14a** and **14b**, which were then converted to aldehydes **15a** and **15b**, respectively using the Dess–Martin oxidation (Scheme 4). Condensation of methyl amine with **15a** followed by reduction with $LiAlH_4$ afforded **16a**. O-demethylation of **16a** using BBr_3 yielded the phenol **17a**. Similarly, **17b** was obtained from **14b.** Treatment of the phenols **17a–b** with NaH and substituted isocyanates yielded the desired physostigmine analogs **18a–c**.

Treatment of compounds **10a–k** and **14a–d** with $LiAlH_4$ in THF under reflux yielded the compounds with physovenine core structures **19a–j**. O-demethylation of compounds **19a–j** followed by reaction with substituted isocyanates furnished the desired physovenine analogs **21a–g**. The biological activities of the physostigmines (**18a–c**) and physovenines (**21a–g**) analogs are summarized in Table 1.

It is evident from the IC_{50} values reported in Table 1 that in the physostigmine series the benzyl substituted analogs **18a**, **18b**, and **18c** were inactive against hAChE but active against hBuChE. In the physovenine series benzyl substituted compounds **21a**, **21b**, and **21c** were selective hBuChE inhibitors with inhibitory potencies in the submicromolar range. The most active against hBuChE being **21a** with an IC_{50} value of 70 nM and selectivity around 58 folds. In the alkyl substituted derivatives, **21d**, **21e**, **21f**, and **21g** were slightly selective toward hAChE. Comparing compounds **21f** and



Scheme 2. Preparation of radical precursors.



Scheme 3. Radical cyclizations.

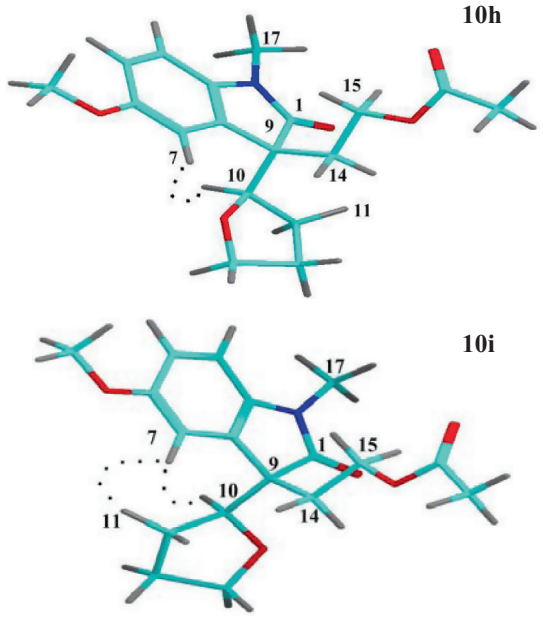
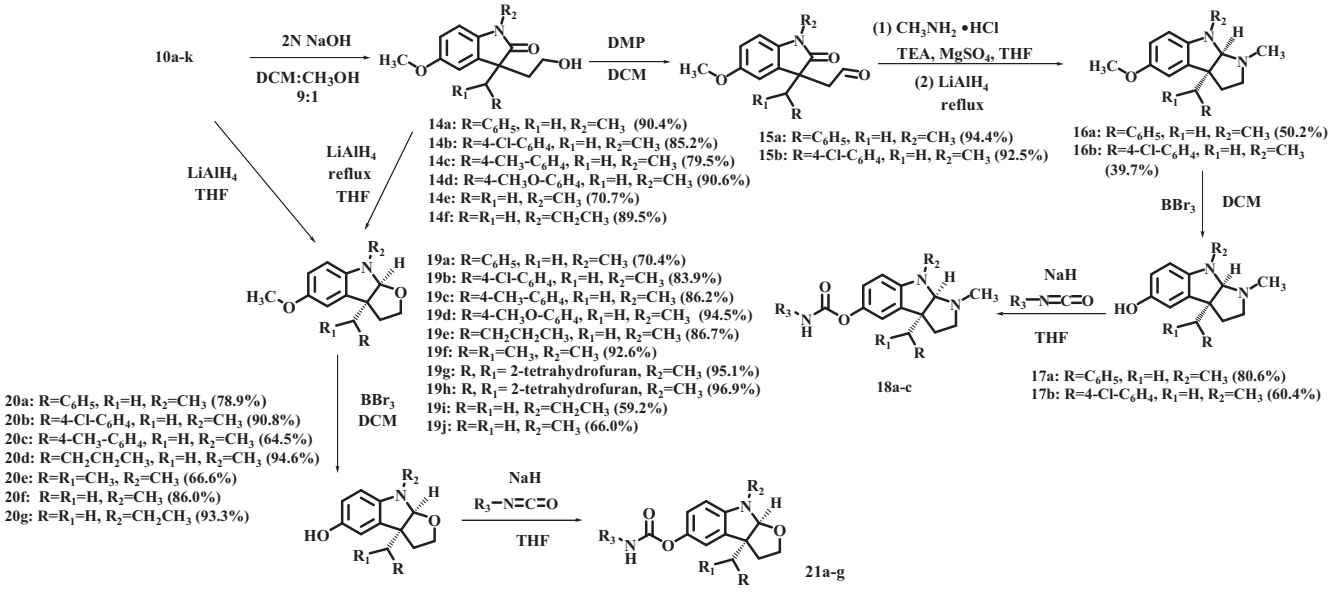


Figure 2. NOE correlations of **10h** and **10i**.

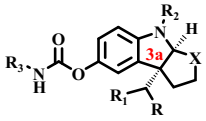
21g (at their maximum inhibition, according to the inhibition kinetics) it is apparent that **21g** was five-fold more active than the aromatic carbamate **21f** against hAChE and two-fold more active against hBuChE. In general physovenine and physostigmine analogs, **18a–c** and **21a–e** with alkyl substituents were more active against hAChE than the arylalkyl substituents at the quaternary carbon center **3a**. Indeed, it is evident that the size of the substituent at **3a** has a strong effect on the inhibitory activity against hAChE. For physovenine analogs, the inhibitory activity toward hAChE decreased by about 2.8 times upon substitution of a methyl group with a butyl group (compare **21d** with **21e**), and 13 times upon introduction of a benzyl group (compare **21a** with **21e**), while the introduction of a *p*-chloro-benzyl substituent led to an inactive derivative (**21b**). This trend can be likely ascribed to the narrow AChE's gorge which might not allow to accommodate physovenine derivatives with bulky substituents at the quaternary carbon center **3a**. Conversely, due to the larger dimensions of the BuChE catalytic gorge (approximately 340 $Å^3$ larger than the corresponding portion of the hAChE gorge) (Zha et al.), BuChE could easily accommodate analogs **21a–c**. The evident effect of the size of the substituent at **3a** on the inhibitory potency toward hAChE also determined a change of the selectivity profile. In fact while physovenine analogs **21e–g** (R = R_1 = H) and **21d** (R = $CH_2CH_2CH_3$; R_1 = H) turned out to be selective hAChE inhibitors, analogs **21a–c** with bulky aryl alkyl substituent at **3a** were highly selective BuChE inhibitors.

In conclusion, the most potent among the alkyl substituted compounds was **21g** with an IC_{50} value of 53 nM against hAChE and selectivity of 18 fold in activities between the two enzymes. We believe the present work helps to establish the trend in SAR among physostigmine and physovenine analogs for activity against hAChE and hBuChE.



Scheme 4. Synthesis of physovenine and physostigmine analogs.

Table 1
hAChE and hBuChE of the analogs



	Yield (%)	X	R	R_1	R_2	R_3	IC_{50}[a] hAChE[b] (μM) ± SEM	IC_{50}[a] hBuChE[b] (μM) ± SEM
18a	75.0	N-CH_3	C_6H_5	H	CH_3	$C(CH_3)_3$	Not active	4.35 ± 0.24
18b	63.7	N-CH_3	C_6H_5	H	CH_3	3-Cl-4-CH_3-C_6H_3	>30[c]	7.17 ± 0.54
18c	41.6	N-CH_3	4-Cl-C_6H_4	H	CH_3	3-Cl-4-CH_3-C_6H_3	Not active	13.7 ± 1.3
21a	62.9	O	C_6H_5	H	CH_3	3-Cl-4-CH_3-C_6H_3	4.04 ± 0.45	0.070 ± 0.007
21b	43.1	O	4-Cl-C_6H_4	H	CH_3	3-Cl-4-CH_3-C_6H_3	Not active	0.173 ± 0.019
21c	48.8	O	4-CH_3-C_6H_4	H	CH_3	3-Cl-4-CH_3-C_6H_3	9.55 ± 0.51	0.231 ± 0.018
21d	15.0	O	$CH_2CH_2CH_3$	H	CH_3	3-Cl-4-CH_3-C_6H_3	0.638 ± 0.049	1.20 ± 0.20
21e	59.1	O	H	H	CH_3	3-Cl-4-CH_3-C_6H_3	0.226 ± 0.022	0.985 ± 0.125
21f	48.7	O	H	H	C_2H_5	3-Cl-4-CH_3-C_6H_3	0.361 ± 0.025	1.87 ± 0.39
21g	54.6	O	H	H	C_2H_5	Cyclohexane	0.182 ± 0.010 0.053 ± 0.005[d]	2.74 ± 0.11 0.959 ± 0.052[e]

[a] IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of two independent measurements, each performed in duplicate. According to the inhibition kinetics, IC_{50} values were determined following a previously developed protocol (Zha[8] et al) based on Ellman's method (Ellman[9] et al.) and a standard incubation time of 20 min, if not otherwise specified.
[b] Human recombinant AChE and BuChE from human serum were used.
[c] % inhibition at 30 μM = 27.2 ± 4.6.
[d] IC_{50} value determined after a 120 min incubation period, according to the slower inhibition kinetics of **21g** against hAChE.
[e] IC_{50} value determined after a 60 min incubation period, according to the slower inhibition kinetics of **21g** against hBuChE.

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