Synthesis and Screening for Antiacetylcholinesterase Activity of (1-Benzyl-4-oxopiperidin-3-ylidene)methylindoles and -pyrroles Related to Donepezil

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2001

Impact Factor: 5.45 · DOI: 10.1021/jm0109356

10 AUTHORS, INCLUDING:

Alberto Leoni
University of Bologna
106 PUBLICATIONS **1,497** CITATIONS
SEE PROFILE

Rita Morigi
University of Bologna
65 PUBLICATIONS **890** CITATIONS
SEE PROFILE

Maurizio Recanatini
University of Bologna
152 PUBLICATIONS **4,585** CITATIONS
SEE PROFILE

Aldo Roda
University of Bologna
486 PUBLICATIONS **8,768** CITATIONS
SEE PROFILE

Brief Articles

Synthesis and Screening for Antiacetylcholinesterase Activity of (1-Benzyl-4-oxopiperidin-3-ylidene)methylindoles and -pyrroles Related to Donepezil

Aldo Andreani,* Andrea Cavalli, Massimiliano Granaiola, Massimo Guardigli, Alberto Leoni, Alessandra Locatelli, Rita Morigi, Mirella Rambaldi, Maurizio Recanatini, and Aldo Roda

Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Received May 21, 2001

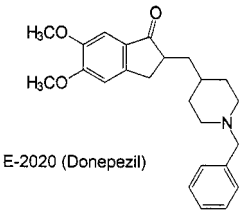
The design, synthesis, and rapid evaluation of a new class of acetylcholinesterase (AChE) inhibitors related to donepezil are reported. A molecular dynamics simulation of the complex between AChE and one representative compound of the series showed a possible inhibitor binding mode in which favorable interactions are formed between the benzylpiperidinone moiety and some active-site residues. The biochemical evaluation of this newly synthesized series was performed using a chemiluminescent method suitable for high-throughput screening.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the central nervous system (CNS) characterized especially by premature mental deterioration. A wide range of evidence shows that acetylcholinesterase (AChE) inhibitors can interfere with the progression of AD.[1,2] The successful development of these compounds was based on the well-accepted hypothesis that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. Donepezil (E2020, Chart 1)[3] inaugurates a new class of AChE inhibitors with longer and more selective action with manageable adverse effects. The recently published[4] crystal structure of donepezil bound to AChE revealed that donepezil binds along the active-site gorge where interactions with the enzyme occur through the benzylpiperidine and indanone moieties. Donepezil forms no direct contact with the protein via hydrogen bonds/salt bridge; all hydrogen bonds are mediated via water molecules.[4]

In an attempt to obtain analogues of donepezil that could maintain its main spatial and physicochemical characteristics while being more compact and less flexible, we applied a strategy to synthesize and rapidly evaluate a series of molecules to be eventually developed as AChE inhibitors for use in AD. We designed the small library of benzylpiperidinone derivatives **4a**−**17** (Schemes 1−3), where the indanone group was replaced by a bioisosteric indole or pyrrole ring bearing different substituents. The biochemical evaluation of the newly synthesized series was performed using a chemiluminescent (CL) method suitable for high-throughput screening (HTS) of AChE inhibitors.

Chart 1



E-2020 (Donepezil)

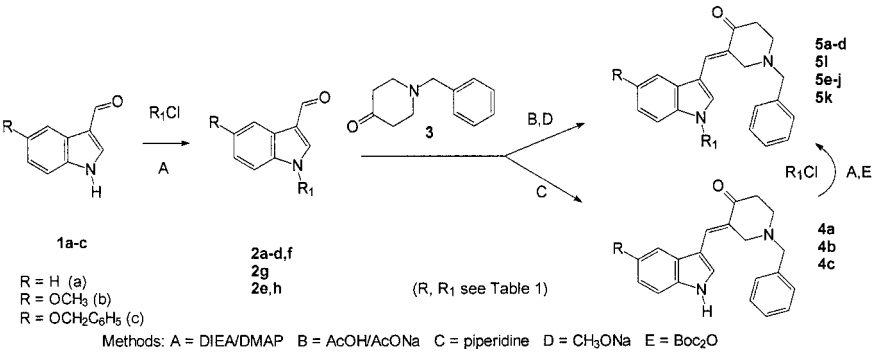
Results and Discussion

The potent AChE inhibitor donepezil was modified by replacing the indanone moiety with an indole and by introducing on the piperidin(on)e ring a double bond connecting the basic ring to the indole fragment (Table 1). This modification was aimed at obtaining less flexible molecules devoid of stereogenic centers. To verify the possibility that such compounds can bind into the AChE gorge, we built a three-dimensional model of the docking complex between the N-benzoylated derivative **5b** and AChE. The inhibitor was positioned into the AChE gorge in an orientation similar to that of donepezil, as revealed by the X-ray crystallographic analysis[4] (Figure 1a). The benzyl moiety was stacked against the indole ring of Trp84, and the charged nitrogen of the piperidinone ring was oriented in a position suitable for a π-cation interaction with the phenyl ring of Phe330. However, after 100 ps of molecular dynamics simulations, the inhibitor assumed a different orientation (Figure 1b) with respect to that of donepezil, even if it still maintained a position in the gorge compatible with the possibility of binding. The resulting enzyme−inhibitor interaction seems to be of an electrostatic nature and involves only the benzylpiperidinone moiety. The π-stacking interaction of the benzyl ring with Trp84 is maintained, but the positive charge of the protonated nitrogen atom seems to be neutralized by the negative

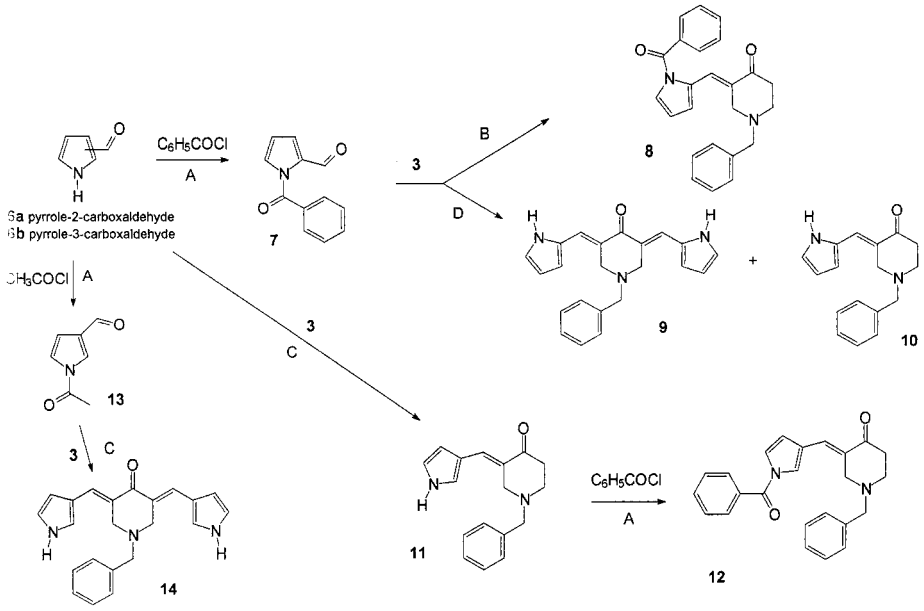
* To whom correspondence should be addressed. Phone: +39-051-2099714. Fax: +39-051-2099734. E-mail: aldoandr@alma.unibo.it.

10.1021/jm0109356 CCC: $20.00
Published on Web 10/02/2001

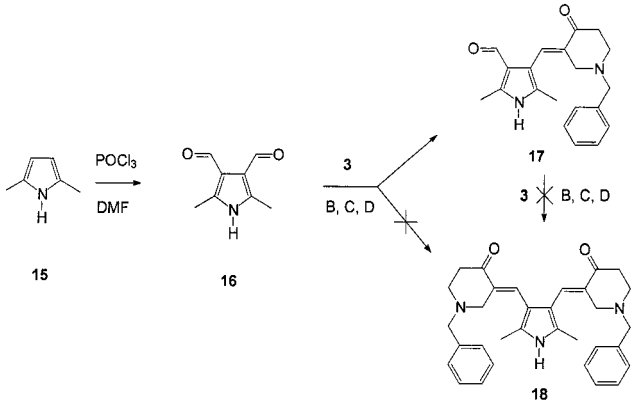
Scheme 1



Scheme 2



Scheme 3



side chains of Asp72 and, eventually, Tyr334. Compared to donepezil, the important π-stacking interaction of the indanone ring with Trp279 is lost because neither the indole nor the benzoyl fragments of **5b** are correctly oriented to be in contact with that residue. On the basis of this docking model, we concluded that the [(1-benzyl-4-oxopiperidin-3-ylidene)methyl]-1H-indole scaffold was suitable for the exploration of a new class of AChE inhibitors analogues of donepezil. Their syntheses and Table 2 containing IR and NMR data are in Supporting Information.

The CL method used for the evaluation of the biological activity of the newly synthesized compounds is based on a series of coupled enzymatic reactions involving AChE, choline oxidase, and horseradish peroxidase, with luminol as the chemiluminescent substrate. Such a CL system was previously used for the measurement of AChE activity[5] and ACh concentration;[6] preliminary results concerning its use for the evaluation of AChE inhibition were also published.[7] The assay is performed in 384-well microtiter plates, and a low-light imaging luminograph is used in order to acquire the CL signal

Table 1. Compounds **2**, **4**, **5**, **8**−**12**, **14**, and **17**

compd	R	R_1	formula (MW)	method[a] or lit.	mp, °C	yield, %	solvent[b]
2a	H	$CH_2C_6H_5$		19, 20			
2b	H	COC_6H_5		21, 22			
2c	H	$CO(C_6H_4)$F-*p*	$C_{16}H_{10}FNO_2$ (267.3)	A	135−138	82	F
2d	H	$CO(C_6H_4)$Cl-*p*		23, 24			
2e	$OCH_2C_6H_5$	COC_6H_5	$C_{23}H_{17}NO_3$ (355.4)	A	147−149	70	F
2f	H	$COCH_3$		25			
2g	OCH_3	$COCH_3$		26			
2h	$OCH_2C_6H_5$	$COCH_3$	$C_{18}H_{15}NO_3$ (293.3)	A	114−117	70	F
4a	H		$C_{21}H_{20}N_2O$ (552.5)	C	198−200	15	H
4b	OCH_3		$C_{22}H_{22}N_2O_2$ (346.4)	C	175−178	15	I
4c	$OCH_2C_6H_5$		$C_{28}H_{26}N_2O_2$ (422.5)	C	160−163	15	H
5a	H	$CH_2C_6H_5$	$C_{28}H_{26}N_2O$ (406.5)	D	170−172	60	H
5b	H	COC_6H_5	$C_{28}H_{24}N_2O_2$ (420.5)	B	160−163	15	H
5c	H	$CO(C_6H_4)$F-*p*	$C_{28}H_{23}FN_2O_2$ (438.5)	B	131−133	20	I
5d	H	$CO(C_6H_4)$Cl-*p*	$C_{28}H_{23}ClN_2O_2$ (455.0)	B	155−158	15	H
5e	H	$CO(C_6H_4)OCH_3$-*p*	$C_{29}H_{26}N_2O_3$ (450.5)	A	205−210 dec	15	H
5f	H	$CO(C_6H_4)NO_2$-*p*	$C_{28}H_{23}N_3O_4$ (465.5)	A	210−213 dec	30	I
5g	H	$CO(C_6H_4)$CN-*p*	$C_{29}H_{23}N_3O_2$ (445.5)	A	142−145	45	I
5h	H	$SO_2C_6H_5$	$C_{27}H_{24}N_2O_3S$ (456.6)	A	158−161	80	G
5i	H	$COOC(CH_3)_3$	$C_{26}H_{28}N_2O_3$ (416.5)	E	139−142	15	I
5j	H	$COCH_3$	$C_{23}H_{22}N_2O_2$ (358.4)	A	128−131	20	H
5k	OCH_3	COC_6H_5	$C_{29}H_{26}N_2O_3$ (450.5)	A	144−148	50	I
5l	$OCH_2C_6H_5$	COC_6H_5	$C_{35}H_{30}N_2O_3$ (526.6)	B	130−133	15	H
8			$C_{24}H_{22}N_2O_2$ (370.4)	B	153−156	50	H
9			$C_{22}H_{21}N_3O$ (343.4)	D	189−192	5	H
10			$C_{17}H_{18}N_2O$ (266.3)	D	175−176	10	H
11			$C_{17}H_{18}N_2O$ (266.3)	C	156−158 dec	40	J
12			$C_{24}H_{22}N_2O_2$ (370.4)	A	122−124	32	H
14			$C_{22}H_{21}N_3O$ (343.4)	C	132−134	10	I
17			$C_{20}H_{22}N_2O_2$ (322.4)	B, C, D	185−186	35−40	H

[a] See Scheme 1; literature references available in Supporting Information. [b] Recrystallization solvent (F = acetone/petroleum ether, G = ethyl acetate) or eluent for column chromatography (H = petroleum ether/acetone 80/20; I = petroleum ether/ethyl acetate 80/20; J = petroleum ether/ethyl ether 90/10).

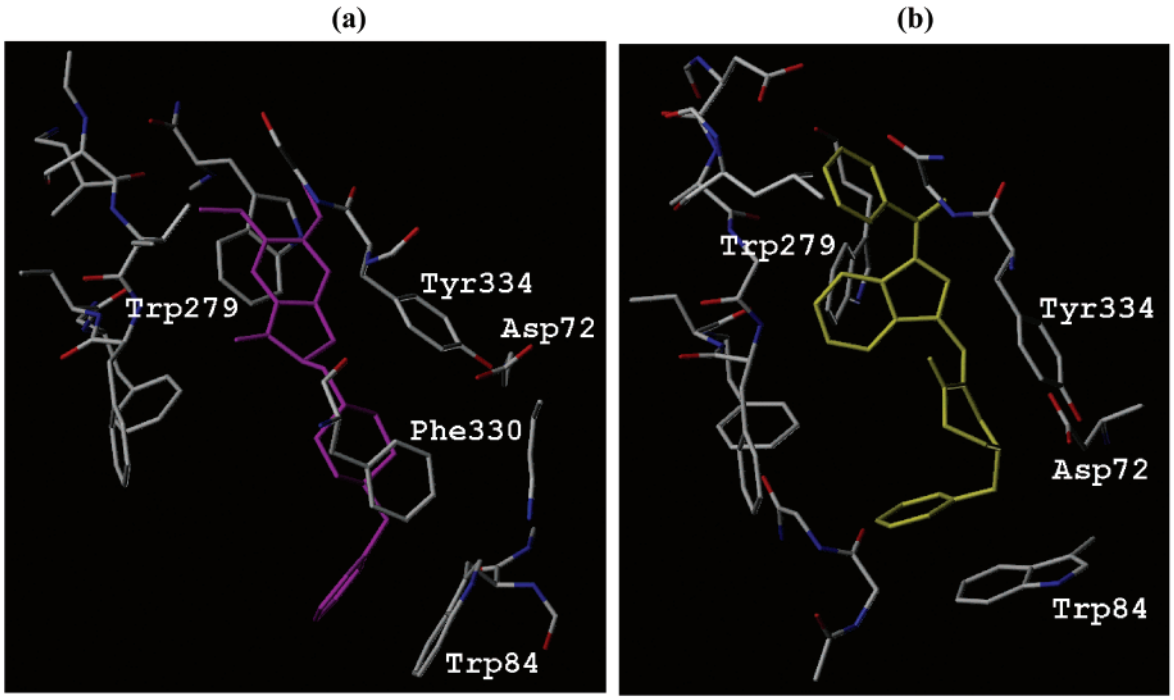


Figure 1. Interactions of (a) donepezil (magenta) and (b) **5b** (yellow) with some selected residues of the AChE gorge. Hydrogen atoms are not shown for clarity.

from the whole plate, making it possible to examine up to 30 compounds in the same analytical session.

The analytical procedure relies on the analysis of the kinetics of the CL emission. It was proven that the initial slope of the luminescence kinetic profile is directly proportional to the activity of AChE in the sample. Therefore, the residual AChE activity in the presence of an AChE inhibitor is simply given by the ratio of the slope of the luminescence kinetic profile in the presence of the inhibitor to that in the absence of

Table 3. Antiacetylcholinesterase Activity[a]

compd	IC_{50}, μM	compd	IC_{50}, μM
4a	8 ± 2	**5j**	11 ± 2
4b	6 ± 2	**5k**	80 ± 10
4c	25 ± 5	**5l**	insoluble
5a	>100	**8**	30 ± 10
5b	70 ± 15	**9**	18 ± 4
5c	60 ± 10	**10**	11 ± 3
5d	50 ± 20	**11**	6 ± 2
5e	70 ± 5	**12**	30 ± 5
5f	50 ± 5	**14**	6 ± 3
5g	20 ± 10	**17**	30 ± 10
5h	insoluble	tacrine	0.18 ± 0.05[b]
5i	30 ± 12	donepezil	0.04 ± 0.01[c]

[a] Mean ± SD of at least three independent measures. [b] IC_{50} values reported in the literature: 4−0.05 μM. [c] IC_{50} values reported in the literature: 0.014−0.005 μM.

the inhibitor. Evaluation of AChE inhibition in the presence of different concentrations of inhibitor allows one to obtain the dose/inhibition curve, and thus the IC_{50} value of the inhibitor. The method was used to determine the IC_{50} values of the well-known AChE inhibitors tacrine and donepezil. These inhibitors, as well as the other compounds, were tested as free bases. The result obtained for tacrine (Table 3) was in good agreement with those reported in the literature, whereas the IC_{50} value obtained for donepezil was somewhat higher than those previously reported, which ranged from 0.005 to 0.014 μM.

Table 3 shows the IC_{50} values for the newly synthesized compounds measured using the described HTS method; the data reported represent the mean ± SD of at least three independent measures; compounds **5h** and **5l** could not be measured because of their low solubility. In many cases the coefficients of variation were lower than 20%, which can be considered a satisfying value for a first-level screening method.

As regards the AChE inhibition data (Table 3), we observe that the parent compound 3-[(1-benzyl-4-oxopiperidin-3-ylidene)methyl]-1H-indole (**4a**) showed activity in the micromolar range. This is a degree of potency lower than that of both tacrine and donepezil, and the loss of interaction with the Trp279 residue might be one of the reasons for the decreased inhibitory activity. Another reason for the low potency displayed by the series with respect to donepezil might be the relative rigidity of the [(4-oxopiperidin-3-ylidene)methyl]indole scaffold, which could hinder the penetration into the AChE gorge to reach the binding site. However, the new derivatives confirmed the possibility of a binding interaction with the target enzyme, maintaining also an appropriate degree of lipophilicity (calculated log P [8] for **4a** is 3.35) in view of the distribution into the CNS.

The CL method allowed us to determine the IC_{50} values of the newly synthesized compounds in very short times (5−10 min) and to consume small quantities of samples and reagents, thanks to the use of 384-well microtiter plates and the high detectability and fast kinetics of the CL signal. Moreover, the possibility of using a DMSO/water 1:10 (v/v) mixed solvent in the preparation of AChE inhibitor solutions facilitates the analysis of compounds with a relatively low solubility in water. The procedure proved to be reliable in terms of reproducibility of the data, and the obtained IC_{50} values were in reasonable agreement with those reported in the literature for the known compounds.

In conclusion, we have explored an integrated approach for the design, synthesis, and rapid evaluation of a new class of AChE inhibitors structurally related to donepezil. The availability of a simple, rapid, and sensitive CL high-throughput screening method for AChE inhibitors facilitated the study of the biological activity of such compounds and the identification of the most promising molecules. The new derivatives show a rather low degree of potency against the enzyme, but the molecular skeleton allows for structural modifications that are presently under study to explore the potential of the series toward new cholinergic agents useful in the treatment of AD.

Acknowledgment. This work was supported by University of Bologna (funds for selected research topics) and MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica).

Supporting Information Available: Procedure for the synthesis of AChE compounds and Table 2 containing IR and NMR data; literature references for Table 1. This material is available free of charge via the Internet at http://www.pub.acs.org.

Note Added after ASAP

Literature references for Table 1 have been added to the Supporting Information. The Supporting Information paragraph has been corrected to reflect this edition and additional Supporting Information has been posted on October 5, 2001.

References

(1) Siddiqui, M. F.; Levey, A. I. Cholinergic Therapies in Alzheimer's Disease. *Drugs Future* **1999**, *24*, 417−424.
(2) See, for example, the whole issue of the following: *Curr. Med. Chem.* **2000**, *7* (3).
(3) E-2020. *Drugs Future* **1991**, *16*, 16−18.
(4) Kryger, G.; Silman, I.; Sussman, J. L. Three-Dimensional Structure of a Complex of E2020 with Acetylcholinesterase from Torpedo Californica. *J. Physiol. (Paris)* **1998**, *92*, 191−194.
(5) Birman, S. Determination of Acetylcholinesterase Activity by a New Chemiluminescence Assay with the Natural Substrate. *Biochem. J.* **1985**, *225*, 825−828.
(6) Ternaux, J. P.; Chamoin, M. C. Enhanced Chemiluminescent Assays for Acetylcholine. *J. Biolumin. Chemilumin.* **1994**, *9*, 65−72.
(7) Pasini, P.; Musiani, M.; Russo, C.; Valenti, P.; Aicardi, G.; Crabtree, J. E.; Baraldini, M.; Roda, A. Chemiluminescence imaging in bioanalysis. *J. Pharm. Biomed. Anal.* **1998**, *18*, 555−564.
(8) *C-QSAR*, version 1.87; BioByte: Claremont, CA, 1994.

JM0109356