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Carbamate Derivatives of Indolines as Cholinesterase Inhibitors and Antioxidants for the Treatment of Alzheimer's Disease

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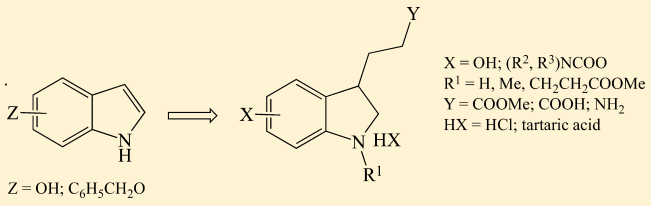
Supporting Information

ABSTRACT: The cascade of events that occurs in Alzheimer's disease involving oxidative stress and the reduction in cholinergic transmission can be better addressed by multifunctional drugs than cholinesterase inhibitors alone. For this purpose, we prepared a large number of derivatives of indoline-3-propionic acids and esters. They showed scavenging activity against different radicals in solution and significant protection against cytotoxicity in cardiomyocytes and primary cultures of neuronal cells exposed to H_2O_2 species and serum deprivation at concentrations ranging from 1 nM to 10 μM depending on the compound. For most of the indoline-3-propionic acid derivatives, introduction of *N*-methyl-*N*-ethyl or *N*-methyl-*N*-(4-methoxyphenyl) carbamate moieties at positions 4, 6, or 7 conferred both acetyl (AChE) and butyryl (BuChE) cholinesterase inhibitory activities at similar concentrations to those that showed antioxidant activity. The most potent AChE inhibitors were **120** (3-(2-aminoethyl) indolin-4-yl ethyl(methyl)carbamate dihydrochloride) and **94** (3-(3-methoxy-3-oxopropyl)-4-(((4-methoxyphenyl)(methyl) carbamoyl)oxy)indolin-1-ium hydrochloride) with IC_{50}s of 0.4 and 1.2 μM, respectively.



INTRODUCTION

The sporadic form of Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is characterized by progressive neurodegeneration resulting in impaired cognition and behavioral abnormalities. Cognitive dysfunction is associated with a reduction in cholinergic transmission in cortical and hippocampal neurons.[1] This observation led to the introduction of cholinesterase (ChE) inhibitors in an attempt to maintain cholinergic transmission.[2] Of these, donepezil and galantamine are reversible, selective inhibitors of acetylcholinesterase (AChE), while rivastigmine contains a carbamate moiety that binds covalently to the enzyme and remains attached to it until it is removed slowly by hydrolysis.[3] Rivastigmine inhibits both AChE and butylcholinesterase (BuChE).[4] As AD progresses levels of AChE in the brain decline, but those of BuChE, found mainly in subcortical neurons and glial cells,[5] remain unchanged or increase.[6] Inhibition of BuChE can increase levels of ACh in the brain and may also be able to impede the formation of abnormal βamyloid,[7] which can further impair neuronal activity. In addition, it was shown that treatment with the dual AChE and BuChE inhibitor rivastigmine in women with the wild-type BuChE gene slowed progression of cognitive impairment and reduced white matter loss in the brain.[8] Therefore, inhibitors of both AChE and BuChE may have additional benefits in AD and also in dementias associated with subcortical pathology, such as that of Parkinson's disease, Lewy body's disease, and also vascular dementia.[9]

Oxidative stress is one of the earliest events in the pathogenesis of AD and may even occur before the onset of memory loss[10] and the appearance of senile plaques and neurofibrillary tangles.[11] Oxidative stress has also been shown to mediate tau-induced neurodegeneration.[12] The latter is more closely correlated with cognitive impairment than the number or size of β-amyloid plaques.[13] Oxidative and nitrative stress results from the formation of reactive oxygen (ROS) and reactive nitrogen (RNS) species, respectively. Nitric oxide (NO) released from activated microglia reacts with superoxide anion ($O_2^{-\bullet}$) to form peroxinitrite ($ONOO^-$), which can damage proteins by causing nitration of tyrosine residues. This can be detected in the brain with an antibody to 3-nitrotyrosine in subjects with mild cognitive impairment, a prodromal phase of AD.[14] Thus, drugs that combine inhibition of AChE and BuChE with the ability to reduce oxidative and nitrative stress may be more efficacious than those that only inhibit ChE in preventing and/or treating AD.[15]

The naturally occurring compound, indole-3-propionic acid (IPA) was shown to possess radical scavenging properties and to protect primary neurons in culture against oxidative damage

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induced by β-amyloid.[16] In previous research from our laboratory, it was found that introduction of an OH group into the 4- or 6-positions of the indole ring increased its antioxidant potential.[17] Carbamoylation of the OH group with *N*,*N*-dialkyl- or *N*-alkyl-*N*-aryl-carbamoyl chlorides confers AChE and BuChE inhibitory activity on these molecules. Chyan et al.[16] claimed that an electron-rich aromatic ring system in IPA is essential for antioxidant activity. Zaikin (2007)[17] found that indoline-3-propionic acid, the reduced analogue of IPA, was more potent and effective than IPA as an antioxidant, probably because it has an anilinic type of amino group that should enable it to undergo a facile oxidation to the corresponding quinoidal type of compound (Figure 1), whereas the aromatic indole may be more resistant to oxidation. Thus, as the indolines are oxidized they act as reducing agents more readily than the corresponding indoles.

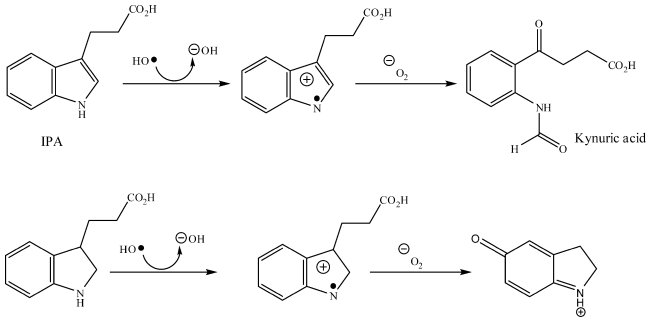


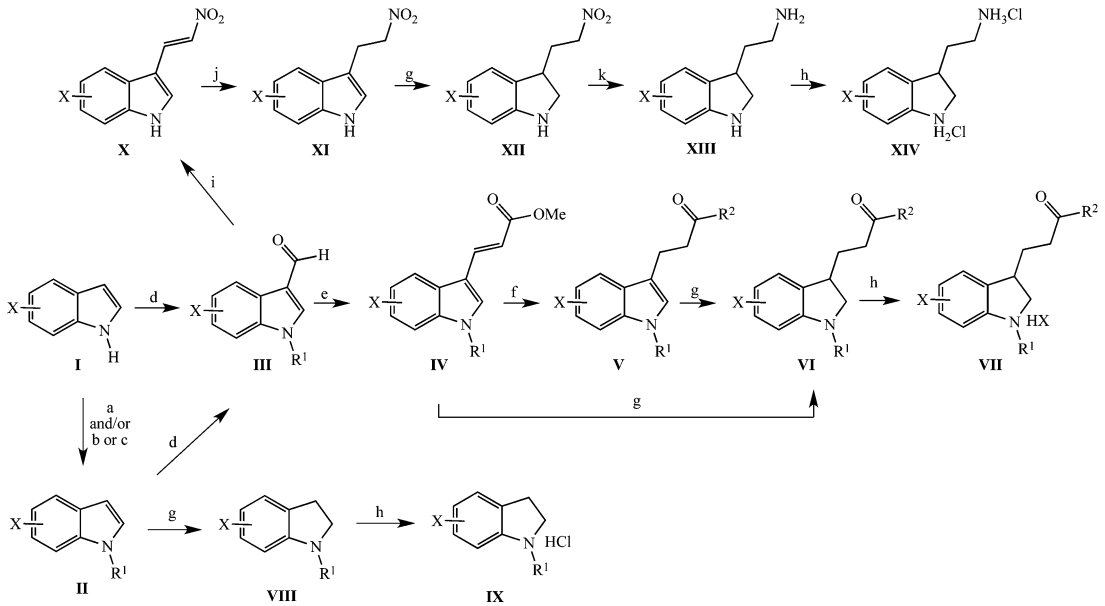
Figure 1. Interaction of indoles and indolines with ROS[16]

The current study describes the preparation and in vitro activities of carbamate derivatives of indoline-3-propionic acid and esters as inhibitors of AChE and BuChE, and ROS and RNS radical scavengers. Their potential as protectants against cytotoxicity induced in cardiomyocytes and neuronal cells by oxidative stress was also assessed. The goal was to identify compounds that were equipotent in protecting cells against cytotoxicity induced by ROS and RNS and inhibiting AChE and BuChE that could be developed for the treatment of AD and other neurodegenerative diseases.

CHEMISTRY

The syntheses of the various indoline derivatives are described in the Scheme 1, and the synthetic intermediates and final products are listed in Table 1. The starting materials were the commercially available hydroxy or benzyloxy substituted indoles 1−5 of general formula **I**. Initial carbamoylation of the phenolic OH with various R^1R^2NCOCl reagents gave the corresponding carbamates **II**. Vilsmeier−Haack formylation of the indolic carbamates led to compounds **III**, which were subsequently converted into the α,β-unsaturated esters **IV** via a Knoevenagel condensation reaction. Selective reduction of the side chain double bond of **IV** gave compounds **V**. In view of the poor aqueous solubility of the latter and their low basicity that prevented the formation of salts, the compounds were further reduced to give the corresponding indolines **VI**, which were basic enough to form water-soluble salts **VII**. Some of the esters were hydrolyzed to their corresponding acids, which in some cases were converted into the amides. To evaluate the importance of the propionic acid or ester side chain linked at position 3 of the indolines, several derivatives of formula **VIII**, prepared by reduction of the indoles **II**, were converted into the corresponding water-soluble salts **IX**. To compare the activity of the NH indolinic compounds to their corresponding N-R analogues, some of the compounds were *N*-methylated with MeI or alkylated with methyl acrylate to give compounds **113** and **114** belonging to category **IX**. To compare the biological activity of the indole-3-propionic acid derivatives to that of compounds having a pattern of substitution analogous to that of melatonin (without the acetamido group), compound **120** belonging to category **XIV** was prepared from the nitro

Scheme 1. General Synthetic Scheme: Indolines and Their Synthetic Intermediates[a]



[a]Reagents and conditions: (a) $R^1R^2NCOCl/Et_3N/DMAP/CH_2Cl_2$; (b) MeI/50% NaOH/$Bu_4N^+Br^-$; (c) $CH_2{=}CH{-}COOMe/DBU/CH_3CN$; (d) $POCl_3$/DMF; (e) $HOOCCH_2COOMe$/Py/piperidine; (f) 10% Pd/C/HCO_2NH_4; (g) $NaBH_3CN$/AcOH or $NaBH_4$/TFA; (h) HCl; (i) NH_4OAc/CH_3NO_2; (j) $NaBH_4$/silica gel/*i*-PrOH/$CDCl_3$; (k) 10% Pd/C/H_2 1−3 atm/4 h 3 d/MeOH

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Table 1. Indolines and Their Synthetic Intermediates

compd	X	R^1	R^2	HX
I				
1	4-HO			
2	6-HO			
3	4-$C_6H_5CH_2O$			
4	6-$C_6H_5CH_2O$			
5	7-$C_6H_5CH_2O$			
II				
6	4-Me_2NCOO	H		
7	4-(Et,Me)NCOO	H		
8	4-(Bu,Me)NCOO	H		
9	4-[(4-MeO-C_6H_4),Me)NCOO	H		
10	4-(Et,Me)NCOO	Me		
11	4-(Bu,Me)NCOO	Me		
12	4-[(4-MeO-C_6H_4),Me)NCOO	Me		
13	6-(Et,Me)NCOO	H		
14	6-(Bu,Me)NCOO	H		
15	6-[(4-MeO-C_6H_4),Me)NCOO	H		
16	6-(Bu,Me)NCOO	Me		
17	6-[(4-MeO-C_6H_4),Me)NCOO	Me		
18	4-(Et,Me)NCOO	CH_2CH_2COOMe		
19	6-(Et,Me)NCOO	CH_2CH_2COOMe		
III				
20	4-$C_6H_5CH_2O$	H		
21	6-$C_6H_5CH_2O$	H		
22	6-HO	H		
23	4-(Et,Me)NCOO	H		
24	4-(Et,Me)NCOO	Me		
25	4-[(4-MeO-C_6H_4),Me)NCOO	H		
26	4-[(4-MeO-C_6H_4),Me)NCOO	Me		
27	4-(Bu,Me)NCOO	Me		
28	6-(Et,Me)NCOO	H		
29	6-(Bu,Me)NCOO	H		
30	6-(Bu,Me)NCOO	Me		
31	6-[(4-MeO-C_6H_4),Me)NCOO	H		
32	6-[(4-MeO-C_6H_4),Me)NCOO	Me		
33	7-$C_6H_5CH_2O$	H		
IV				
34	4-OH	Me		
35	4-$C_6H_5CH_2O$	H		
36	4-$C_6H_5CH_2O$	Me		
37	4-(Et,Me)NCOO	H		
38	4-(Et,Me)NCOO	Me		
39	4-(Bu,Me)NCOO	Me		
40	4-[(4-MeO-C_6H_4),Me)NCOO	H		
41	4-[(4-MeO-C_6H_4),Me)NCOO	Me		
42	6-OH	H		
43	6-$C_6H_5CH_2O$	H		
44	6-(Et,Me)NCOO	H		
45	6-(Bu,Me)NCOO	H		
46	6-(Bu,Me)NCOO	Me		
47	6-[(4-MeO-C_6H_4),Me)NCOO	H		
48	6-[(4-MeO-C_6H_4),Me)NCOO	Me		
49	7-$C_6H_5CH_2O$	H		
V				
50	4-OH	H	OMe	
51	4-(Et,Me)NCOO	H	OMe	
52	4-(Et,Me)NCOO	H	OH	
53	4-(Et,Me)NCOO	Me	OMe	
54	4-(Et,Me)NCOO	H	OEt	
55	4-(Et,Me)NCOO	H	NH_2	
56	4-(Bu,Me)NCOO	Me	OMe	

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Table 1. continued

compd	X	R^1	R^2	HX
V				
57	4-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	
58	4-[(4-MeO-C_6H_4),Me)NCOO	Me	OMe	
59	4-[(4-MeO-C_6H_4),Me)NCOO	Me	OH	
60	6-OH	H	OMe	
61	6-TBSO	H	OMe	
62	6-OH	H	OH	
63	6-TBSO	Me	OMe	
64	6-OH	Me	OH	
65	6-(Et,Me)NCOO	H	OMe	
66	6-(Et,Me)NCOO	H	OH	
67	6-(Bu,Me)NCOO	H	OMe	
68	6-(Bu,Me)NCOO	Me	OMe	
69	6-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	
70	6-[(4-MeO-C_6H_4),Me)NCOO	Me	OMe	
71	7-OH	H	OMe	
72	7-(Et,Me)NCOO	H	OMe	
VI				
73	4-OH	H	OMe	
74	6-OH	H	OMe	
75	4-(Et,Me)NCOO	H	OMe	
76	4-(Et,Me)NCOO	H	OEt	
77	4-(Et,Me)NCOO	Me	OMe	
78	4-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	
79	6-(Et,Me)NCOO	H	OMe	
80	6-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	
VII				
81	4-OH	H	OMe	HCl
82	4-OH	H	OH	HCl
83	4-OH	Me	OMe	HCl
84	6-OH	H	OMe	HCl
85	6-OH	H	OH	HCl
86	6-OH	Me	OH	HCl
87	4-(Et,Me)NCOO	H	OMe	HCl
88	4-(Et,Me)NCOO	Me	OMe	HCl
89	4-(Et,Me)NCOO	H	OEt	tartrate
90	4-(Et,Me)NCOO	H	OH	HCl
91	4-(Et,Me)NCOO	Me	OH	HCl
92	4-(Et,Me)NCOO	H	NH_2	HCl
93	4-(Bu,Me)NCOO	Me	OMe	HCl
94	4-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	HCl
95	4-[(4-MeO-C_6H_4),Me)NCOO	H	OH	HCl
96	4-[(4-MeO-C_6H_4),Me)NCOO	Me	OMe	HCl
97	4-[(4-MeO-C_6H_4),Me)NCOO	Me	OH	HCl
98	6-(Et,Me)NCOO	H	OMe	HCl
99	6-(Et,Me)NCOO	H	OH	HCl
100	6-(Bu,Me)NCOO	H	OMe	HCl
101	6-(Bu,Me)NCOO	Me	OMe	HCl
102	6-[(4-MeO-C_6H_4),Me)NCOO	H	OMe	HCl
103	6-[(4-MeO-C_6H_4),Me)NCOO	Me	OMe	HCl
104	7-(Et,Me)NCOO	H	OMe	HCl
VIII				
105	4-OH	H		
106	4-(Et,Me)NCOO	H		
107	4-(Et,Me)NCOO	CH_2CH_2COOMe		
108	6-(Et,Me)NCOO	H		
109	6-(Et,Me)NCOO	CH_2CH_2COOMe		
IX				
110	4-OH	H	H	HCl
111	4-Me_2NCOO	H		HCl
112	4-(Et,Me)NCOO	H		HCl

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Table 1. continued

compd	X	R^1	R^2	HX
IX				
113	4-(Et,Me)NCOO	CH_2CH_2COOMe		HCl
114	6-(Et,Me)NCOO	Me		HCl
115	6-(Et,Me)NCOO	CH_2CH_2COOMe		HCl
X				
116	4-(Et,Me)NCOO	H		
XI				
117	4-(Et,Me)NCOO	H		
XII				
118	4-(Et,Me)NCOO	H		
XIII				
119	4-(Et,Me)NCOO	H		
XIV				
120	4-(Et,Me)NCOO	H		2HCl

derivative **116**, which underwent three stages of reduction (Scheme 1). Compound **120** was isolated as the dihydrochloride salt.

RESULTS AND DISCUSSION

Chemical and Metabolic Stability Studies. The chemical stability in solution of two carbamates **87** and **98** assessed by high performance liquid chromatography showed that the mesylate salt of **87** is stable in aqueous solution of 1 mg/mL at room temperature (25 °C) for at least 9 days. In the presence of human plasma at 37 °C, 40% of **87** was hydrolyzed to **90** within 90 min, but the carbamate group remained intact. A concentrated solution of the citrate salt of **98** (100 mg/mL) maintained at room temperature was hydrolyzed to the acid **99** within a few days. Dilute solutions of 0.5 mg/mL were stable as solutions of 50 mg/mL or more if they were stored at −20 °C. In the presence of human or mouse liver microsomes, **87** or **98** were rapidly converted to their respective acids, **90** and **99**. No other metabolic changes were detected such as the removal of the alkyl radicals, Et and Me, from the carbamate in contrast to what was shown for (6-(*N*-ethyl-*N*-methylcarbamyloxy)-*N*-propargyl-1(*R*)-aminoindan.[18]

AChE and BuChE Inhibitory Activity. Three indole derivatives substituted with a carbamate group (**51**, **53**, and **69**) that were tested for AChE inhibitory activity were found to be 2.5−20 times weaker than their indoline analogues[19] (Table 2). They were also much less active as antioxidants, and therefore no further indoles were prepared or tested. We have previously reported that *N*,*N*-dimethyl carbamate derivatives of phenylethylamine and *N*-propargyl-aminoindan are more potent inhibitors of AChE than those in which one alkyl is methyl and the second has a longer chain, such as ethyl, propyl, or butyl, but their duration of action is much shorter because of the more rapid hydrolysis of the carbamate.[3,20] When the second substituent is 4-methoxyphenyl, the AChE inhibitory activity is restored to that of the dimethyl analogue but the rate of decarbamoylation of the enzyme is much slower, thereby increasing the duration of enzyme inhibition.[3]

In preliminary studies, we found that introduction of a carbamate into position 5 of the indoline structure resulted in very weak AChE inhibitors with IC_{50}s >500 μM because they readily undergo oxidation to quinoidal structures.[19] Therefore, in the current series, the carbamate moiety was sited in positions 4 or 6 and in one compound, **104**, in position 7. Again, the *N*,*N*-dimethylamino (**111**) and the *N*-methyl-*N*-(4-methoxyphenyl) (**94**) derivatives were the most potent AChE inhibitors. The greater AChE inhibitory activity of **94** than that of carbamates with ethyl or butyl alky chains may be explained by the π−π interaction of the aryl substituent with phenylalanines 288 and 290 located at the bottom of the gorge adjacent to the active site of AChE.[3] By contrast, compounds

Table 2. Cholinesterase Inhibition by Carbamate Derivatives of Indoles and Indolines[a]

	IC_{50} ± SD (μM)		AChE selectivity
compd	AChE	BuChE	IC_{50} BuChE/AChE
51	19.6 ± 0.1	9.5 ± 0.5	0.48
53	180 ± 1.0	NT	
69	>600	NT	
87	7.40 ± 0.03	0.30 ± 0.04	0.04
88	58.7 ± 0.7	6.80 ± 0.15	0.11
89	40.2 ± 0.3	0.56 ± 0.01	0.21
90	19.0 ± 1.1	0.30 ± 0.01	0.016
91	124 ± 17	9.70 ± 0.05	0.078
92	62.0 ± 1.7	8.50 ± 0.50	0.14
93	68.1 ± 6.9	197 ± 1	2.89
94	1.20 ± 0.01	4.60 ± 0.04	3.8
95	37.8 ± 0.2	417 ± 9	11.0
96	246 ± 18	>600	>2.4
97	125 ± 7	>600	>4.8
98	55.2 ± 0.1	3.90 ± 0.22	0.07
99	175 ± 1	10.0 ± 0.5	0.06
100	62.3 ± 5.2	404 ± 1	6.5
101	122 ± 1	66.8 ± 0.1	0.55
102	38.2 ± 1.1	54.0 ± 1.5	1.4
103	17.4 ± 2.2	372 ± 3	4.1
104	61.6 ± 0.5	0.008 ± 0.001	1.2×10^{-4}
111	1.20 ± 0.14	1.3 ± 0.17	1.08
112	7.20 ± 0.02	0.7 ± 0.01	0.1
113	5.60 ± 0.34	0.70 ± 0.13	0.125
114	418 ± 6	58.7 ± 1.9	0.14
115	473 ± 5	6.80 ± 0.03	0.014
120	0.40 ± 0.04	0.20 ± 0.01	0.5
rivastigmine	0.62 ± 0.01	0.16 ± 0.01	0.26
ladostigil	30.3 ± 1.1	0.48 ± 0.02	0.06

[a]Values represent the mean ± STD of 3-4 experiments and were computed after incubation of the enzyme with the compounds for 120 min. NT = not tested. Ladostigil is (3*R*)-3-(2-propyn-1-ylamino)-2,3-dihydro-1*H*-inden-5-yl ethyl(methyl) carbamate.

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with *N*-butyl or *N*-methoxyphenyl substituents (**93**, **94**, **95**, **96**, **97**, **100**, **102**, and **103**) were much weaker inhibitors of BuChE than their respective *N*-methyl-*N*-ethyl analogues (**87**, **88**, **89**, **90**, **98**, and **99**) (Table 2). This may be because at the active site of BuChE the two phenylalanines of AChE are replaced by leucine and valine,[21] thereby eliminating the $\pi-\pi$ interaction. The gorge of BuChE is larger than that of AChE, enabling the *N*-methyl-*N*-ethyl carbamates to interact more efficiently with the active site than with that of AChE. However, this does not enable us to explain why compounds **93** and **100** were so much weaker as BuChE inhibitors than their *N*-methyl-*N*-ethyl analogues. This reduction in BuChE inhibitory activity relative to that of the *N*-methyl-*N*-ethyl analogues was not seen with the *N*-methyl-*N*-butyl derivatives of aminoindan or phenylethylamine.[3,22]

The propionic acid derivatives (**90**, **91**, **95**, and **99**) were found to be weaker AChE inhibitors than their respective methyl esters. Substitution of methyl ester by ethyl (**89**) decreased AChE inhibitory activity more than that of BuChE, while conversion of the ester to an amide (**92**) caused a much larger reduction in BuChE inhibitory activity. With the exception of compound **103**, the *N*-methyl indolines were all weaker AChE and BuChE inhibitors than their respective N−H analogues. On the other hand, substitution of the propionic acid methyl ester side chain by a 2-aminoethyl group resulted in the most potent AChE inhibitor of this series (**120**). Its greater activity than that of the compounds with an acidic or ester group in the same position may result from the interaction of the basic NH_2 group with the peripheral anionic site situated at the entrance to the narrow gorge of the enzyme, thereby causing improved alignment of the molecule to the catalytic site situated near its base.[23] The ability to interact with the peripheral anionic site could confer an added advantage for treatment of AD as other AChE inhibitors that also bind to the peripheral site of the enzyme can prevent the aggregation of amyloid-β-peptide into fibrils and plaques.[24]

As previously reported for aminoindans and phenylethylamines, compounds with the carbamate moiety in position 6 were 7−9 times less potent as AChE inhibitors than their counterparts in position 4 and 13−33 times less potent as BuChE inhibitors.[3] The reduction in AChE activity that was obtained by moving the carbamate from position 4 to 6 was 9-fold (**90**−**99**), 35-fold, (**94**−**102**), and 88-fold (**113**−**115**). The 7-substituted *N*-methyl-*N*-ethyl carbamate (**104**) was also 8-fold weaker as an AChE inhibitor than that in position 4. The IC_{50}s for AChE and BuChE of **98** were also somewhat higher than that of ladostigil, (3*R*)-3-(2-propyn-1-ylamino)-2,3-dihydro-1H-inden-5-yl ethyl(methyl) carbamate. The most active BuChE inhibitor of all the compounds tested was **104** which also showed the highest selectivity toward this enzyme (770-fold). The lower AChE activity of 6- and 7-substituted carbamates may result from a change in the overall shape of the molecules, thereby making it more difficult for them to align correctly in the gorge leading to the active site, as previously shown for derivatives of aminoindan. Such steric constraints are less critical in the interaction with BuChE.

Antioxidant Activity. Two Luminol-dependent chemiluminescence-inducing cocktails were used to quantify the antioxidant scavenging abilities of indolines.[25] Cocktail A generates a constant wave of light due to H_2O_2 and Co^{2+}-catalyzed $OH^{\bullet}$ at a degree and rate that can be measured by chemiluminescence (Figure 2). Cocktail B generates $O_2^{-\bullet}$, NO, and a constant wave of light due to $ONOO^-$.[25] Although H_2O_2 is not very reactive,[26] it may be converted to a number of ROS, including $OH^{\bullet}$ and $HOO^{\bullet}$.[27] Melatonin, a derivative of IPA, which has a methoxy group in position 5 of the indole ring, was shown to scavenge ROS and RNS in solution.[26,28] In a study that also used a chemiluminometric method to measure its antioxidant activity, the effective concentrations of melatonin (IC_{50}) were 537 and 127 μM for $OH^{\bullet}$ radical and peroxynitrite, respectively.[29] In our system, melatonin gave an IC_{50} for scavenging H_2O_2 and $OH^{\bullet}$ generated by glucose oxidase (GO) of 95.8 μM, and for NO released by morpholino sydononimine (Sin1), the IC_{50} was 1.02 mM. Many of carbamates tested were much more potent than melatonin, with IC_{50}s for scavenging of H_2O_2 and $OH^{\bullet}$ ranging from 70 nM to 24 μM and for peroxinitrite formed by NO from 0.57 to 8.5 μM (Table 3).

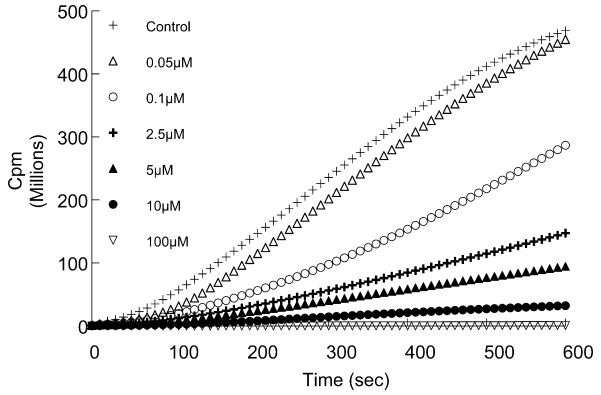

Figure 2. Concentration dependent quenching of luminescence (radical scavenging) by compound **91**.

Table 3. Radical Scavenging Properties of Indoline Carbamates[a]

compd	glucose oxidase $IC_{50} \pm$ SD (μM)	Sin1 $IC_{50} \pm$ SD (μM)	DPPH % radicals scavenged ± SD at conc of 10 μM
87	1.46 ± 0.05	4.55 ± 0.48	24.8 ± 0.7
88	3.13 ± 0.36	NT	NT
90	1.59 ± 0.39	NT	NT
91	1.64 ± 0.06	NT	NT
92	2.39 ± 0.40	NT	NT
93	6.84 ± 0.05	NT	NT
94	19.0 ± 0.6	3.18 ± 0.20	30.4 ± 0.4
95	4.33 ± 0.06	NT	NT
96	15.3 ± 0.01	NT	NT
97	10.2 ± 4.1	NT	NT
98	1.45 ± 0.05	2.36 ± 0.05	31.7 ± 2.3
99	1.14 ± 0.09	NT	NT
100	24.1 ± 0.18	NT	NT
101	6.27 ± 0.05	NT	NT
102	3.41 ± 0.02	NT	NT
103	2.24 ± 0.03	NT	NT
111	0.77 ± 0.03	NT	NT
112	1.38 ± 0.10	0.57 ± 0.01	15.0 ± 1.0
113	0.30 ± 0.01	8.49 ± 0.003	27.8 ± 1.2
114	1.15 ± 0.01	NT	NT
115	0.22 ± 0.04	2.18 ± 0.17	NT
120	0.07 ± 0.01	2.13 ± 0.18	36.3 ± 3.5
melatonin	95.8 ± 0.03	1024 ± 2	NT

[a]Values represent mean ± SD from three independent experiments. NT = not tested.

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Moreover, with the exception of **94**, all the compounds tested had antioxidant activity at concentrations at or below those that inhibited AChE. While most of the compounds tested reduced luminescence induced by the GO and Sin1 cocktails at similar concentrations, compounds **120** and **113** were about 30 times more effective against ROS generated by GO than by Sin1. Moreover, both compounds showed similar antioxidant activity to that of the other carbamates when tested against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The apparently selective effect against ROS generated by GO may have resulted from inhibition of the GO enzyme thereby preventing their formation.

Attachment of the carbamate to AChE or BuChE and consequent carbamoylation of the enzyme releases the respective 4- or 6-hydroxy indoline analogues (leaving groups). These leaving groups, compounds **81**, **84**, and **110**, were found to be as good as, or even better than the parent carbamates at scavenging either ROS or RNS (Table 4). In general, compounds with a carbamate or an OH group in position 4 were better scavengers of ROS but not of RNS than those in position 6.

Table 4. Radical Scavenging Properties of OH Indoline Compounds[a]

	IC_{50} (μM) ± SD		% radicals scavenged ± SD with conc of 10 μM	
compd	glucose oxidase	Sin1	DHR	DPPH
81	0.67 ± 0.05	0.25 ± 0.03	10.6 ± 1.5	32.4 ± 7.0
82	1.44 ± 0.04	NT	19.1 ± 0.8	NT
83	0.45 ± 0.03	NT	15.4 ± 0.9	NT
84	1.34 ± 0.01	1.38 ± 0.01	52.1 ± 0.8	28.2 ± 6.6
85	2.34 ± 0.02	1.34 ± 0.17	41.9 ± 1.9	NT
86	0.84 ± 0.05	3.25 ± 0.03	66.3 ± 2.3	NT
110	1.15 ± 0.01	NT	NT	NT
quercetin	0.06 ± 0.01	0.61 ± 0.22	33.6 ± 2.1	19.2 ± 1.7
gallic acid	NT	NT	NT	31.4 ± 1.4

[a]Values represent mean ± SD from three independent experiments. NT = not tested.

The antioxidant activity of the 4- and 6-hydroxy indolines was also seen when ROS were generated in the presence of Fe^{3+} and measured by a fluorescence method or against the radical DPPH that does not depend on the presence of ROS, Se^{4+} and Co^{2+}.

Protection against Cytotoxicity Induced by Oxidative Stress in Cardiomyocytes. The H9c2 cardiomyocytes were chosen as a model in which to test the potential protective effect of the compounds against cytotoxicity induced by ROS because they are more than 10 times more sensitive than neuronal cells to apoptosis induced by H_2O_2.[30,31] H9c2 cardiomyocyte cells derived from rat cardiac myoblasts treated with 100 μM H_2O_2 for 1 h, showed 30−50% cell mortality. Many of the compounds tested against H_2O_2 cytotoxicity caused a significant reduction in cell death at two concentration ranges, 1 pm to 100 nM and 10−100 μM (Table 5 and Figure 3). At a concentration of 100 μM, none of the compounds showed any significant reduction of cell viability. Only two indole derivatives were tested for antioxidant activity in cardiomyocytes. Of these, **67** was more than 10000 times weaker and **69** about 100 times weaker than their respective indoline analogues (Table 5). With the exception of **91**, the most potent indoline compounds that significantly reduced apoptosis at concentrations of 10−100 pM were all carbamates and methyl esters of propionic acid (**87**, **94**, **98**, and **102**). The corresponding acid derivatives of three of these carbamates (**92**, **96**, and **99**) were much less potent or inactive at concentrations below 100 nM (Table 5). The exception was **91**, which maximally reduced cytotoxicity by 43−78% at concentrations of 1 pM to 1 nM and has a propionic acid group and a methyl on the indoline nitrogen. The only other *N*-methyl derivatives tested (**88** and **103**) were both methyl indoline-3-propionates and were much less active than **91**. The carbamates were also more potent than their respective leaving groups with an OH in positions 4, **81**, or 6, **84**. This protective effect could have resulted from an action inside the cell in which the compounds prevent the fall in the mitochondrial potential ($\Delta\Psi$m) induced by oxidative stress as was shown by Zaikin (2007)[17] and was also reported for ladostigil, a carbamate derivative of amino-indan.[32] Ladostigil also protected cardiomyocites at a concentration range of 0.1−10 nM but to a somewhat lesser extent than the *N*-methyl *N*-ethyl carbamate derivatives of indoline methyl esters **87** and **98**. The fall in $\Delta\Psi$m is thought to occur through the formation of pores in the mitochondria by dimerized pro-apoptotic proteins Bax or activated Bid, Bak, or Bad.[33] Activation of these pro-apoptotic proteins is accompanied by the release of cytochrome c into the cytoplasm. The more lipophilic carbamates and methyl indoline-3-propionate derivatives are likely to penetrate the cell and mitochondrial membranes more readily than their respective OH or acids derivatives.

As the concentration of the compounds increased above 100 nM, their protective effect declined but reappeared at 1 μM, or more frequently at 10−100 μM. The concentrations constituting the second range of protective activity are compatible with those that scavenged ROS and RNS. None of the compounds caused any measurable cytotoxicity in cardiomyocytes at concentrations up to 100 μM (Table 5).

Protection against Apoptosis Induced by Oxidative Stress through Serum Deprivation in Chick Neuronal Cells. This experiment was performed in order to obtain evidence of a protective effect of selected compounds against another insult that induces oxidative stress in a primary neuronal cell culture. Serum deprivation induces apoptosis because of a lack of necessary nutrients and trophic factors.[34] Oxidative stress activates the intrinsic (caspase 9-dependent) apoptotic pathway, causing the release of cytochrome c, and can be prevented by drugs that scavenge or inactivate ROS.[35] Four compounds comprising two carbamates and their leaving groups (**112**, **110**, **87**, and **81**) showed significant protection at a concentration of 10 nM and also at those ranging from 1 to 10 μM against cytotoxicity induced in a primary culture of chick neuronal cells by serum deprivation (Figure 4). This protective effect may also result from different actions of the drugs at low and higher concentrations to reduce apoptosis.

CONCLUSION

We have described the synthesis and activity in vitro of indolines substituted at position 3 with propionic acids, their ester analogues or a 2-aminoethyl group, and substituted on the aromatic ring with an *N*-methyl-*N*-ethyl, *N*-methyl-*N*-butyl, or *N*-methyl-*N*-4-methoxyphenyl carbamate moieties in positions 4, 6, or 7. These compounds were found to act as inhibitors of AChE and BuChE and as antioxidants. Compounds with the carbamate in position 4 are more potent AChE and BuChE inhibitors than those in positions 6 or 7, and the methyl esters

Table 5. Antioxidant Activity of Indoles and Indolines in Cardiomyocyte Cell Culture[a]

	prevention of apoptosis[b]				
compd	first range (nM)	second range (μM)	% protection at first peak	% viability 100 μM drug in absence of H_2O_2	reduction in fall of $\Delta\Psi$m % reduction[c]
67	100	1−100	28.8	NT	56 ± 28
69	100	1−10	43.1	NT	48.2 ± 6.8
81	1−100	1−100	57.3 ± 8.2	120.9 ± 21.1	NT
84	1−100	10	50.0 ± 7.6	NT	87 ± 33
85	>100	>100	none	NT	NT
87	0.001−100	100	73.0 ± 1.14	99.9 ± 3.4	NT
88	>100	1−100	none	97.3 ± 7.4	NT
90	>100	>100	none	93.4 ± 2.1	NT
91	0.001−1	100	57.4 ± 7.4	106.1 ± 8.6	NT
92	100	1−100	55.2 ± 13.9	96.0 ± 9.6	NT
93	1−100	100	31.5 ± 8.4	93.9 ± 3.8	NT
94	0.001−1	100	43.1 ± 4.0	84.7 ± 5.6	NT
95	>100	100	none	95.5 ± 4.5	NT
96	>100	1−100	none	107.1 ± 12.1	NT
97	>100	10−100	none	100.6 ± 22.0	NT
98	0.01−100	100	58.5 ± 14.4	100.0 ± 1.9	77 ± 44
99	1−100	100	56.1 ± 6.4	102.4 ± 3.6	NT
100	1−100	10−100	40.1 ± 9.8	87.8 ± 2.9	54 ± 21
102	0.001−100	100	43.8 ± 11.9	NT	NT
103	10	10−100	33.3 ± 9.3	106.3 ± 4.8	NT
110	1−1000	10−100	46.8 ± 14.1	NT	NT
111	0.1−10	1−100	35.3 ± 5.7	NT	NT
112	0.1−100	100	58.5 ± 7.8	NT	NT
ladostigil	0.1−10	100	38 ± 6.7	NT	32 ± 5
quercetin	100−100000		45.1 ± 5.7	NT	NT
NAC	100−100000		25.2 ± 6.4	NT	20 ± 12

[a]NT = not tested. NAC = *N*-acetylcysteine. $\Delta\Psi$m = mitochondrial potential. Values represent mean ± SD of at least three independent experiments. [b]Concentrations causing >25% protection at a concentration of 100 nM. [c]Concentration of 100 nM.

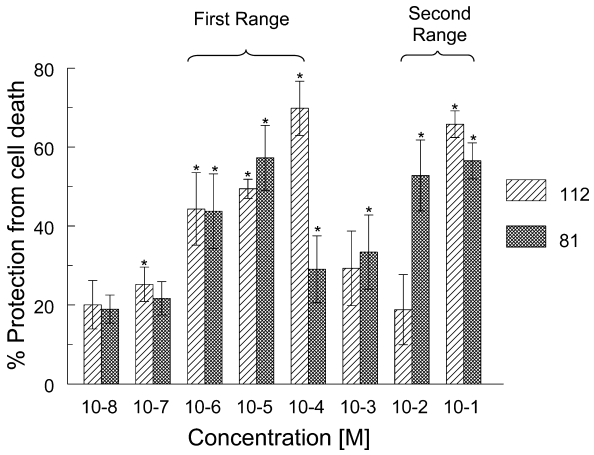

Figure 3. Protection by two indoline derivatives against cytotoxicity induced in cardiomyocytes by oxidative stress. Shows the biphasic concentration activity of a carbamate (**112**) and 4-OH indoline methyl ester (**81**) in protection against cytotoxicity of cardiomyocytes exposed to H_2O_2. Significantly different from value in absence of drug, * $p < 0.05$.

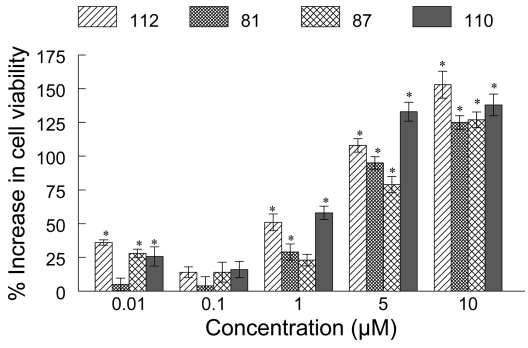

Figure 4. Protection by indoline derivatives against cytotoxicity induced in chick neuronal cells by serum deprivation. Significantly different from value in absence of drug, * $p < 0.05$.

are more potent than their respective acids. Compounds with an (*N*-methyl-*N*-ethyl)carbamate are more potent inhibitors of BuChE than of AChE and those with longer alkyl or aryl substituents. With the exception of **94**, which has a (*N*-methyl-*N*-methoxyphenyl)carbamate in position 4, all the compounds tested, at concentrations similar to or lower than those inhibiting AChE, show antioxidant activity against ROS and RNS generated by GO and Sin1 in the presence of Se^{4+} and Co^{2+}. Carbamoylation of AChE releases the respective 4- or 6-OH indoline analogues (leaving groups). These leaving groups (compounds **81**, **84**, and **110**) were as good as, or better than, the parent carbamates at scavenging either ROS and/or RNS. Many of the compounds, particularly the methyl indoline-3-propionate with a carbamate moiety, are able to reduce cytotoxicity induced by H_2O_2 in cardiomyocytes at concentrations ranging from 1 pm to 100 nM and act by reducing the fall in the mitochondrial potential induced by H_2O_2. At higher concentrations, compatible with those that scavenge ROS, almost all the compounds also protect against cytotoxicity. Thus, several of these novel compounds possess advantages

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over existing AChE inhibitors for the treatment of AD because they interact at relevant concentrations with several important targets to reduce the pathological changes occurring in this disease.

EXPERIMENTAL SECTION

Chemistry. *General Remarks.* ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Avance-200, Avance-DPX-300, Avance-DMX-600, and Avance-III-700 spectrometers. Chemical shifts are expressed in ppm downfield from Me_3Si (TMS) used as internal standard. The values are given in δ scale. The "t" is indicative of a multiplet similar to a triplet with second-order characteristics. Mass spectra (MS) were obtained on a Varian Mat 731 spectrometer (CI^+ = chemical ionization). HRMS were obtained on an AutoSpec spectrometer (Water Company, UK) (CI^+ CH_4). Electron spray ionization (ESI) was obtained on a Micromass Q-TOF Micro mass spectrometer (Micromass (Waters) UK). MALDI were obtained on Autoflex III in TOF/TOF mode (Bruker, Germany). Elemental analyses (CHNO) were obtained using a CHNS-O analyzer using a Flash EA model (Italy). Progress of the reactions was monitored by TLC on silica gel (Merck, Art. 5554). All the flash chromatographic procedures were carried out on silica gel (Merck, Art. 9385). All moisture sensitive reactions were carried out in flame-dried vessels. Melting points were determined on a Fisher–Johns apparatus. The nomenclature of the compounds was assigned according to ChemDraw Ultra version 11.0.1 and 12 (CambridgeSoft). The chemical shifts of compounds containing the carbamate moiety are assigned to both rotamers. The 4- and 6-hydroxyindoles **1** and **2**, and the 4-, 6-, and 7-benzyloxyindoles **3**, **4**, and **5**, respectively, were commercial products. Experimental procedures for compounds, the biological activity of which was assessed are listed below, and data of the synthetic intermediates, which were not biologically tested, are found in the Supporting Information. The purity of the compounds was determined by elemental analyses, confirming ≥95% purity.

General Procedures. *Procedure A: Synthesis of Carbamates.*[36] Carbamoyl chloride (15 mmol) was added to a solution of a 4-, 5-, or 6-hydroxyindole or to a 4-, 5-, 6-, or 7-hydroxyindole-3-propanoic acid (or ester) (7.5 mmol) in dry CH_2Cl_2 (50 mL) containing Et_3N (9.0 mmol) and 4-DMAP (10% mol). The mixture was stirred at room temperature for 24–72 h. The reaction was quenched by addition of water, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residual carbamates were purified either by chromatography or by extraction with 2N NaOH and CH_2Cl_2 to remove traces of unreacted hydroxyindoles and carbamoyl chlorides, followed by elution of the organic phase through a plug of silica gel, which was washed with EtOAc–hexane (1:1). The filtrate was evaporated, and the residue was crystallized from CH_2Cl_2 and hexane.

Procedure B: Vilsmeier Formylation.[37] Phosphorus oxychloride ($POCl_3$) (5.38 mmol) was added dropwise with stirring to DMF (20 mmol) under N_2, the temperature being kept at 10–20 °C. A carbamate (4.9 mmol) in DMF (3 mL) was slowly added with stirring while maintaining the temperature of the mixture at 20–30 °C. The mixture was then stirred at 35 °C for 45 min and was then cooled to room temperature. Cold water (10 mL) was added, followed by addition of a solution of 20% NaOH in water, until pH ∼ 13. Although the reaction was exothermic, the mixture was further heated to its boiling point for 1 min and was then stirred and allowed to cool to room temperature. In some cases, the product, which solidified at this stage, was filtered and the crystals were washed with water. In other cases, water was added followed by extraction with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, and evaporated to give the 3-formylindoles.

Procedure C: Knoevenagel Condensation.[38] To a solution of malonic acid monomethyl ester (2.18 mmol) in pyridine (8.09 mmol) containing a catalytic amount of piperidine (2–3 drops) was added a 3-formylindole (1.46 mmol). The mixture was refluxed for 5–6 h, cooled to room temperature, diluted with EtOAc, and acidified with 3N HCl to pH 7.0. The organic phase was separated, washed with brine, dried over $MgSO_4$, and evaporated and the product was isolated as the α,β-unsaturated ester.

Procedure D: Reduction of Indoles. Method I.[39] $NaBH_3CN$ (4.23 mmol) was added portionwise over 10 min at 0 °C to a solution of an α,β-unsaturated ester or to an indole (1.06 mmol) in AcOH (7.1 mL), and the mixture was stirred at room temperature from 1 h to overnight. Water (3 mL) was added, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residual indoline was converted into the hydrochloride salt upon dissolution in EtOAc followed by extraction with 2N HCl. The aqueous phase was evaporated, and the product was isolated as the salt. Alternatively, the hydrochloride salts were obtained upon addition of HCl gas to a solution of the indolines in dry ether.

Method II.[40] To an ice-cold stirred solution of an indole (1.64 mmol) in TFA (12.5 mL), $NaBH_4$ (3.30 mmol) was added and the mixture was stirred for 22–77 h at room temperature. Water was then added, followed by the addition of saturated $NaHCO_3$ until a pH = 7–8. The mixture was then extracted with EtOAc and brine, dried over Na_2SO_4, and concentrated and the residue was purified by chromatography.

Procedure E: Removal of the Benzyl Group and Reduction of α,β-Unsaturated System. Method I.[41] To a solution of an indole substituted at position 3 with an α,β-unsaturated ester/acid/amide or/and a phenolic compound protected with an *O*-benzyl group (6.72 mmol) in EtOH (50 mL) was added 10% Pd/C (0.16 g) and HCO_2NH_4 (67 mmol). The mixture was refluxed for 1 h. The catalyst was removed by filtration through Celite, the filtrate was concentrated, and the product was isolated as the α,β-saturated and/or the debenzylated product.

Method II. To a solution of an α,β-unsaturated ester/acid/amide and/or a compound protected with *O*-benzyl group or a compound containing a nitro group (1 equiv) in EtOH, was added 10% Pd/C (10% w/w). The mixture was stirred under H_2 at a 1–3 atm pressure for 48 h at room temperature. The mixture was then filtered through Celite and concentrated.

Procedure F: N-Methylation of Indoles.[42] To a solution of methyl iodide (3 mmol) and an indole (3 mmol) in CH_2Cl_2 (50 mL) was added a solution of 50% NaOH (18 mmol) in water (0.72 mL), followed by the addition of $Bu_4N^+Br^-$ (9 mmol). The mixture was stirred at room temperature for 16 h, extracted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, evaporated, and the product was isolated as the *N*-methylated indole.

Procedure G: Hydrolysis of Methyl Esters. Method I.[43] To a solution of a methyl ester (0.133 mmol) in MeOH (3.36 mL) a 2 N solution of NaOH (0.46 mL) was added, and the obtained solution was stirred at room temperature overnight, evaporated, and the residue was dissolved in EtOAc to which 1N HCl was added until a pH = 1–2. The aqueous phase was separated, extracted with EtOAc (×3), and the organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated to give the acid.

Method II.[44] To a solution of a methyl ester (0.1 mmol) in MeOH (0.1 mL) and H_2O (0.5 mmol), KOH (0.5 mmol) was added and the mixture was stirred at room temperature for 18 h. The solution was concentrated to remove the MeOH and to the residue EtOAc was added, and the mixture was extracted with 1N HCl. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to give the acid.

Method III.[45] To an ice-cold solution of a methyl ester (0.65 mmol) in MeOH (15 mL) and H_2O (2.5 mL), LiOH (6.5 mmol) was added. The obtained solution was refluxed for 3.5 h and was then diluted with EtOAc and extracted with 1N HCl. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and evaporated to give the acid.

Procedure H: Coupling with Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium Hexafluorophosphate (BOP).[46] To a solution of an indole-3-propionic acid (0.3 mmol) in DMF (6 mL), under N_2, at room temperature was added Et_3N (0.3 mmol) and BOP (0.3 mmol). The mixture was stirred at room temperature for 45 min followed by addition of $NaNH_2$ (0.3 mmol). The mixture was stirred

at room temperature overnight, diluted with EtOAc and washed with water (×2), 1N HCl (×2), $NaHCO_{3(aq)}$ (×2), brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure.

Procedure I: Preparation of Tartrate Salts. To a stirred solution of an amine (0.04 mmol) in EtOH/MeOH (1.5 mL) was added L-(+)-tartaric acid (0.04 mmol). The mixture was stirred for 30 min at rt, and evaporated and the product was isolated as the tartrate salt.

Procedure J: Preparation of Nitro Compounds.[47] NH_4OAc (0.44 mmol), was added to a stirred solution of an aldehyde (0.9 mmol) in $MeNO_2$ (0.03 mmol) and the solution was refluxed for 1−3 h, while being monitored by TLC (EtOAc−Hex (1:2)) until disappearance of the starting aldehyde. The mixture was then diluted with EtOAc and extracted with water. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated to give the nitro compound.

Procedure K: Reduction of an α,β-Unsaturated Nitro Compounds with $NaBH_4$.[48] To a stirred mixture of a nitrostyrene (0.44 mmol), silica gel (1.3 g), isopropanol (1.8 mL) and $CHCl_3$ (9.6 mL) was added $NaBH_4$ (1.8 mmol). The mixture was stirred at room temperature for 30−90 min. The excess $NaBH_4$ was decomposed with 1N HCl and the mixture was filtered. The filtrate was washed with CH_2Cl_2 and separated, the organic phase was washed with brine, dried over Na_2SO_4, filtered and evaporated to give the nitroethane.

Procedure L: N-Alkylation of an Indole with Methyl Acrylate. To a stirred solution of an indole, substituted at positions 4 or 6 with an *N*-ethyl-*N*-methyl carbamate (0.46 mmol), and methyl acrylate (0.69 mmol) in CH_3CN (2 mL) was added DBU (0.23 mmol). The mixture was stirred at 50 °C for 19 h, extracted with EtOAc, washed with 1N HCl, dried over Na_2SO_4 and evaporated.

3-(2-(Methoxycarbonyl)ethyl)-1*H*-indol-4-yl Ethyl(methyl)-carbamate (51). **51** was synthesized from **37** or from **50** by procedure E, **51** was crystallized from EtOAc−hexane and was isolated as a white solid in 92% yield, mp 122−124 °C. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 10.16 (bs, 1H, H-1), 7.22 (d, *J* = 7.5 Hz, 1H, H-7), 7.09 (bs, 1H, H-2), 7.03 (t, *J* = 7.5 Hz, 1H, H-6), 6.70 (d, *J* = 7.5 Hz, 1H, H-5), 3.67 (s, 3H, H-17), 3.37−3.56 (m, 2H, H-11), 3.17 + 3.03 (s, 3H, H-13), 3.08 (t, *J* = 6.7 Hz, 2H, H-14), 2.65 (t, *J* = 6.7 Hz, 2H, H-15), 1.22−1.31 (m, 3H, H-12). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.74, 155.24, 145.98, 139.89, 123.50, 123.34, 122.17, 113.81, 113.14, 112.93, 109.54, 51.47, 44.59, 44.42, 36.18, 36.05, 34.16, 34.09, 22.61, 13.56, 12.66. MS (CI$^+$) *m*/*z* 305.149 (MH$^+$, 90.07), 304.139 (M$^{\bullet+}$, 81.83), 333.180 ([M + C_2H_5]$^+$, 9.63), 273.120 ([MH − CH_3OH]$^+$, 18.86), 231.105 ([M$^{\bullet+}$ − $C_3H_5O_2$], 8.38). HRMS calcd for $C_{16}H_{20}N_2O_4$ (M$^{\bullet+}$, DCI$^+$/CH_4) 304.1423, found 304.1385, for $C_{16}H_{21}N_2O_4$ (MH$^+$, DCI$^+$/CH_4) 305.1501, found 305.1488. Anal. Calcd for $C_{16}H_{20}N_2O_4$ (304.34 g/mol): C, 63.14; H, 6.62; N, 9.20. Found C, 63.299; H, 6.822; N, 8.966.

3-(2-(Methoxycarbonyl)ethyl)-1-methyl-1*H*-indol-4-yl Ethyl-(methyl)carbamate (53).[42] **53** synthesized from **38** by procedure E was isolated as a white solid (yield 96%), mp >270 °C, and was used as such without further purification; ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 7.18 (dd, *J* = 8, 1 Hz, 1H), 7.10 (t, *J* = 8 Hz, 1H), 6.98 (bs, 1H), 6.72 (bd, *J* = 7 Hz, 1H), 3.74 (s, 3H), 3.63 (s, 3H), 3.63−3.55 + 3.43−3.36 (m, 2H), 3.16 + 2.97 (s, 3H), 3.04 ("t", *J* = 8 Hz, 2H), 2.66−2.61 (m, 2H), 1.28 + 1.17 (t, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.69, 155.19, 146.21, 146.05, 140.26, 127.99, 122.12, 113.18, 112.97, 107.57, 51.52, 44.63, 44.45, 36.23, 36.15, 34.12, 32.87, 22.46, 13.60, 12.70. MS (CI$^+$) *m*/*z* 319.164 (MH$^+$, 71.12), 318.160 (M$^{\bullet+}$, 58.45), 287.132 ([MH − CH_3OH]$^+$, 15.07), 245.114 ($C_{14}H_{17}N_2O_2^+$, 38.93), 86.053 ($C_4H_8NO^+$, 100). HRMS calcd for $C_{17}H_{23}N_2O_4^+$ (MH$^+$) 319.1658; found 319.1645.

3-(2-(Methoxycarbonyl)ethyl)-1*H*-indol-6-yl Butyl(methyl)-carbamate (67). Compound **67** prepared from **45** by procedure E and purified by chromatography eluted with EtOAc−hexane (1:2) was isolated as a yellow oil in 52% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 9.98 (bs, 1H), 7.51 (d, *J* = 8.52 Hz, 1H), 7.14 (d, *J* = 1.70 Hz, 1H), 7.08 (m, 1H), 6.80 (dd, *J* = 8.52, 1.70 Hz, 1H), 3.61 (s, 3H), 3.30−3.50 (m, 2H), 3.09 + 2.96 (s, 3H), 3.04 (t, *J* = 8.06 Hz, 2H), 2.68 (t, *J* = 8.06 Hz, 2H), 1.57−1.67 (m, 2H), 1.29−1.42 (m, 2H), 0.89−0.99 (m, 3H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.84, 155.70, 148.37, 137.46, 125.65, 123.32, 118.99, 114.48, 105.40, 51.55, 49.40, 34.40, 33.67, 33.87, 29.32, 21.31, 20.51, 14.15. MS (CI$^+$) *m*/*z* 332.171 (M$^+$, 81.29). HRMS calcd for $C_{18}H_{24}N_2O_4$ (M$^+$, DCI$^+$/CH_4) 332.1736, found 332.1707.

Methyl 3-(6-((4-Methoxyphenyl)(methyl)carbamoyloxy)-1*H*-indol-3-yl)propanoate (69). **69** was synthesized from **47** or from **60** by procedure E and purified by chromatography eluted with EtOAc−hexane (1:1) was isolated as a white solid in 63% yield, mp 120−124 °C. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 10.00 (bs, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.37 (dd, *J* = 6.7, 2.5 Hz, 2H), 7.12−7.13 (m, 2H), 6.96 (dd, *J* = 6.7, 2.5 Hz, 2H), 6.80 (bd, *J* = 8.4 Hz, 1H), 3.80 (s, 3H), 3.60 (s, 3H), 3.34 (bs, 3H), 3.02 (t, *J* = 7.3 Hz, 2H), 2.67 (*J* = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.83, 158.92, 155.12, 148.23, 137.39, 128.29, 127.77, 125.74, 123.41, 119.03, 114.91, 114.67, 114.33, 105.30, 55.69, 51.55, 38.64, 35.31, 21.27. MS (CI$^+$) *m*/*z* 382.151 (M$^+$, 56.01), 164.072 ([M$^+$ − $C_{12}H_{12}NO_3$]$^+$, 93.42), 136.077 ([M$^+$ − $C_{13}H_{12}NO_4$]$^+$, 99.97). HRMS calcd for $C_9H_{10}NO_2$ ([M$^+$ − $C_{12}H_{12}NO_3$]$^+$, DCI$^+$/CH_4) 164.0712, found 164.0724, for $C_8H_{10}NO$ ([M$^+$ − $C_{13}H_{12}NO_4$]$^+$, DCI$^+$/CH_4) 136.0762, found 136.0773. Anal. Calcd for $C_{21}H_{22}N_2O_5$ (MW 382.41 g/mol): C, 65.96; H, 5.80; N, 7.33; O, 20.92. Found C, 65.848; H, 5.968; N, 6.959.

Methyl 3-(4-Hydroxyindolin-3-yl)propanoate Hydrochloride (81). Upon addition of HCl gas to a solution of **73** in ether, compound **81** was obtained as a hygroscopic white solid in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.27 (t, *J* = 9.6 Hz, 1H), 6.89 (t, *J* = 9.6 Hz, 1H), 3.87 (ABq, *J* = 14.5, 4.8 Hz, 1H), 3.72−3.69 (m, 1H), 3.67 (s, 3H), 3.60 (dd, *J* = 14.5, 4.8 Hz, 1H), 2.48 (t, *J* = 9.6 Hz, 2H), 2.28−2.24 (m, 1H), 1.97−1.92 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 175.27, 156.69, 138.97, 131.38, 131.28, 131.17, 125.24, 117.06, 110.46, 52.17, 51.88, 40.87, 32.39, 28.99.

3-(4-Hydroxyindolin-3-yl)propanoic Acid Hydrochloride (82). Compound **82** was isolated as a yellow oil in quantitative yield from **73** upon addition of 3N HCl to a solution of **73** in MeOH. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.27 (t, *J* = 8.2 Hz, 1H), 6.92 (bt, *J* = 8.2 Hz, 2H), 3.89 (bt, *J* = 8.1 Hz, 1H), 3.71−3.61 (m, 2H), 2.44 (t, *J* = 8.1 Hz, 2H), 2.35−2.20 (m, 1H), 1.97−1.87 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 173.72, 154.58, 137.98, 131.32, 125.59, 117.49, 110.91, 51.68, 40.84, 32.49, 28.98.

Methyl 3-(4-Hydroxy-1-methylindolin-3-yl)propanoate Hydrochloride (83). **83** was synthesized from **34** by procedure D, method I. The crude product consisted of a mixture of the methyl ester and the acid. Thus, it was dissolved in MeOH and a few drops of concentrated HCl were added. The mixture was stirred overnight. The solution was evaporated, and the ester was isolated as an oil (yield 69%). ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.34 (t, *J* = 8 Hz 1H), 7.05 (d, *J* = 8 Hz, 1H), 6.94 (d, *J* = 8 Hz, 1H), 3.97−3.91 (bm, 1H), 3.84−3.81 + 3.60 (m, 2H), 3.67 (s, 3H), 3.26 (s, 3H), 2.51 ("t", *J* = 7.5 Hz, 2H), 2.40−2.29 + 2.00−1.88 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 175.11, 156.62, 143.14, 131.74, 125.04, 118.05, 109.90, 62.76, 52.18, 43.31, 39.79, 32.48, 28.68.

Methyl 3-(6-Hydroxyindolin-3-yl)propanoate Hydrochloride (84). **84** synthesized from **42** or from indoline **74** by procedure D was stirred at room temperature for 2 h. The residue was crystallized from MeOH/Et_2O to provide the **84** as a green hygroscopic solid in 68% yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.29 (bd, *J* = 8.4 Hz, 1H), 6.92 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.91 (s, 1H), 3.96 (ABq, *J* = 0.8 Hz, 1H), 3.66 (s, 3H), 3.51−3.53 (m, 2H), 2.45 (t, *J* = 7.7 Hz, 2H), 2.13 (td, *J* = 14.0, 7.4 Hz, 1H), 1.89 (*J* = 14.0, 7.4 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 174.93, 159.60, 137.60, 129.79, 127.13, 118.46, 107.34, 52.48, 52.25, 41.53, 32.31, 30.28. MS (CI$^+$) *m*/*z* 222.110 (MH$^+$, 100), 221.103 (M$^+$, 46.37), 190.087 ([MH$^+$ − CH_3OH], 26.79), 134.076 ([M$^+$ − $C_4H_7O_2$]$^+$, 42.54). HRMS calcd for $C_{12}H_{16}NO_3$ (MH$^+$, DCI$^+$/CH_4) 222.1130, found 222.1104.

3-(6-Hydroxyindolin-3-yl) Propanoic Acid Hydrochloride (85). **85** was synthesized from **62** or from **74** by procedure D and was isolated as a brown hygroscopic oil in 23% yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.31 (d, *J* = 8.0 Hz, 1H), 6.88−6.97 (m, 2H), 3.97 (m, 1H), 3.46−3.68 (m, 2H), 2.45 (t, *J* = 8.0 Hz, 2H), 2.01−2.18 (m, 1H), 1.77−1.95 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 173.62, 158.39, 136.34, 128.40, 125.75, 117.14, 116.88, 105.89, 51.50,

51.24, 40.26, 39.04, 30.80, 28.94, 28.58. MS (CI^-) m/z 207.090 ($M^{\bullet+}$, 69.86), 148.033 ($C_9H_{10}NO^-$, 34.36). HRMS calcd for $C_{11}H_{13}NO_3$ (M^+, DCI^-/CH_4) 207.0895, found 207.0904.

3-(6-Hydroxy-1-methylindolin-3-yl)propanoic acid) Hydrochloride (86). **86** was synthesized from **64** by procedure D, stirred at room temperature for 2 h, and isolated as a colorless hygroscopic oil in 63% yield. ^1H NMR (300 MHz, CD_3OD) ppm δ 7.33 (bs, 1H), 6.98 (m, 1H), 4.08 (t, J = 10.8 Hz, 1H), 3.67 (t, J = 10.8 Hz, 1H), 3.55 (m, 1H), 3.25 (s, 3H), 2.48 (t, J = 7.2 Hz, 2H), 2.15−2.26 (m, 1H), 1.80−1.94 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 176.35, 160.09, 142.74, 129.32, 127.16, 106.33, 63.71, 43.18, 40.45, 32.35 + 32.24, 29.91 + 29.80. MS (CI^+) m/z 221.105 ($M^{\bullet+}$, 71.83), 222.112 (MH^+, 70.38). HRMS calcd for $C_{12}H_{16}NO_3$ (MH^+, DCI^+/CH_4) 222.1130, found 222.1123, for $C_{12}H_{15}NO_3$ (M^+, DCI^+/CH_4) 221.1052, found 221.1046.

3-(2-(Methoxycarbonyl)ethyl)indolin-4-yl Ethyl(methyl)-carbamate Hydrochloride (87). **87** was synthesized from **37** or **51** or from **75** by procedure D, stirred at room temperature overnight, and isolated as a yellow oil in 40% yield. ^1H NMR (300 MHz, CD_3CN) ppm δ 7.16−7.48 (m, 3H), 3.70−3.86 (m, 1H), 3.60 (s, 3H), 3.46−3.62 (m, 3H), 3.35 + 3.37 (m, 1H), 3.06 + 2.95 (s, 3H), 2.36−2.40 (m, 2H), 2.05−2.11 (m, 1H), 1.80−1.85 (m, 1H), 1.15−1.36 (m, 3H). ^{13}C NMR (75 MHz, CD_3CN) ppm δ 173.79, 153.98, 149.63, 138.13, 131.93, 130.78, 124.69, 125.29, 118.24, 117.68, 52.14, 50.45, 44.92, 44.78, 40.52, 34.64, 34.35, 31.73, 28.51, 13.45, 12.61. MS (CI^+) m/z 335.204 ($[M + C_2H_5]^+$, 28.34), 307.170 (MH^+, 100), 306.164 (M, 46.78), 275.143 ($[MH^+ - CH_3OH]$, 27.57). HRMS calcd for $C_{16}H_{23}N_2O_4$ (MH^+, DCI^+/CH_4) 307.1658, found 307.1702.

3-(2-(Methoxycarbonyl)ethyl)-1-methylindolin-4-yl Ethyl-(methyl)carbamate Hydrochloride (88). Compound **88** was prepared from **53** by procedure D, method I. The crude product consisted of a mixture of the methyl ester and the acid, then 43% of the crude was dissolved in MeOH, and 6 drops of 3N HCl were added. The mixture was stirred over three nights. The solution was evaporated and **88** was isolated as an oil (yield 62%). ^1H NMR (300 MHz, D_2O) ppm δ 7.57 (t, J = 8 Hz, 1H), 7.47 (d, J = 8 Hz, 1H), 7.28 (d, J = 8 Hz, 1H), 4.08−4.01 (m, 1H), 3.87−3.77 (m, 2H), 3.67 (s, 3H), 3.58−3.51 + 3.42−3.35 (m, 2H), 3.30 (s, 3H), 3.13 + 2.99 (s, 3H), 2.49 (t, J = 7.5 Hz, 2H), 2.22−2.13 + 1.99−1.86 (m, 2H), 1.25 + 1.17 (t, J = 7 Hz, 3H). ^{13}C NMR (75 MHz, D_2O) ppm δ 175.78, 154.94, 147.98, 142.23, 131.02, 129.95, 124.65, 124.50, 116.07, 61.30, 52.40, 44.53, 44.41, 42.81, 38.53, 34.081, 30.99, 26.88, 12.41, 11.68. MS (CI^+) m/z 321.181 (MH^+, 100), 320.175 ($M^{\bullet+}$, 69.90), 86.070 ($C_4H_8NO^+$, 58.93). HRMS calcd for $C_{17}H_{25}N_2O_4^+$ (MH^+) 321.1814, found 321.1813.

Ethyl 3-(4-(Ethyl(methyl)carbamoyloxy)indolinium Citrate (89). **89** was synthesized from **76** by procedure I and isolated as a yellow oil in quantitative yield. ^1H NMR (300 MHz, CD_3OD) ppm δ 7.05 (t, J = 7.9 Hz, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.47 (bd, J = 7.9 Hz, 1H), 4.10 (q, J = 7.6 Hz, 2H), 3.66−3.59 (m, 1H), 3.57−3.48 (m, 1H), 3.43−3.36 (m, 2H), 3.30−3.25 (m, 1H), 2.98 + 3.11 (s, 3H), 2.85 (ABq, J = 14.0 Hz, 4H), 2.34 (t, J = 7.9 Hz, 2H), 2.10−1.99 (m, 1H), 1.87−1.75 (m, 1H), 1.23 (t, J = 6.7 Hz, 3H), 1.33−1.15 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 176.84, 174.97, 173.48, 153.31, 149.68, 141.50, 130.04, 125.78, 114.82, 114.60, 110.21, 109.48, 74.13, 61.46, 52.89, 52.79, 45.24, 45.15, 43.84, 41.19, 34.54, 34.38, 32.35, 29.31, 29.23, 14.54, 13.52, 12.69. MS (CI^+) m/z 320.178 ($M^{\bullet+}$, 67.08), 321.178 (MH^+, 38.14). HRMS calcd for $C_{17}H_{24}N_2O_4$ (M^+, DCI^+/CH_4) 320.1736, found 320.1775.

3-(3-Oxobutyl)indolin-4-yl Ethyl(methyl)carbamate Hydrochloride (90). **90** was synthesized from **52** by procedure D, stirred at room temperature overnight, and isolated as an off-white hygroscopic crystals in 56% yield. ^1H NMR (300 MHz, CD_3CN) ppm δ 7.17−7.46 (m, 3H), 3.82 ("t", J = 9.90 Hz, 1H), 3.62−3.63 (m, 1H), 3.58 ("t", J = 9.9 Hz 1H), 3.38 + 2.99 (bs, 5H), 2.35 ("t", J = 7.4 Hz, 2H), 2.02−2.09 (m, 1H), 1.73−1.85 (m, 1H), 1.15 + 1.34 (m, 3H). ^{13}C NMR (75 MHz, CD_3CN) ppm δ 175.37, 154.36, 149.38, 137.70, 131.99, 130.96, 124.93, 118.33, 50.97, 44.96, 39.46, 34.59, 31.84, 28.34, 13.30, 12.73. MS (CI^+) m/z 293.150 (MH^+, 100), 292.144 ($M^{\bullet+}$, 76.31), 275.138 ($[MH - H_2O]^+$, 56.34), 219.115 ($[M^+ - C_3H_5O_2]$, 13.73). HRMS calcd for $C_{15}H_{20}N_2O_4$ (M^+, DCI^+/CH_4) 292.1423, found 292.1438, for $C_{15}H_{21}N_2O_4$ (MH^+, DCI^+/CH_4) 293.1501, found 293.1497, for $C_{15}H_{19}N_2O_3$ ($[MH - H_2O]^+$, DCI^+/CH_4) 275.1396, found 275.1384.

3-(4-((Ethyl(methyl)carbamoyl)oxy)-1-methylindolin-3-yl)-propanoic Acid Hydrochloride, (91). Compound 77 was dissolved in CH_2Cl_2 and stirred with 3N HCl at room temperature for 48 h. The aqueous phase was evaporated to provide **91** as a colorless oil in quantitative yield. ^1H NMR (300 MHz, CD_3OD) ppm δ 7.59−7.50 (m, 2H), 7.31 (d, J = 7.5 Hz, 1H), 4.04−3.97 (m, 1H), 3.92−3.85 (m, 1H), 3.79−3.77 (bs, 1H), 3.56 + 3.42 (q, J = 7.2 Hz, 2H), 3.40 (s, 3H), 3.15 + 3.01 (s, 3H), 2.44 (t, J = 7.2 Hz, 2H), 2.30−2.20 (m, 1H), 1.92−1.84 (m, 1H), 1.28 + 1.20 (t, J = 7.2 Hz, 3H). ^{13}C NMR (175 MHz, CD_3OD) ppm δ 174.58, 154.87, 149.91, 149.87, 143.40, 143.34, 131.81, 131.63, 131.54, 131.53, 125.91, 125.74, 116.83, 62.35, 62.24, 45.51, 45.30, 43.35, 40.20, 34.72, 34.49, 32.98, 31.91, 28.52, 28.45, 13.50, 12.61. MS (CI^+) m/z 306.159 ($M^{\bullet+}$, 57.80), 307.167 (MH^+, 100). HRMS calcd for $C_{16}H_{23}N_2O_4$ (MH^+ DCI^+/CH_4) 307.1658, found 307.1668.

3-(3-Amino-3-oxopropyl)indolin-4-yl Ethyl(methyl)-carbamate Hydrochloride (92). **92** was synthesized from **55** by procedure D, stirred at room temperature overnight, and isolated as a colorless oil in 38% yield. ^1H NMR (300 MHz, CD_3OD) ppm δ 7.24−7.60 (m, 3H), 3.94−4.22 (m, 1H), 3.70−3.78 (m, 2H), 3.40−3.56 (m, 2H), 3.14 + 3.00 (s, 3H), 2.43 ("t", J = 7.9 Hz, 2H), 2.27−2.34 (m, 1H), 1.85−1.91 (m, 1H), 1.22−1.46 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 178.65, 154.65, 150.02, 138.26, 132.50, 132.14, 131.70, 131.41, 125.27, 126.02, 117.93, 51.37, 51.16, 45.45, 45.30, 40.59, 34.74, 34.56, 33.07, 32.95, 29.67, 29.39, 13.59, 12.66. MS (CI^+) m/z 362.239 ($[M + C_2H_{11}]^+$, 49.69), 334.217 ($M + C_3H_7]^+$, 99.97), 320.20 ($[M + C_2H_5]^+$, 79.22), 292.165 (MH^+, 23.11), 275.136 ($[MH^+ - NH_3]$, 23.50), 86.01 ($C_4H_8NO^+$, 88.33). HRMS calcd for $C_{17}H_{26}N_3O_3$ ($[M + C_2H_5]^+$, DCI^+/CH_4) 320.1974, found 320.2004, for $C_{15}H_{22}N_3O_3$ (MH^+, DCI^+/CH_4) 292.1661, found 292.1655.

3-(2-(Methoxycarbonyl)ethyl)-1-methylindolin-4-yl Butyl-(methy)lcarbamate Hydrochloride (93). **93** synthesized from **56** by procedure D, method I, was dissolved in MeOH, and 6 drops of 3N HCl were added. The mixture was stirred for 72 h. The obtained solution was evaporated, and the product was isolated as an oil (33% yield). ^1H NMR (300 MHz, CD_3OD) ppm δ 7.42 (bs, 1H), 7.31−7.30 (bm, 1H), 7.14−7.09 (m, 1H), 3.82 (bm, 1H), 3.76−3.70 (bm, 2H), 3.67 (s, 3H), 3.51 + 3.38 (bm, 2H), 3.21 (s, 3H), 3.15 + 3.01 (s, 3H), 2.48−2.34 (bt, J = 7 Hz, 2H), 2.20 + 1.88 (bs, 2H), 1.70−1.57 (m, 2H), 1.45−1.33 (m, 2H), 1.02−0.94 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 173.34, 154.01, 148.38, 145.10, 144.98, 130.00, 129.90, 129.80, 121.56, 121.16, 112.92, 112.71, 60.99, 60.87, 50.85, 48.93, 40.40, 38.74, 33.93, 33.660, 30.67, 30.60, 29.89, 29.12, 27.34, 27.22, 19.60, 19.50, 12.81. MS (CI^+) m/z 349.212 (MH^+, 70.32), 348.205 ($M^{\bullet+}$, 44.07), 114.091 ($C_6H_{12}NO^+$, 100). HRMS calcd for $C_{19}H_{29}N_2O_4^+$ (MH^+) 349.2127, found 349.2124.

3-(3-Methoxy-3-oxopropyl)-4-(((4-methoxyphenyl)(methyl)-carbamoyl)oxy) Indolin-1-ium Hydrochloride (94). Compound **94** was isolated as a hygroscopic yellow solid in quantitative yield upon addition of HCl gas to a solution of **78** in ether. ^1H NMR (300 MHz, CD_3OD) ppm δ 7.47 (bt, J = 7.3 Hz, 1H), 7.36 (d, J = 9.3 Hz, 2H), 7.37−7.32 (m, 2H), 6.97 (d, J = 9.3 Hz, 2H), 3.93−3.81 (m, 1H), 3.81 (s, 3H) 3.67 (s, 3H), 3.62−3.54 (m, 2H), 3.33 (bs, 3H), 2.27 (bt, J = 5.5 Hz, 2H), 1.89 (bs, 1H), 1.66 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) ppm δ 174.60, 160.37, 154.37, 149.99, 138.37, 136.48, 131.84, 131.35, 128.91, 124.88, 117.54, 115.58, 55.96, 52.24, 51.35, 41.21, 39.19, 31.96, 28.69. MS (CI^+) m/z 384 ($M^{\bullet+}$, 10.22), 385 (MH^+, 22.77), 386 (MH_2^+, 6.92).

3-(4-((4-Methoxyphenyl)(methyl)carbamoyl)indolin-3-yl)-propanoic Acid Hydrochloride (95). Compound **94** was dissolved in 3N HCl and stirred over two nights and then evaporated. The HCl salt was isolated in 93% yield. ^1H NMR (300 MHz, D_2O) ppm δ 7.49−7.25 (m, 5H), 6.99 (d, J = 8.5 Hz, 2H), 3.95−3.86 (m, 1H), 3.78 (s, 3H), 3.63 + 3.57 (m, 2H), 3.43 + 3.27 (bs, 3H), 2.20 (m, 2H), 1.74 + 1.61 (m, 2H). ^{13}C NMR (50 MHz, D_2O) ppm δ 178.90, 160.31, 151.64, 149.83, 138.81, 137.15, 132.66, 132.38, 129.77, 125.93, 119.27, 116.87, 57.70, 54.31, 41.40, 40.36, 32.93, 29.11.

3-(2-(Methoxycarbonyl)ethyl)-1-methylindolin-4-yl 4-Methoxyphenyl(methyl) Carbamate Hydrochloride (96). Compound **96** prepared from **58**, was dissolved in MeOH, and a few drops of concentrated HCl were added. The mixture was stirred overnight, and the obtained solution was evaporated. The product was isolated as an oil (yield 99%). ^{1}H NMR (300 MHz, CD_3CN) ppm δ 7.45−7.27 (m, 5H), 6.95 (d, J = 9 Hz, 2H), 3.79 (s, 3H), 3.73 + 3.45 (bs, 3H), 3.65 (s, 3H), 3.27 + 3.07 (s, 3H), 2.24 (bs, 2H), 1.87 + 1.71 (bs, 2H). ^{13}C NMR (75 MHz, CD_3CN) ppm δ 173.75, 157.76, 153.54, 149.38, 143.11, 136.34, 131.13, 128.88, 124.83, 116.30, 115.25, 61.26, 56.12, 52.21, 42.57, 39.58, 39.19, 31.94, 28.19.

3-(4-((4-Methoxyphenyl)(methyl)carbamoyl)-1-methylindolin-3-yl)propanoic Acid Hydrochloride (97). **97** synthesized from **59** by procedure D, method I, was isolated in 65% yield. ^{1}H NMR (300 MHz, D_2O) ppm δ 7.43−7.31 (m, 2H), 7.25 (d, J = 9 Hz, 2H), 7.16 (d, J = 8 Hz, 1H), 6.86 (d, J = 9 Hz, 2H), 3.89 + 3.82 (m, 1H), 3.66 (bs, 4H), 3.47 (bs, 1H), 3.31 + 3.21 (bs, 3H), 3.16 (bs, 3H), 2.17−2.12 (m, 2H), 1.69 + 1.51 (bm, 2H). ^{13}C NMR (50 MHz, D_2O) ppm δ 178.56, 160.25, 156.16, 149.61, 143.72, 137.08, 132.67, 132.78, 131.67, 129.68, 126.39, 118.20, 116.80, 63.14, 57.65, 45.10, 40.39, 33.03, 28.86 (C-18).

3-(2-(Methoxycarbonyl)ethyl)-1-indoline-6-yl Ethyl(methyl)-carbamate Hydrochloride (98). **98** was synthesized from **99** or **79**. A solution of compound **99** in MeOH containing a few drops of 1N HCl was stirred overnight, the solvent was then evaporated, and the product was isolated as brown crystals in 43% yield, mp 100−105 °C. Alternatively, the HCl salt was obtained by bubbling HCl gas into a solution of indoline **79** in ether or CH_2Cl_2. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.53 (bd, J = 6.4 Hz, 1H), 7.35 (bs, 1H), 7.18 (bd, J = 6.4 Hz, 1H), 4.05 (m, 1H), 3.68 (s, 3H), 3.56−3.64 (m, 1H), 3.35−3.52 (m, 2H), 3.35−3.41 (m, 1H), 3.11 + 2.99 (s, 3H), 2.52 ("t", J = 7.3 Hz, 2H), 2.21 (bs, 1H), 1.94 (bs, 1H), 1.18 + 1.26 + 1.41 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 174.74, 155.43, 152.85, 136.84, 127.09, 125.83, 124.97, 114.95, 52.56, 52.33, 45.21, 41.75, 34.72, 34.46, 32.15, 29.99, 12.62, 13.47. MS (CI$^+$) m/z 219.100 ([M$^+$ − $C_4H_7O_2$]$^+$, 62.63), 306.157 (M$^+$,100). HRMS calcd for $C_{16}H_{22}N_2O_4$ (M$^+$, DCI$^+$/CH_4) 306.1580, found 306.1567.

3-(6-(Ethyl(methyl)carbamoyloxy)indolin-3-yl)propanoic Acid Hydrochloride (99). **99** was synthesized from **66** by procedure D, stirred at room temperature for 2 h, and isolated as a white crystals in 23% yield, mp 117−119 °C. ^{1}H NMR (300 MHz, D_2O) ppm δ 7.51 (bd, J = 8.1 Hz, 1H), 7.31 (bd, 1H), 7.24 (dd, J = 8.1, 1.8 Hz, 1H), 4.05−4.15 (m, 1H), 3.59 + 3.68 (m, 2H), 3.30−3.48 (m, 1H), 3.06 + 2.94 (s, 3H), 2.50 ("t", J = 7.7 Hz, 2H), 2.11−2.16 (m, 1H), 1.87−1.94 (m, 1H), 1.11−1.36 (m, 3H). ^{13}C NMR (75 MHz, D_2O) ppm δ 177.44, 155.79, 151.27, 150.97, 135.95, 126.34, 124.04, 113.73, 51.35, 44.23. MS (CI$^+$) m/z 219.096 ([M$^+$ − $C_3H_5O_2$]$^+$, 17.24), 292.142 (M$^+$, 18.23). HRMS calcd for $C_{15}H_{20}N_2O_4$ (M$^+$, DCI$^+$/CH_4) 292.1423, found 292.1424.

3-(2-(Methoxycarbonyl)ethyl)-1-indoline-6-yl Butyl(methyl)-carbamate Hydrochloride (100). **100** was synthesized from **67** by procedure D, stirred at room temperature for 1 h, and isolated as a white hygroscopic crystals in 60% yield, mp hygroscopic salt. ^{1}H NMR (300 MHz, D_2O) ppm δ 7.53 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 1.8 Hz, 1H), 7.25 (dd, J = 3.0, 1.8 Hz, 1H), 4.05−4.12 (m, 1H), 3.66 (s, 3H), 3.60−3.73 (m, 1H), 3.47 (t, J = 7.4 Hz, 1H), 3.14−3.34 (m, 1H), 3.08 + 2.95 (s, 3H), 2.52 (t, J = 7.9 Hz, 2H), 2.13−2.20 (m, 2H), 1.89−1.99 (m, 2H) 1.45−1.65 (m, 2H), 1.19−1.38 (m, 2H), 0.83−0.94 (m, 3H). ^{13}C NMR (75 MHz, D_2O) ppm δ 177.50, 150.97, 147.5, 126.36, 124.00, 113.67, 109.16, 51.35, 49.74, 48.87, 40.06, 31.04, 29.11, 28.60, 28.19, 19.16, 13.05. MS (CI$^+$) m/z 334.188 (M$^+$, 6.29), 335.198 (MH$^+$, 4.36). HRMS calcd for $C_{18}H_{26}N_2O_4$ (M$^+$, CH_4) 334.1893, found 334.1877, for $C_{18}H_{27}N_2O_4$ (MH$^+$, CH_4) 335.1971, found 335.1976.

Methyl 3-(6-(Butyl(methyl)carbamoyloxy)-1-methylindolin-3-yl)propanoate Hydrochloride (101). **101** was synthesized from **68** by procedure D, stirred at room temperature overnight, and isolated as a white hygroscopic crystals in 34% yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.48−7.53 (m, 2H), 7.29 (bs, 1H), 4.15 (bs, 1H), 3.68−3.81 (m, 1H), 3.68 (s, 3H), 3.35−3.48 (m, 2H), 3.21−3.26 (m, 4H), 3.12 + 2.99 (s, 3H), 2.53 (bs, 2H), 2.27 (bs, 1H), 1.95 (bs, 1H), 1.61−1.67 (m, 2H), 1.28−1.39 (m, 2H), 0.97−0.99 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 174.61, 155.60, 153.20, 142.40, 136.18, 127.18, 125.52, 114.21, 63.82, 52.45, 50.16, 43.61, 40.71, 35.37, 35.21, 32.34, 31.09, 30.38, 29.53, 20.78, 14.17. MS (CI$^+$) m/z 348.208 (M$^+$, 82.42), 349.214 (MH$^+$, 100), 261.168 ([M$^+$ − $C_4H_7O_2$]$^+$, 30.49), 114.107 ([MH$^+$ − $C_{13}H_{17}NO_3$]$^+$, 38.56). HRMS calcd for $C_{19}H_{28}N_2O_4$ (M$^+$, DCI$^+$/CH_4) 348.2049, found 348.2080, for $C_{19}H_{29}N_2O_4$ (MH$^+$, DCI$^+$/CH_4) 349.2127, found 349.2141.

Methyl 3-(6-((4-Methoxyphenyl)(methyl)carbamoyloxy)-indolin-3-yl)propanoate Hydrochloride (102). **102** was synthesized from **69** or from **80**. When prepared from **69** by procedure D, the solution was stirred at room temperature overnight, and the product was isolated as white crystals in 18% yield, mp dec >150 °C. Alternatively, the HCl salt was obtained by bubbling HCl gas to a solution of indoline **80** in ether or CH_2Cl_2. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.51 (bd, J = 7.4 Hz, 1H), 7.41 (bs, 1H), 7.31 (dd, J = 8.9, 2.2 Hz, 2H), 7.20 (bs, 1H), 6.96 (dd, J = 8.9, 2.2 Hz, 2H), 4.01−4.13 (m, 1H), 3.81 (s, 3H), 3.68 (s, 3H), 3.46−3.61 (m, 5H), 2.52 (t, dd, J = 7.7 Hz, 2H), 2.10−2.29 (m, 1H), 1.78−1.94 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 173.51, 158.50, 151.64, 136.04, 135.62, 135.20, 127.33, 127.10, 125.83, 123.54, 114.08, 113.42, 54.62, 51.35, 50.92, 40.51, 37.74, 30.77, 28.65. MS (CI$^+$) m/z 385.179 (M$^+$, 3.5). HRMS calcd for $C_{21}H_{25}N_2O_5$ (M$^+$, DCI$^+$/CH_4) 385.1763, found 385.1786.

Methyl 3-(6-((4-Methoxyphenyl)(methyl)carbamoyloxy)-1-methylindolin-3-yl)propanoate Hydrochloride (103). **103** was synthesized from **70** by procedure D, stirred at room temperature overnight, and isolated as a white crystals in 47% yield, mp dec >150 °C. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.42−7.55 (m, 2H), 7.33 (bd, J = 8.4 Hz, 2H), 7.25 (bs, 1H), 6.96 (bd, J = 8.4 Hz, 2H), 4.12 (m, 1H), 3.79 (s, 3H), 3.67 (s, 3H), 3.56−3.37 (m, 1H), 3.43−3.45 (m, 1H), 3.25−3.35 (m, 6H), 2.51 (t, J = 6.7 Hz, 2H), 2.15−2.30 (m, 1H), 1.85−2.01 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 174.74, 159.80, 153.24, 142.32, 136.95, 136.43, 128.73, 128.13, 127.17, 125.48, 115.42, 114.04, 63.56, 56.01, 52.28, 43.17, 40.68, 39.12, 32.18, 29.47. MS (CI$^+$) m/z 400.195 (MD$^+$, 100), 399.190 (MH$^+$, 72.16), 164.078 ([MH$^+$ − $C_{13}H_{17}NO_3$]$^+$, 71.13), 136.083 ([MH$^+$ − $C_{14}H_{17}NO_4$]$^+$, 57.04). HRMS calcd for $C_{22}H_{27}N_2O_5$ (MH$^+$, DCI$^+$/CH_4) 399.1920, found 399.1898, for $C_{22}H_{26}N_2O_5D$ (MD$^+$, DCI$^+$/CH_4) 400.1983 found 400.1952, for $C_9H_{10}NO_2$ ([MH$^+$ − $C_{13}H_{17}NO_3$]$^+$, DCI$^+$/CH_4) 164.0712 found 164.0784, for $C_8H_{10}NO$ ([MH$^+$ − $C_{14}H_{17}NO_4$]$^+$, DCI$^+$/CH_4) 136.0762, found 136.0831.

Methyl 3-(7-(Ethyl(methyl)carbamoyloxy)indolin-3-yl)-propanoate Hydrochloride (104). **104** was synthesized from **72** by procedure D, stirred at room temperature overnight, and crystallized from MeOH−ether and was isolated as a white solid in 4.2% yield, mp dec >180 °C. ^{1}H NMR (300 MHz, D_2O) ppm δ 7.44−7.48 (m, 1H), 7.38 (t, J = 8.2 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 4.09−3.98 (m, 1H), 3.79−3.67 (m, 1H), 3.69 (s, 3H), 3.64−3.50 (m, 2H), 3.46- 3.33 (s, 1H), 3.01 + 3.14 (s, 3H), 2.53 (qd, J = 8.1, 2.7 Hz, 2H), 2.24−2.16 (m, 1H), 1.93−2.04 (m, 1H), 1.24−1.19 (t, J = 7.4 Hz 3H). ^{13}C NMR (75 MHz, CD_3CN) ppm δ 173.86, 153.37, 143.84, 141.93, 131.60, 128.16, 122.87 + 122.42, 118.20, 52.18, 51.47, 44.99, 41.93, 34.69, 31.85, 29.65, 13.54 + 12.6. MS (CI$^+$) m/z 335.207 ([M + C_2H_5]$^+$, 9.77), 307.169 (MH$^+$, 100), 306.163 (M$^+$, 36.98), 275.147 ([M$^{\bullet+}$ − MeOH], 12.94). HRMS calcd for $C_{16}H_{23}N_2O_4$ (MH$^+$, DCI$^+$/CH_4) 307.1658, found 307.1690.

Indolin-4-ol Hydrochloride (110). **110** was synthesized from 4-hydroxyindole or from **105** by procedure D, stirred at room temperature for 2 h, and isolated as a green solid in 60% yield, mp 168−170 °C. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.24 (t, J = 8.3 Hz, 1H) 6.95 (d, J = 8.3 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 3.84 (t, J = 9.0 Hz, 2H), 3.23 (t, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 156.21, 138.21, 130.78, 122.80, 117.15, 110.86, 46.90, 27.37. MS (CI$^+$) m/z 136.073 (MH$^+$, 99.98), 135.066 (M$^{\bullet+}$, 85.67), 118.066 ([MH$^+$ − H_2O], 18.51). HRMS calcd for $C_8H_{10}NO$ (MH$^+$, DCI$^+$/CH_4) 136.0762, found 136.0735, for C_8H_9NO (M$^{\bullet+}$, DCI$^+$/CH_4) 135.0684, found 135.0656.

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Indolin-4-yl Dimethylcarbamate Hydrochloride (111). 111 was synthesized from **6** by procedure D, stirred at room temperature for 2 h, and isolated as a colorless oil in 69% yield. ^{1}H NMR (300 MHz, D_2O) ppm δ 7.35−7.29 (m, 2H), 7.09 (bd, J = 7.1 Hz, 1H), 3.78 (bt, J = 7.9 Hz, 2H), 3.10 (bt, J = 7.9 Hz, 2H), 2.97 (s, 3H), 2.83 (s, 3H). ^{13}C NMR (75 MHz, D_2O) ppm δ 155.17, 147.77, 136.63, 129.86, 128.80, 123.41, 116.97, 46.00, 36.20, 26.29. MS (CI^+) m/z 207.117 (MH^+, 54.82), 206.106 ($M^{\bullet+}$, 100). HRMS calcd for $C_{11}H_{14}N_2O_2$ ($M^{\bullet+}$, DCI^+/CH_4) 206.1055, found 206.1062.

Indolin-4-yl Ethyl(methyl)carbamate Hydrochloride (112). 112 was synthesized from **7** or from **105** by procedure D, stirred at room temperature for 2 h, and isolated as a colorless hygroscopic oil in 63% yield. ^{1}H NMR (300 MHz, CD_3CN) ppm δ 7.35 (t, J = 7.6 Hz, 1H), 7.26 (d, J = 7.6 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H) 3.73 (t, J = 7.9 Hz, 2H), 3.36−3.43 (m, 2H), 3.11 (t, J = 7.9 Hz, 2H), 2.97 + 3.04 (s, 3H), 1.18 (bs, 3H). ^{13}C NMR (75 MHz, CD_3CN) ppm δ 153.94, 149.60, 138.31, 130.33, 129.91, 123.93, 118.28, 117.63, 46.40, 44.89, 34.63, 27.62, 13.38, 12.67. MS (CI^+) m/z 221.126 (MH^+, 100), 220.120 ($M^{\bullet+}$, 58.49). HRMS calcd for $C_{12}H_{17}N_2O_2$ (MH^+, DCI^+/CH_4) 221.1290, found 221.1260, for $C_{12}H_{16}N_2O_2$ ($M^{\bullet+}$, DCI^+/CH_4) 220.1212, found 220.1203.

Methyl 3-(4-((Ethyl(methyl)carbamoyl)oxy)indolin-1-yl)-propanoate Hydrochloride (113). 113 was synthesized from **107** by addition of a solution of HCl (g)/ether. The hydrochloride salt was isolated as a yellow viscous oil. ^{1}H NMR (200 MHz, CD_3OD) ppm δ 7.55−7.37 (m, 2H), 7.23 (bd, J = 7.8 Hz, 1H), 3.98 (t, J = 7.5 Hz, 2H), 3.83 (t, J = 6.9 Hz, 2H), 3.74 (s, 3H), 3.53 + 3.42 (q, J = 7.2 Hz, 2H), 3.24 (t, J = 7.5 Hz, 2H), 3.00 + 3.13 (bs, 3H), 2.95 (t, J = 6.9 Hz, 2H), 1.34−1.13 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD) ppm δ 172.23, 154.73, 149.90, 143.98, 131.08, 129.25, 123.94, 116.06, 55.46, 52.79, 45.33, 34.72, 34.44, 30.46, 26.78, 13.44, 12.60. MS (CI^+) m/z 306.165 ($M^{\bullet+}$, 88.18), 307.169 (MH^+, 99.99). HRMS calcd for $C_{16}H_{23}N_2O_4$ (MH^+, CI/CH_4) 307.1658, found 307.1689.

Indolin-6-yl Ethyl(methyl)carbamate Hydrochloride (114). 114 was synthesized from **13** or from **108** after dissolving the indoline in EtOAc and extracted with 1N HCl. Separation and evaporation of the aqueous phase gave the product as a yellow oil in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.51 (d, J = 8.2 Hz, 1H), 7.33 (d, J = 2.2 Hz, 1H), 7.24 (dd, J = 8.2, 2.2 Hz, 1H), 3.92 (t, J = 7.8 Hz, 2H), 3.52 + 3.41 (q, J = 7.8 Hz, 2H), 3.33 (t, J = 7.8 Hz, 2H), 3.11 + 2.99 (s, 3H), 1.26 + 1.19 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 155.70, 152.70, 137.58, 133.89, 127.26, 124.28, 114.84, 47.89, 45.30, 45.20, 34.64, 34.34, 29.71, 13.42, 12.58. MS (CI^+) m/z 220 ($M^{\bullet+}$, 92.36), 221 (MH^+, 1.89).

Methyl 3-(6-((Ethyl(methyl)carbamoyl)oxy)indolin-1-yl)-propanoate Hydrochloride (115). The hydrochloride salt **115** was obtained by addition of a solution of HCl (g)/ether to **109**, and the precipitate was crystallized from MeOH−ether as a white solid, mp 131−134 °C, isolated in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.47 (d, J = 8.4 Hz, 1H), 7.37 (s, 1H), 7.19 (bd, J = 8.4 Hz, 1H), 3.99 (t, J = 7.5 Hz, 2H), 3.81 (t, J = 7.0 Hz, 2H), 3.74 (s, 3H), 3.57−3.38 (m, 2H), 3.30 (t, J = 7.5 Hz, 2H) 3.11 + 2.99 (bs, 3H), 2.93 (t, J = 7 Hz, 2H), 1.31−1.15 (m, 3H). ^{3}C NMR (75 MHz, CD_3OD) ppm δ 172.37, 155.781, 152.88, 143.11, 133.00, 127.59, 124.07, 113.23, 56.19, 52.792, 52.663, 45.32, 45.22, 34.64, 34.33, 30.44, 28.59,13.42, 12.60; .MS (CI^+) m/z 306.167 ($M^{\bullet+}$,38.43), 307.167 (MH^+, 67.12), 233.149 ($M - C_3H_5O_2$, 39.42). HRMS calcd for $C_{16}H_{23}N_2O_4$ (MH^+, CI/CH_4) 307.1658, found 307.1669.

3-(2-Aminoethyl)indolin-4-yl Ethyl(methyl)carbamate Dihydrochloride (120). 120 was synthesized from **119** by procedure E, method II. The amine was converted to HCl salt after workup with 2N HCl and evaporation of the aqueous phase and isolated as an off-white solid in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD) ppm δ 7.54 (t, J = 8.1 Hz, 1H, H-6), 7.42 (d, J = 8.1 Hz, 1H), 7.30 + 7.28 (d, J = 8.1 Hz, 1H), 4.05−3.95 (m, 1H), 3.88−3.73 (m, 2H), 3.65−3.40 (m, 2H), 3.17 + 3.02 (s, 3H), 3.10−3.00 (m, 2H), 2.22 (bs, 1H), 2.02 (m, 1H), 1.30 + 1.22 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) ppm δ 153.25, 153.12, 148.36, 136.62, 130.03, 129.69, 123.70, 116.23, 49.71, 43.82, 43.75, 37.71, 37.65, 36.87, 33.09, 32.98, 30.14, 11.92, 10.93. MS (CI^+) m/z 263.163 ($M^{\bullet+}$, 1.52), 264.171 (MH^+, 22.59). HRMS calcd for $C_{14}H_{22}N_3O_2$ (MH^+, DCI^+/CH_4) 264.1712, found 264.1706.

Chemical and Metabolic Stability. *Chemical and Metabolic Stability Studies.* The chemical stability in solution was assessed for two carbamates (**87** and **98**) by means of high performance liquid chromatography. An X-Terra C18 column was used with MeCN/water as eluent and 0.1% formic acid modifier. Detection was by UV at 254 nm. For metabolic studies, compound **87** (0.1 mM final concentration) was incubated in 50 mM potassium phosphate buffer, pH 7.4, with human or mouse liver microsomes (1 mg/mL), in the presence or absence of NADPH regenerating system, freshly mixed solution A (26.1 mM $NADP^+$, 66 mM glucose-6-phosphate, 66 mM $MgCl_2$ in H_2O) and solution B (40 U/mL glucose-6-phosphate dehydrogenase in 5 mM sodium citrate) in a 400 μL reaction volume. Microsomes were added, and the reaction was terminated after a 0, 30, 60, and 90 min incubation at 37 °C by adding 600 μL of ice cold acetonitrile; samples were centrifuged at 14000 rpm for 5 min to remove insoluble material. The clear supernatant was evaporated to dryness in SpeedVac and reconstituted in 300 μL of 0.1% trifluoroacetic acid. The samples were centrifuged and filtered as appropriate before 50 μL aliquots were injected onto the LC system.

Biology. *AChE and BuChE Inhibition.* The enzyme sources were bovine erythrocytes for AChE and equine serum for BuChE (Sigma Israel). AChE was dissolved in phosphate buffer pH 8 containing 0.01% NaN_3, 1 mM ethylenediamintetraacetic acid (EDTA), and 0.5 mg/mL bovine serum albumin (BSA) and diluted to give stock solution of 8.8 U/mL. BuChE was dissolved in 0.1 M phosphate buffer, pH 8, containing 0.01% NaN_3 and 1 mM EDTA to give a stock solution with a final concentration of 10 U/mL. ChE inhibition by carbamates develops relatively slowly depending on the nature of the alkyl substituents.[3] Therefore, the stock solution of AChE was diluted in phosphate buffer containing 0.01% NaN_3, 1 mM EDTA, and 0.5 mg/mL BSA, pH 8, to give a final concentration of 4.4 U/mL and incubated at 37 °C with 6−10 concentrations of each compound. Samples of 10 μL were collected after 15, 30, 60, and 120 min and added to a mixture of acetylthiocholine iodide (ATC) 75 mM, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) 0.01 M, and phosphate buffer pH 7 at a ratio of 1:1:2 in 0.2 mL wells. The rate of development of the yellow color was measured by means of a multiscan microplate reader (Labsystems) at 414 nm according to the method of Ellman.[49] The percentage inhibition of AChE by each concentration was calculated by comparison with the activity of the enzyme in the absence of drug. The concentration of each drug at each time point that inhibited enzyme activity by 50% (IC_{50}) was calculated. For measurement of BuChE inhibition, experiments were performed like those for AChE, but butylthiocholine iodide 75 mM was substituted for ATC.

Antioxidant Activity. This activity was determined by the reduction in Luminol-dependent chemiluminescence (LDCL) as previously described.[50] This method uses two different cocktails. Cocktail A is comprised of Luminol (10 μM), GO (2.3 units), sodium selenite (2 mM), and Co^{2+} (10 μM) added to 800 μL of Hank's Balance Salt Solution (HBSS). In the presence of glucose, GO generates a constant flux of H_2O_2 and Co^{2+} catalyzes the generation of H_2O_2 and $OH^{\bullet}$. Cocktail A produces a burst of LDCL within 30−60 s and lasts for 7−10 min that was measured at 22−23 °C on Lumac 2500 M Biocounter (Landgraaf, The Netherlands). The LDCL was expressed as cpm. Cocktail B is composed of a mixture of Luminol (10 μM), sodium selenite (2 mM), Co^{2+} (10 μM), and Sin1, all added to 800 μL of HBBS. This cocktail generates a constant flux of light due mainly to the formation of ONOO.

*Conversion of Dihydrorhodamine **123** to Rhodamine **123**.* The probe dihydrorhodamine 123 (DHR) acts as a radical scavenger in solution. DHR is widely used to detect certain ROS ($OH^{\bullet}$, $OHOO^-$, and NO_2^-) and is poorly responsive to $O_2^{-\bullet}$, H_2O_2, or $NO^{\bullet}$.[30a,51] DHR, that is not fluorescent, is oxidized to rhodamine 123, which gave a high fluorescence at 535 nm when excited at 500 nm. The rate of appearance of fluorescence was measured on a Safire-Tecan plate reader (Männedorf, Austria). To each well was added 20 μL of tested compound and 180 μL from stock solution containing: 0.2 μM Fe^{3+}−

NTA (1:3) (mol:mol), 4.4 mM ascorbic acid, and 5.5 mM DHR all diluted in HBSS. Ascorbic acid was used as a reducing agent of Fe^{2+} that was created in this reaction in order to renew the supply of Fe^{3+}. Immediately following the addition of the reagent, the kinetics of fluorescence development was followed at 37 °C for 40 min with readings taken every 2 min. The slopes of DHR fluorescence intensity as a function of time were calculated from measurements taken between 24 and 40 min.

2,2-Diphenyl-picrylhydrazyl Radical (DPPH) Assay. This assay measures hydrogen atom (or one electron) donating activity and hence provides an evaluation of antioxidant activity due to free radical scavenging. DPPH, a purple-colored stable free radical, was reduced to a yellow-colored diphenylpicryl hydrazine by antioxidants. The spectrophotometric assay was carried out as described by Koren et al.,[52] then 10 μL of a sample solution containing 5 or 10 μM of the tested compound were added to 20 μL of DPPH (from 10 mM stock), and 1.6 mL of absolute MeOH solution were then added. Following 30 s of incubation at room temperature and protection from light, the absorbance at 517 nm was determined using a Cecil CE 1011 spectrophotometer (Cecil, London, UK).

Cardiomyocytes Cell Culture. H9c2 cells were cultured in Dulbecco's Modified Eagle's Medium 4500 mg/L D-glucose with 10% fetal calf serum (FCS), 10000 U/mL penicillin, 100 mg/mL streptomycin, and 25 μg/mL amphotericin B at 37 °C with 95% air and 5% CO_2. One day before the experiment, cells were seeded in 96 well-plates at a density of 5×10^3 cells/well and incubated with 100 μM H_2O_2 for 1 h. Cells were incubated with the compounds at concentrations ranging from 1 pM to 100 μM for 1 h prior to addition of H_2O_2. Cell viability was measured by means of Alamar Blue (AbD Serotec, BUF012A). To be able to compare the protective effect of the compounds tested at different times, the degree of cell death (30–50%) was normalized to 100% in each experiment. The effect of the drugs was expressed as percent reduction of this value.

Prevention of Fall in Mitochondrial Potential. To determine whether the drugs reduced cytotoxicity induced by oxidative stress in cardiomyocytes by preventing the fall in the mitochondrial membrane potential (ΔΨm), the sensitive fluorophore 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide (JC-1) was used. JC-1 has the unique property of developing large shifts in its fluorescence signal from low to high levels of ΔΨm characteristic of energized mitochondria. The monomer emitted a green fluorescence (535 nm) in the cytosol. In the presence of oxidative stress, red fluorescent (595 nm) J-aggregates of the molecule were formed in the mitochondrial matrix. The reciprocal behavior at the two emission wavelengths, which can be measured simultaneously, resulted in large changes in the 595/535 nm ratio as the ΔΨm falls.[31] Drugs that prevented the fall in ΔΨm lower this ratio. The cardiomyocytes were plated in DMEM in 12-well plates placed overnight in an incubator (37 °C, 5% CO_2). A concentration of 100 nM of compound to be tested was added and left in contact with the cells for 1 h. The cells were then washed and exposed to 50 μM H_2O_2 for 1 h and the cells washed again. JC-1 in HBSS buffer was added to give a final concentration of 2 μM and the cells kept for 30 min at 37 °C. The cells were washed twice with HBSS buffer and fluorescence measured by means of a Nikon TE2000 fluorescent microscope. The images were taken by cooled CCD cameras (PCO sensicam, Hamamatsu orca ER), with a fluorescein long pass filter (appropriate for DHR). After a baseline reading was taken (for 15 min), 50 μM H_2O_2 was added and the changes measured every 5–10 min for 1 h.

Cultured Chick Telencephalon Neurons. Chick embryos aged 8–9 days were transferred to a plastic dish, and decapitated. Both hemispheres were removed, collected, and cleaned from any loose tissue. Hemispheres were mechanically dissociated and 2 mL of the resulting cell suspension, containing 9×10^5 cells/mL medium, were added to each well. For assessment of protection against reduced neurotrophic support (low serum), the cells were grown in Eagle's Minimum Essential Medium with 1 g of glucose/L, 2% FCS, 0.01% gentamycin, and 2 mM L-glutamine. Different concentrations of the drugs were applied when the neurons were plated out on day one (division, DIV1). At DIV8, viability of neurons was analyzed by the 3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazole-3-ium bromide (MTT) assay. We chose to use a different method to assess the action of the drugs against oxidative stress because this was easier to use than Alamar blue in primary neuronal cells. MTT measures the activity of mitochondrial dehydrogenase in viable cells and is based on the reduction of yellow MTT to dark-blue formazan crystals by mitochondrial dehydrogenases. For determination of cell viability, MTT solution was added to each well to a final concentration of 0.5 mg/mL. After 2 h, the MTT containing medium was aspired. Cells were lysed with 3% SDS, and formazan crystals were dissolved in 2-propanol/HCl. To determine optical density, a plate-reader (Anthos HT II) was used at a wavelength of 570 nm. Cell viability was expressed in optical density units, and the protective effect of the compounds expressed as % increased in viability as described for cardiomyocytes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for starting materials and intermediates which were not tested biologically. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖Equal contribution. The synthetic procedures described were taken from the Ph.D. dissertation of I.Y. "Indole Derivatives as Neuroprotective Agents", Bar Ilan University, 2012. The biological evaluations described were taken from the Ph.D. dissertation of E. F.-G. "Novel multifunctional drugs for the treatment of neurodegenerative diseases", The Hebrew University, 2012.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACh, acetylcholine; ATC, acetylthiocholine iodide; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; BSA, bovine serum albumin; ChE, cholinesterase; DHR, dihydrorhodamine 123; DIV, division; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; GO, glucose oxidase; HBSS, Hank's Balance Salt Solution; IPA, indole-3-propionic acid; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide; LDCL, Luminol-dependent chemiluminescence; ΔΨ*m*, mitochondrial membrane potential; MTT, 3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazole-3-ium bromide; NO, nitric oxide; OD, optical density; RNS, reactive nitrogen species; ROS, reactive oxygen species; Sin1, morpholino sydononimine

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