Journal of Medicinal Chemistry

Design, Synthesis, and Biological Evaluation of *Erythrina* Alkaloid Analogues as Neuronal Nicotinic Acetylcholine Receptor Antagonists

François Crestey,^{†,§} Anders A. Jensen,^{†,||} Morten Borch,[†] Jesper Tobias Andreasen,[†] Jacob Andersen,[†] Thomas Balle,[‡] and Jesper Langgaard Kristensen^{*,†}

[†]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

[‡]Faculty of Pharmacy, University of Sydney, Sydney, New South Wales 2006, Australia

Supporting Information



ABSTRACT: The synthesis of a new series of *Erythrina* alkaloid analogues and their pharmacological characterization at various nicotine acetylcholine receptor (nAChR) subtypes are described. The compounds were designed to be simplified analogues of aromatic erythrinanes with the aim of obtaining subtype-selective antagonists for the nAChRs and thereby probe the potential of using these natural products as scaffolds for further ligand optimization. The most selective and potent nAChR ligand to come from the series, 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (3c) (also a natural product by the name of *O*-methylcorypalline), displayed submicromolar binding affinity toward the $\alpha 4\beta 2$ nAChR with more than 300-fold selectivity over $\alpha 4\beta 4$, $\alpha 3\beta 4$, and $\alpha 7$. Furthermore, this lead structure (which also has inhibitory activity at monoamine oxidases A and B and at the serotonin and norepinephrine transporters) showed antidepressant-like effect in the mouse forced swim test at 30 mg/kg.

INTRODUCTION

The neuronal nicotinic acetylcholine receptors (nAChRs) have been proposed to be involved in a number of different disease states of the central nervous system (CNS), including depression, schizophrenia, attention deficit hyperactivity disorder, Alzheimer's and Parkinson's diseases, substance abuse, and pain, and this has made the receptors popular targets in drug discovery.¹ A common feature of the vast majority of nAChR compounds developed to date is that they are either agonists or positive allosteric modulators of the receptors.² In contrast, much less attention has been directed toward the development of antagonists targeting the neuronal nAChRs.³ Most of the available selective nAChR antagonists are natural products, and many of them, e.g., D-tubocurarine, methyllycaconitine (MLA), and α -conotoxins, are not optimal as lead compounds for CNS drug discovery due to their large sizes and unfavorable physicochemical properties. A notable exception is dihydro- β -erythroidine (DH β E). DH β E is a member of the Ervthrina alkaloid family characterized by their unique tetracyclic spiroamine framework (Figure 1).4 Erythrinanes were first isolated from Erythrina species at the end of the 19th century. Extracts from these plants were found to have curare-like neuromuscular blocking effects, and systematic phytochemical examination of the genus Erythrina has identified more than 100 erythrinanes that can be subdivided into several classes. Of these, the most abundant classes are the aromatic erythrinanes, including, e.g., erysotrine,

erysopine, erysodine, erysovine, and erythraline (Figure 1). The activities of some of these compounds, erysotrine, erysopine, erysodine, and also DH β E, have been investigated at the nAChRs.⁵ Erysodine was found to be the most potent competitive antagonist of the four at the $\alpha 4\beta 2$ nAChR, displaying a binding affinity of 50 nM in a [³H]cytisine binding assay and a K_i value of 7.5 μ M at the α 7 nAChR.^{6,7} Moreover, erysodine has also been compared with DH β E in vivo where it prevented both the early developing decrease and the late-developing increase in locomotor activity produced by (*S*)-nicotine in rats, and it produced a greater separation between the effective dose and the dose where toxic motor effects were observed.⁸ The latter observation can presumably be ascribed to erysodine having a greater selectivity for neuronal nAChR over the muscle-type receptor than DH β E.

The potent antagonism exerted by erysodine together with its ability to enter the brain after systemic administration makes this natural product an interesting pharmacological tool for studies of the nAChRs in vivo. The limited availability of erysodine and analogues from natural sources precludes the widespread applications of these ligands, but at the same time this makes them very interesting lead structures in the pursuit of simpler and more accessible analogues. Whereas previous "deconstruction" of other natural product like MLA has

Received: September 4, 2013 Published: November 4, 2013



Figure 1. The erythrinane scaffold, DH β E, and structure of aromatic erythrinanes.



Figure 2. Aromatic erythrinanes and new ligands in which the structural complexity is gradually reduced.





^{*a*}Reagents and conditions: (i) benzyl bromide (1.2 equiv), K_2CO_3 (1.5 equiv), EtOH, 70 °C, 97–98%; (ii) isopropyl bromide (1.2 equiv), K_2CO_3 (1.5 equiv), EtOH, 70 °C, 56–70%; (iii) NO₂Me (3.6 equiv), NH₄OAc (3 equiv), AcOH, 120 °C, 49–81%; (iv) LiAlH₄ (5 equiv), dry THF, 0 °C to reflux conditions, 84–99%.

Scheme 2. Synthesis of *cis*-Erythrinane Derivatives $1a-g^{a}$



"Reagents and conditions: (i) method A; (a) AlMe₃ (2 equiv), ethyl 2-cyclohexanoneacetate (1.05 equiv), Sc(OTf)₃ (12.5 mol %), CH₃CN, 0 °C to rt, (b) TfOH (3.5 equiv), 0 °C to rt; method B, (a) ethyl 2-cyclohexanoneacetate (1.05 equiv), PhMe, reflux conditions, (b) aq HI (57%), rt. (ii) LiAlH₄ (2.5 equiv), dry THF, 0 °C to rt, 20–56% over two steps. (iii) concd H_2SO_4 (8.8 equiv), AcOH, reflux conditions, 79–80%. (iv) BBr₃ (3 equiv), DCM, 0 °C to rt, 64%.

provided some interesting small molecule nAChR ligands,⁹ reports on structure–activity relationships of the *Erythrina* alkaloids on the nAChRs are very scarce.¹⁰

In the search for new competitive nAChR antagonists, we embarked on the synthesis of ligands in which the structure of the aromatic erythrinanes is simplified step by step in order to identify the structural elements in the molecules essential for their nAChR activity. Bermudez et al. have investigated the binding mode of several erythrinanes to the nAChR using a combination of mutational studies and molecular modeling, and shown that the substitution pattern on the catecol unit has a profound effect on the affinity of the different alkaloids.⁶ Thus, we decided to focus our study on two parameters: number of rings in the molecules and substitution pattern on the catecol unit. Ease of synthesis was given priority so that the ligands could be accessed rapidly. First, the methoxy group on the Aring was omitted and the double bonds in the A- and B-ring were saturated, giving the first series of analogues, then the A-





^{*a*}Reagents and conditions: (i) γ -butyrolactone (1.1 equiv), PhMe, reflux conditions, 46–72%; (ii) (a) POCl₃ (10 equiv), PhMe, reflux conditions, (b) NaBH₄ (2 equiv), EtOH–AcOH (10:1), 0 °C to rt, 42–69%; (iii) H₂, Pd/C (10 mol %), MeOH, rt, 72–75%; (iv) BBr₃ (3 equiv), DCM, 0 °C to rt, 83%.

and B-rings were deleted sequentially, giving the final two series of analogues, see Figure 2. We included all possible permutations of H, CH_3 , and $-CH_2$ - on the catechol unit that is also present in the natural products, thus giving 15 ligands in the initial set of compounds. Following the pharmacological characterization of these ligands, we synthesized a further six ligands based on the most promising scaffold. However, none of these ligands showed an improved profile. Given the evidence for a role of nAChRs in depression and preclinical studies demonstrating antidepressant-like action of nAChR antagonists in rodents, the present study was finalized by evaluating the pharmacological properties of the initial lead compound at monoamine transporters and investigate its possible antidepressant activity in the mouse forced swim test.

CHEMISTRY

First, the synthesis of the required substituted phenethylamines 4a-f, common intermediates for the preparation of all the designed ligands, is outlined in Scheme 1. Benzyl protection of the hydroxyl group of commercially available isovanillin 5a and vanillin 5b furnished aldehydes 6a and 6b in 98% and 97% yields, respectively. The same conditions were applied for the isopropyl protection reaction, giving aldehydes 6c and 6d in good yields. The nitrostyrenes 7a-f were obtained from the condensation of the corresponding protected aldehydes 6a-f with nitromethane in 49-81% yields, and a subsequent reduction with LiAlH₄ yielded amines 4a-f.¹¹

The syntheses of the cis-erythrinanes 1a-d have been performed via two different pathways as shown in Scheme 2.^{12,13} Following a modified procedure (method A) recently published by Tietze and co-workers,¹⁴ the spirocyclic core of the Erythrina alkaloids was formed by a Lewis acid mediated domino reaction of phenethylamines 4c and 4f with ethyl 2cyclohexanoneacetate, forming successively three new bonds in one process. The resulting crude intermediates were reduced with an excess of LiAlH₄, providing erythrinane derivatives 1a and 1d in 20% and 56% yields over two steps, respectively. Tetracyclic aza-spiro analogues 1b and 1c were obtained from phenethylamines 4d and 4e in moderate yields by applying the procedure (method B) described by Isobe and co-workers,¹⁵ followed by the subsequent reduction with LiAlH₄ of the crude products. The deisopropylation of compounds 1a and 1b were performed in AcOH/ H_2 SO₄ at reflux,¹⁶ leading to the desired cis-erythrinane derivatives 1e and 1f. The treatment of 1c with 3 equivalents of BBr₃ in dichloromethane afforded the dihydroxy analogue 1g as a hydrobromide in 64% yield.

The racemic pyrrolo[2,1-*a*]isoquinoline derivatives 2a-g were prepared in a two-step protocol described by Bailey and co-workers as depicted in Scheme 3.¹⁷ Phenethylamines 4a-b and 4e-f reacted with γ -butyrolactone, providing amides 8a-d which cyclized upon treatment with POCl₃. Subsequent reduction of the intermediate iminium salts with NaBH₄ gave isoquinoline substrates 2a-d with 42-69% yields. Debenzylation of 2a and 2b using H₂/Pd(c) in methanol gave tricyclic analogues 2e and 2f in 72% and 75% yields, respectively. Compound 2g was obtained from derivative 2c as a hydrobromide by following the same procedure as previously described above.

The *N*-methyl-1,2,3,4-tetrahydroisoquinolines 3a-d were obtained in a single-step procedure by performing a Pictet–Spengler reaction with the appropriate phenethylamines 4 using 5 equiv of formaldehyde in formic acid.¹⁸ The subsequent debenzylation using the same conditions as described above furnished 3e and 3f in 83% and 89% yields, respectively. The treatment of dimethoxyisoquinoline 3c with BBr₃ afforded dihydroxyisoquinoline 3g as its hydrobromic salt in 69% yield (Scheme 4).





^aReagents and conditions: (i) HCOH 37% aq (5 equiv), HCO₂H, 100 °C, 58–99%; (ii) H₂, Pd/C (10 mol %), MeOH, rt, 83–89%; (iii) BBr₃ (5 equiv), DCM, 0 °C to rt, 69%.

Subsequent to the pharmacological characterization of the 15 ligands detailed above, six analogues of 3c were synthesized (9a-f) in which substituents were introduced at the 1-position of the tetrahydroisoquinoline ring system. Indeed, treatment of *N*-monomethylated amine 10 with the appropriate acyl chlorides provided the corresponding amides which underwent a Bischler–Napieralski cyclodehydration in the presence of POCl₃. Subsequent reduction of the iminium salts with NaBH₄ led to 9a-f in 78–88% yield over three steps (Scheme 5).

Scheme 5. Synthesis of 1-Substituted Tetrahydroisoquinolines $9a-f^a$



^{*a*}Reagents and conditions: (i) (a) $(Boc)_2O$ (1.1 equiv), DCM, rt, (b) LiAlH₄ (1.5 equiv), dry THF, 0 °C to reflux conditions, 83% over 2 steps; (ii) (a) RCOCl (1.1 equiv), TEA (1.1 equiv), dry DCM, 0 °C to rt, (b) POCl₃ (10 equiv), dry PhMe, reflux conditions, (c) NaBH₄ (5 equiv), MeOH, 0 °C to rt, 78–88% over 3 steps.

RESULTS AND DISCUSSION

The ligands were characterized in a [³H]epibatidine binding assay using membranes from HEK293 cells stably expressing

the rat heteromeric nAChR subtypes $\alpha 4\beta 2$, $\alpha 3\beta 4$, and $\alpha 4\beta 4$ and in a [³H]MLA binding assay using membranes from tsA201 transiently expressing the $\alpha 7/5$ -HT_{3A} chimera (composed of the amino-terminal domain of the rat $\alpha 7$ nAChR subunit and the ion channel domain of the murine 5-HT_{3A} receptor subunit), essentially as previously described.¹⁹ The functional characteristics of the analogues at a HEK293T cell line stably expressing the mouse $\alpha 4\beta 2$ nAChR and at a HEK293 cell line stably expressing the rat $\alpha 3\beta 4$ nAChR in the FLIPR Membrane Potential Blue assay was determined as previously described.²⁰ (S)-Nicotine (EC₇₀-EC₈₀ for the respective receptors, i.e., 1 μ M for $\alpha 4\beta 2$ and 3 μ M for $\alpha 3\beta 4$) was used as agonist in the antagonist experiments.

All 15 compounds in the series displaced [³H]epibatidine binding from the rat $\alpha 4\beta 2$ nAChR in a concentrationdependent manner, exhibiting K_i values at the receptor ranging from 0.55 to ~100 μ M. In contrast, the vast majority of the compounds exhibited negligible binding affinity to the rat $\alpha 4\beta 4$ and $\alpha 3\beta 4$ nAChRs and to the $\alpha 7/5$ -HT_{3A} chimera (Table 1). Overall, this SAR was also reflected in the inhibitory potencies



	E B N	A	R'			R"	<u>~</u> 0_	
	Ļ		R L					
		1c-g	2c-g		3c-g	9a-f	-	
				bindi	ng K_i (μ M)		functional IC ₅₀ (μ M)	
compd	R	R′	α4β2	α4β4	$\alpha 3\beta 4$	$\alpha 7/5$ -HT _{3A}	α4β2	$\alpha 3\beta 4$
1c	CH_3	CH ₃	~100	~100	~100	~30	31	~50 ^b
1d	$-CH_2-$		7.3	~50	~30	18	9.2	$\sim 50^{b}$
1e	Н	CH ₃	8.5	~50	~100	20	2.3	$\sim 50^{b}$
1f	CH ₃	Н	18	23	~30	5.5	2.9	33
1g	Н	Н	1.5	19	~30	7.3	0.58	$\sim 50^{b}$
2c	CH ₃	CH ₃	5.5	~ 100	~300	>500	31	$\sim 100^{b}$
2d	$-CH_2-$		0.55	21	~100	~100	3.3	$\sim 100^{b}$
2e	Н	CH ₃	~100	~ 100	~300	~500	$\sim 50^{b}$	$\sim 300^{b}$
2f	CH ₃	Н	3.4	~ 100	~100	~300	23	~300 ^b
2g	Н	Н	11	~50	~100	~300	$\sim 100^{b}$	$\sim 1000^{b}$
3c	CH ₃	CH ₃	0.87	~300	~300	>500	12	$\sim 100^{b}$
3d	$-CH_2-$		1.7	~50	~300	~500	2.6	$\sim 300^{b}$
3e	Н	CH ₃	10	~ 100	~300	>500	33	$\sim 1000^{b}$
3f	CH ₃	Н	1.7	~ 100	~300	>500	4.4	>1000
3g	Н	Н	6.8	~ 100	~100	>500	16	>1000
			binding K_i (μM)				functional IC ₅₀ (µM)	
	R″		α4β2	$\alpha 4\beta 4$	$\alpha 3\beta 4$	$\alpha 7/5$ -HT _{3A}	α4β2	$\alpha 3\beta 4$
9a	ethyl		~300				>300	
9b	<i>n</i> -butyl		~30				>300	
9c	cyclohexyl		~300				>300	
9d	phenyl		~100				>300	
9e	3-methoxyphenyl		~300				>300	
9f	3,4-dimethoxy- phenyl		>300				>300	

^{*a*} The K_i values for the compounds at the rat $\alpha 4\beta 2$, $\alpha 4\beta 4$, and $\alpha 3\beta 4$ nAChR subtypes were obtained in a [³H]epibatidine binding assay and the K_i values for the compounds at the $\alpha 7/5$ -HT_{3A} chimera were determined in a [³H]MLA binding assay. The functional IC₅₀ values for the compounds at the mouse $\alpha 4\beta 2$ nAChR and the rat $\alpha 3\beta 4$ nAChR were obtained in the FLIPR Membrane Potential Blue assay. (*S*)-Nicotine (EC₇₀-EC₈₀ for the respective receptors, i.e., 1 μ M for $\alpha 4\beta 2$ and 3 μ M for $\alpha 3\beta 4$) was used as agonist in the functional experiments. The data were the means of 3–5 individual experiments performed in duplicate. The complete pharmacological data (including SEM values) are given in the Supporting Information. ^bBecause a complete concentration–inhibition curve could not be obtained for the compound in the concentration ranges tested, the IC₅₀ value given is an estimate.



Figure 3. Correlation between binding and functional data for compound series 1-3 at the $\alpha 4\beta 2$ nAChR. In (A), data from all three series are shown together, whereas (B–D) show them individually.

on mouse $\alpha 4\beta 2$ nAChR and rat $\alpha 3\beta 4$ signaling in the FMP Blue assay (Table 1). The overall correlation between binding affinities and functional inhibitory potencies of the 15 compounds at the $\alpha 4\beta 2$ nAChR was not impressive (Figure 3A). Interestingly, considerably more pronounced correlations were observed for each of the three compound classes separately, in particular when the data for **2e** and **3c** (which constituted outliers in the **2c**-**g** and **3c**-**g** series) were omitted from the comparisons, giving R^2 values of 0.99 and 0.97, respectively (Figure 3B,C,D).

In terms of structure—activity relationships, compounds with all four rings intact (1c-g) retain some affinity for α 7, but this is eliminated as the size of the ligands is reduced, as exemplified by the direct analogues of erysodine (ligands 1e-3e): With all four rings intact, the affinity at α 7 of compound 1e of $20 \ \mu$ M is in the same range as the 7.5 μ M reported for erysodine.⁶ In contrast, the binding affinity for erysodine at α 4 β 2 drops from 50 nM to 8.5 μ M for 1e (Table 1). Deleting the A-ring gives 2e with negligible affinity for α 7 and α 4 β 2 (~500 and ~100 μ M, respectively). Removal of the B-ring in 1e (3e) restores affinity for α 4 β 2 to the same level as for 1e.

In general, no clear picture appeared with respect to the effect of the substitution pattern on the catechol unit when it comes to affinity for $\alpha 4\beta 2$. Introduction of a single methoxy group in the R' position yields some of the least active analogues in all three compound series (1e-3e), most pronounced in the virtually inactive 2e analogue. Generally, the dioxolane unit performed well for all three compounds in the series (1d-3d). Compound 2d has the highest affinity for $\alpha 4\beta 2$ of all compounds in this study (0.55 μ M), but this compound also exhibits affinity for $\alpha 4\beta 4$.

The three dimethoxy analogues comprise one of the most potent antagonists in the entire series (3c) where affinity for

 $\alpha 4\beta 2$ is in the nM range with greater than 300-fold selectivity toward the other subtypes tested in this study as well as a virtually inactive analogue (1c). Compound 1c has the lowest affinity for all four subtypes of all compounds with the A- and B-rings intact, but as the A ring is removed to give 2c, affinity for $\alpha 4\beta 2$ increases to 5.5 μ M and deletion of the B-ring gives 3c, which is quite potent and the most selective compound in the series. In comparison, the parent alkaloid erysotrine has been reported to have a binding affinity at $\alpha 4\beta 2$ of 0.6 μ M.⁶ Thus, 3c is an interesting new lead compound with low molecular weight that allows for further optimization.

To probe the potential in 3c, the 1-position of the tetrahydroisoquinoline ring system where the A- and B-rings are attached in the *Erythrina* alkaloids was modified by introducing different substituents: three ligands with an alkyl side chain (9a-c) and three other derivatives bearing an aromatic moiety (9d-f), see Scheme 5. Unfortunately, substitution at this position on 3c was detrimental to activity at $\alpha 4\beta 2$ as shown in Table 1. With the exception of 9b and 9d, the ligands were virtually devoid of affinity for $\alpha 4\beta 2$. This is somewhat perplexing but could be taken as an indication of a different binding mode of 3c to the nAChR than that of the parent alkaloid.

A common feature of all ligands tested in this study was the deletion of the methoxy group on the A ring. This decision was supported by a previously published computational and mutational study on the binding mode of the erythrinanes.⁶ Therein it was concluded that the tertiary nitrogen and the catecol unit are key interaction points for the erythrinanes: both of these structural elements are retained in the present compound series. As mentioned above, no clear picture appeared with respect to the substitution pattern on the catecol unit when it comes to affinity for $\alpha 4\beta 2$. This can be

interpreted in a number of different ways. Assuming that the tetracyclic derivatives 1c-g adopts a similar conformation as the natural products, the deletion of the double bonds and removal of the methoxy group on the A-ring should not be detrimental to the affinity for $\alpha 4\beta 2$, but we see a dramatic decrease. Thus, these structural motifs clearly play an important role in the interaction of erythrinanes with the nAChRs. The three and two ring analogues (2c-g and 3c-g) may adopt a different mode of binding due to the smaller size of the ligands, thereby making a direct comparison with the parent natural products difficult.

Furthermore, we recently obtained an X-ray structure of the acetylcholine binding protein (AChBP) in complex with DH β E, with a different binding mode.²¹ This structure suggests that the methoxy group on the A-ring of DH β E plays a crucial role due to its interaction with a tightly bound water molecule in the protein. With the absence of the methoxy substituent on the A-ring, this interaction is not possible and may account for the reduced affinity of the investigated compounds relative to the parent natural products. It is therefore likely that the compounds presented here adopt a binding mode different to that observed for DH β E in the X-ray structure, and this would explain the lack of affinity of the series **9** compounds.

A link between depression and nicotinic cholinergic neurotransmission is evident from numerous clinical and preclinical studies.²² Antidepressant effects in humans and antidepressant-like effects in rodents have been reported with both agonism and antagonism of nAChRs.²³ Similarly, enhanced antidepressant-like action of conventional antidepressants in rodents has been shown with both nAChR agonists^{24,25} and antagonists.^{26,27} Given the antidepressant effect of the known nAChR antagonist DH β E in vivo,^{26,28} we were intrigued by the possibility of **3c** having similar antidepressant activity.

An interesting feature of compound 3c is that the structural motive of dopamine is imbedded in the C and D rings, see Figure 4. This prompted us to investigate the activity of 3c at



Figure 4. (A) Structure of 3c and dopamine showing that 3c is a restricted analogue of dopamine. (B) Dose–response curves from representative experiments of inhibition by 3c of $[{}^{3}H]$ neurotransmitter uptake in COS7 cells expressing human SERT, NET, or DAT. Data points represent the mean ± SEM from triplicate determinations. The inhibitory potency of 3c was determined from 4–6 independent experiments, each performed in triplicate (mean ± SEM; in μ M): hSERT 46.7 ± 7.1, hNET, 128.9 ± 15.0 hDAT >10000).

some classical targets for antidepressants, the plasma membrane transporters for the monoamine neurotransmitters serotonin, norepinephrine, and dopamine (SERT, NET, and DAT, respectively).²⁹ The inhibitory potency of ligand **3c** was determined at SERT, NET, and DAT in in vitro [³H]-neurotransmitter uptake inhibition assays, and we found **3c** to

have K_i values of 47 and 130 μ M on SERT and NET, respectively (see Figure 4). Surprisingly, no inhibition of DAT could be detected ($K_i > 10$ mM). Thus, the inhibitory potency of **3c** is much lower at SERT and NET compared to prototypical antidepressant drugs such as the dual acting SERT/NET reuptake inhibitors duloxetine and venlafaxine, which have K_i values for SERT and NET in the nM range.³⁰

Tetrahydroisoquinoline 3c has been known in the chemical literature for many years and was also isolated from natural sources in 1986 and named *O*-methylcorypalline.³¹ It is reported to be a weak inhibitor of monoamine oxidase A and B: $K_i = 27$ and 29 μ M, respectively.³² Because inhibitors of monoamine oxidase have proven efficacy in the treatment of depression,³³ 3c has an interesting pharmacological profile for antidepressant activity.

Thus, we decided to investigate the antidepressant-like effect of 3c in vivo using the mouse forced swim test.³⁴ In this behavioral test, a mouse is placed in an inescapable tank filled with water and the distance traveled by the animal is measured. An increase in swimming distance is interpreted as an antidepressant effect of the tested compound. We examined the effect of 3c at 10 and 30 mg/kg and found that it exhibited a significant antidepressant-like effect at 30 mg/kg, see Figure 5.



Figure 5. Results from mouse forced swim test of 3c.

The relatively high dose needed to evoke the antidepressantlike effect raises a question of whether this is mediated solely via nAChRs or via a combination of one or more of the different targets mentioned above. The 30 mg/kg dose showing behavioral activity is equivalent to approximately 10-fold lower DH β E doses (3 mg/kg). Indeed, this dose of DH β E has been demonstrated several times to produce antidepressant-like effects in mice.²⁸ Thus, we speculate that the effect is at least in part mediated via the nAChRs, but this will have to be investigated further before any definite conclusions can be made on the mechanism of action.

CONCLUSIONS

The present study describes the design, synthesis, and biological evaluation of analogues of the tetracyclic *Erythrina* alkaloids as nAChR antagonists. Reducing the complexity of the natural products via the sequential deletion of rings gave an initial series of 15 ligands. Compound **3c** (a previously reported natural product) emerged as a subtype selective competitive antagonist of the $\alpha 4\beta 2$ nAChR with antidepressant effect in vivo. Initial attempts to investigate the potential this scaffold via homologation at the 1-position of the tetrahydroisoquinoline ring systems were detrimental to the affinity toward $\alpha 4\beta 2$, but **3c** remains an interesting and readily manipulated lead structure.

EXPERIMENTAL SECTION

1.1. Medicinal Chemistry: Material and Methods. Starting materials and reagents were obtained from commercial suppliers and used without further purifications. Syringes which were used to transfer anhydrous solvents or reagents were purged with nitrogen prior to use. THF was continuously refluxed and freshly distilled from sodium/benzophenone under nitrogen. Other solvents were analytical or HPLC grade and were used as received. Yields refer to isolated compounds estimated to be >95% pure as determined by ¹H NMR and LC-MS. Thin-layer chromatography (TLC) was carried out on silica gel 60 F₂₅₄ plates from Merck (Germany). Visualization was accomplished by UV lamp (254 nm) or with either ninhydrine dip with heat or iodine on silica as an indicator. Flash column chromatography was performed on chromatography grade, silica 60 Å particle size 35–70 μ m from Fisher Scientific using the solvent system as stated. ¹H NMR and ¹³C NMR spectra were recorded on Varian 300 (Mercury and Gemini) instruments, using CDCl₃ or DMSO- d_6 as solvents and TMS as internal standard. Coupling constants (J values) are given in hertz (Hz). Multiplicities of ¹H NMR signals are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; t, triplet; q, quartet; sept, septet; multiplet; br, broad signal. Melting points (mp) were measured using a MPA100 Optimelt melting point apparatus and are uncorrected. Elemental analyses were performed at the Department of Physical Chemistry, University of Vienna, Austria. High-resolution mass spectra (HRMS) were obtained using a Micromass Q-TOF 2 instrument. The following abbreviations are used: MeOH, methanol; DCM, dichloromethane; THF, tetrahydrofuran; EtOAc, ethyl acetate; AcOH, acetic acid; TEA, triethylamine.

1.2. Typical Procedures and Analytical Data of Representative Synthesized Compounds. *Typical Procedure 1: Synthesis of Tetrahydroisoquinolines* **3.** To a solution of phenethylamine **4** (1 equiv) in formic acid (7–13 mL) was added a 37% aqueous solution of formaldehyde (5 equiv) at room temperature. The mixture was heated at 100 °C for 2 h then cooled to 0 °C and made alkaline by addition of an aqueous solution of NaOH (2 M). The aqueous layer was extracted twice with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel to provide pure tetrahydroisoquinoline **3**.

Typical Procedure 2: Synthesis of Indolizidines 2. To a solution of amide 8 (1 equiv) in toluene (20–150 mL) was added POCl₃ (10 equiv) at room temperature. The mixture was heated under reflux conditions for 4.5 h, then solvents were removed in vacuo. To the crude material dissolved in a mixture EtOH–AcOH (10:1, 11–66 mL) was added NaBH₄ (2 equiv) portionwise at 0 °C. The mixture was allowed to warm up to room temperature and stirred at this temperature for 16 h. After addition of water, volatiles were removed and the aqueous layer was extracted three times with DCM. The combined organic layers were successively washed with an aqueous solution of NaOH (2 M) and brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The resulting crude was purified by column chromatography on silica gel to provide pure indolizidine 2.

Typical Procedure 3: Synthesis of cis-Erythrinanes 1a and 1d. To a solution of the appropriate phenethylamine 4 (1 equiv) in acetonitrile (3-4 mL) were added successively AlMe₃ (2 M in toluene, 2 equiv) dropwise, Sc(OTf)₃ (12.5 mol %), and ethyl 2cyclohexanoneacetate (1.05 equiv) at 0 °C. The mixture was allowed to warm up to room temperature and stirred at this temperature for 17 h. After cooling to 0 °C, TfOH (3.5 equiv) was added dropwise, then the reaction mixture was allowed to warm up to room temperature and stirred at this temperature for 2 h. After addition of a saturated aqueous solution of NaHCO3 at 0 °C, the mixture was extracted twice with EtOAc and the combined organic layers were washed with water, dried over Na2SO4, filtered, and concentrated in vacuo. The resulting crude was then dissolved in dry THF (5 mL) and slowly added to a suspension of LiAlH₄ (2.5 equiv) in dry THF (10 mL) at 0 °C. The mixture was allowed to warm up to room temperature and stirred at this temperature for 12 h then cooled to 0 °C. An aqueous solution of Rochelle salt (2 M) was carefully added and the reaction mixture

stirred at this temperature for 30 min. The solution was made alkaline by addition of an aqueous solution of NaOH (2 M), and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel to provide the pure corresponding *cis*-erythrinane **1**.

Typical Procedure 4: Synthesis of cis-Erythrinanes 1b and 1c. To a solution of the appropriate phenethylamine 4 (1 equiv) in toluene (10-35 mL) was added ethyl 2-cyclohexanoneacetate (1.05 equiv) at room temperature. The mixture was heated under reflux conditions for 12 h. After cooling to room temperature, an aqueous solution of HI (7.6 M) was added dropwise then the reaction mixture was stirred for 2 h. After addition of an aqueous solution of NaHSO₃ (2 M), the mixture was extracted twice with EtOAc and the combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated in vacuo. The resulting crude was then dissolved in dry THF (5 mL) and slowly added to a suspension of LiAlH₄ (2.5 equiv) in dry THF (10 mL) at 0 °C. The mixture was allowed to warm up to room temperature and stirred at this temperature for 12 h then cooled to 0 °C. An aqueous solution of Rochelle salt (2 M) was carefully added and the reaction mixture stirred at this temperature for 30 min. The solution was made alkaline by addition of an aqueous solution of NaOH (2 M), and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel to provide the pure corresponding ciserythrinane 1.

6,7-Dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (**3c**).^{18a} Starting from phenethylamine **4e** (1.00 g, 5.5 mmol), following typical procedure 1 and using EtOAc-MeOH-TEA (40:10:1) as eluent, tetrahydroisoquinoline **3c** was obtained as a pale-yellow solid (1.13 g, 99%); mp 69-71 °C; $R_f = 0.25$ (EtOAc-MeOH-TEA, 40:10:1). ¹H NMR (300 MHz, CDCl₃): δ 6.56 (s, 1H), 6.47 (s, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.47 (br s, 2H), 2.81 (t, J = 5.8, 2H), 2.63 (t, J = 5.8, 2H), 2.42 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 147.5, 147.2, 126.7, 125.8, 111.4, 109.4, 57.9, 56.2, 56.1, 53.3, 46.4, 29.2. Anal. Calcd For C₁₂H₁₇NO₂: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.54; H, 8.24; N, 6.41.

6,7-Methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (**3d**).³⁵ Starting from phenethylamine 4f (0.50 g, 3.0 mmol), following typical procedure 1 and using EtOAc–MeOH–TEA (40:10:1) as eluent, tetrahydroisoquinoline **3d** was obtained as an off-white solid (0.42 g, 72%); mp 53–55 °C; $R_f = 0.35$ (EtOAc–MeOH–TEA, 40:10:1). ¹H NMR (300 MHz, CDCl₃): δ 6.52 (s, 1H), 6.44 (s, 1H), 5.83 (s, 2H), 3.43 (s, 2H), 2.79 (t, J = 5.8, 2H), 2.60 (t, J = 5.8, 2H), 2.40 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 145.8, 145.5, 127.4, 126.6, 108.3, 106.2, 100.5, 58.0, 52.9, 46.1, 29.3. Anal. Calcd For C₁₁H₁₃NO₂: C, 69.09; H, 6.85; N, 7.32. Found: C, 68.89; H, 6.55; N, 7.09.

8,9-Dimethoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-a]isoquinoline (2c).¹⁷ Starting from amide 8c (1.52 g, 5.7 mmol), following typical procedure 2 and using a gradient elution (DCM– MeOH, 6:1 to 4:1), indolizidine 2c was obtained as a off-white solid (0.68 g, 52%); mp 87–89 °C; $R_f = 0.45$ (DCM–MeOH, 4:1). ¹H NMR (300 MHz, CDCl₃): δ 6.60 (s, 1H), 6.56 (s, 1H), 3.85 (s, 6H), 3.52 (br t, J = 8.2, 1H), 3.14–3.23 (m, 1H), 2.95–3.12 (m, 2H), 2.61– 2.81 (m, 3H), 2.29–2.41 (m, 1H), 1.67–2.03 (m, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 147.5, 147.4, 130.5, 126.1, 111.4, 108.9, 63.1, 56.3, 56.2, 53.5, 48.6, 31.0, 28.2, 22.6; Anal. Calcd For C₁₄H₁₉NO₂: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.41; H, 7.94; N, 5.80.

8,9-Methylenedioxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-a]isoquinoline (2d). Starting from amide 8d (0.64 g, 2.4 mmol), following typical procedure 2 and using DCM–MeOH (3:1) as eluent, indolizidine 2d was obtained as a pale-yellowish oil (0.37 g, 69%) which solidified when stored in the refrigerator; mp 62–64 °C; R_f = 0.4 (DCM–MeOH, 10:3). ¹H NMR (300 MHz, CDCl₃): δ 6.57 (s, 1H), 6.54 (s, 1H), 5.88 (s, 2H), 3.33 (br t, *J* = 8.8, 1H), 2.96–3.20 (m, 3H), 2.73 (br dt, *J* = 16.2 and *J* = 3.6, 1H), 2.47–2.64 (m, 2H), 2.23– 2.35 (m, 1H), 1.62–2.02 (m, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 145.9, 145.8, 132.1, 127.4, 108.6, 106.1, 100.8, 63.7, 53.6, 48.8, 30.8,

Journal of Medicinal Chemistry

29.1, 22.4. HRMS (m/z): $[M + H]^+$ calcd for $[C_{13}H_{16}NO_2]^+$ 218.1181; found 218.1179.

16-Isopropoxy-15-methoxy-cis-erythrinane (1a). Starting from phenethylamine 4c (0.35 g, 1.7 mmol), following typical procedure 3 and using DCM–MeOH–TEA (100:15:1) as eluent, *cis*-erythrinane 1a was obtained as a pale-yellow oil (0.1 g, 20%); $R_f = 0.55$ (DCM–MeOH–TEA, 50:5:1). ¹H NMR (300 MHz, CDCl₃): δ 6.67 (s, 1H), 6.49 (s, 1H), 4.45 (sept, J = 6.0, 1H), 3.82 (s, 3H), 2.97–3.23 (m, 4H), 2.84 (dt, J = 10.5 and J = 3.0, 1H), 2.19–2.33 (m, 2H), 1.87–1.98 (m, 1H), 1.34 (d, J = 6.1, 6H), 1.19–1.79 (m, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 148.5, 145.5, 136.1, 126.9, 115.2, 109.9, 71.3, 64.7, 56.5, 46.6, 43.9, 40.8, 36.0, 29.2, 28.9, 25.3, 22.51, 22.47, 21.7, 21.6. HRMS (m/z): [M + H]⁺ calcd for [C₂₀H₃₀NO₂]⁺ 316.2277; found 316.2273.

15-Isopropoxy-16-methoxy-cis-erythrinane (1b). Starting from phenethylamine 4d (0.35 g, 1.7 mmol), following typical procedure 4 and using DCM–MeOH–TEA (100:15:1) as eluent, *cis*-erythrinane 1b was obtained as a pale-yellow oil (0.2 g, 38%); $R_f = 0.55$ (DCM– MeOH–TEA, 50:5:1). ¹H NMR (300 MHz, CDCl₃): δ 6.74 (s, 1H), 6.50 (s, 1H), 4.46 (sept, J = 6.1, 1H), 3.81 (s, 3H), 3.01–3.24 (m, 4H), 2.85 (dt, J = 10.4 and J = 3.0, 1H), 2.16–2.36 (m, 2H), 1.88– 1.98 (m, 1H), 1.35 (dd, J = 6.1 and J = 2.2, 6H), 1.21–1.80 (m, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 148.8, 145.3, 136.2, 128.0, 115.1, 112.0, 72.2, 64.6, 56.1, 46.6, 44.0, 40.8, 36.1, 29.3, 29.0, 25.4, 22.54, 22.51, 21.9, 21.8.HRMS (m/z): [M + H]⁺ calcd for [C₂₀H₃₀NO₂]⁺ 316.2277; found 316.2261.

15,16-Dimethoxy-cis-erythrinane (1c).³⁶ Starting from phenethylamine 4e (1.36 g, 7.5 mmol), following typical procedure 4 and using a gradient elution (DCM–MeOH, 300:15 to DCM–MeOH–TEA, 300:15:1), *cis*-erythrinane 1c was obtained as a pale-orange oil (0.74 g, 34%); $R_f = 0.3$ (DCM–MeOH–TEA, 300:15:1). ¹H NMR (300 MHz, CDCl₃): δ 6.70 (s, 1H), 6.51 (s, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.03–3.22 (m, 4H), 2.87 (dt, J = 10.4 and J = 2.7, 1H), 2.19–2.33 (m, 2H), 1.89–2.03 (m, 1H), 1.23–1.82 (m, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 147.3, 147.1, 135.9, 126.9, 111.4, 110.0, 62.2, 56.4, 56.0, 46.6, 44.0, 40.7, 36.1, 29.4, 29.0, 25.5, 21.8, 21.7. HRMS (m/z): [M + H]⁺ calcd for [$C_{18}H_{26}NO_2$]⁺ 288.1964; found 288.1961. 15,16-Methylenedioxy-cis-erythrinane (1d).^{13c} Starting from

15,16-Methylenedioxy-cis-erythrinane (1d). Starting from phenethylamine 4f (0.25 g, 1.5 mmol), following typical procedure 3 and using DCM–MeOH–TEA (100:15:1) as eluent, *cis*-erythrinane 1d was obtained as a colorless oil (0.23 g, 56%); $R_f = 0.4$ (DCM–MeOH–TEA, 100:15:1). ¹H NMR (300 MHz, CDCl₃): δ 6.71 (s, 1H), 6.48 (s, 1H), 5.87 (d, J = 1.4, 1H), 5.86 (d, J = 1.4, 1H), 2.98–3.23 (m, 4H), 2.84 (dt, J = 10.5 and J = 3.0, 1H), 2.15–2.35 (m, 2H), 1.86–1.97 (m, 1H), 1.22–1.80 (m, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 145.9, 145.4, 137.4, 128.0, 108.4, 106.0, 100.6, 65.1, 46.5, 44.0, 40.6, 36.1, 29.3, 28.8, 25.4, 22.3, 21.8. HRMS (m/z): [M + H]⁺ calcd for [C₁₇H₂₂NO₂]⁺ 272.1651; found 272.1649.

1.3. In Vitro and in Vivo Pharmacology. *nAChR* Binding Assays. The binding properties of the compounds at rat $\alpha 4\beta 2$, $\alpha 4\beta 4$, and $\alpha 3\beta 4$ nAChRs were determined in a [³H]epibatidine binding assay and at the $\alpha 7/5$ -HT_{3A} chimera in a [³H]MLA binding assay as previously described.¹⁹ IC₅₀ values were converted to K_i values using the Cheng–Prusoff equation.

The FLIPR Membrane Potential Assay. The functional properties of the compounds were determined at the mouse $\alpha 4\beta 2$ and rat $\alpha 3\beta 4$ nAChR subtypes essentially as previously described.³⁷

SERT, NET, and DAT Uptake Inhibition Assay. COS7 cells (American Type Culture Collection, Manassas, VA) were cultured and transiently transfected with mammalian expression plasmids containing human SERT (pcDNA3.1-hSERT), human NET (pCI-IRES-neo-hNET), or human DAT (pCI-IRES-neo-hDAT) as detailed previously.³⁸ Uptake inhibition assays were performed 40–48 h after transfection in white 96-well tissue culture plates essentially as described previously.³⁸ Briefly, cells were preincubated in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4) containing 0.1 mM CaCl₂ and 0.5 mM MgCl₂ (PBSCM) with 0 or increasing concentrations of **3c**. In all NET and DAT assays, the PBSCM buffer was supplemented with 1 mM ascorbic acid. After 30 min, PBSCM containing [³H]-serotonin (28

Ci/mmol; SERT assays) or $[{}^{3}H]$ -dopamine (48 Ci/mmol; NET and DAT assays) were added, giving a final substrate concentration of 50 nM. Uptake was allowed to proceed for 10 min and terminated by aspiration and rapid washing of wells three times with PBSCM. Amount of accumulated radioligand was determined by solubilizing cells in scintillant (MicroScint20), followed by counting of plates in a Packard TopCounter. Nonspecific uptake was determined in parallel by measuring uptake in nontransfected cells. IC₅₀ values were converted to K_i values using the Cheng–Prusoff equation.

Mouse Forced Swim Test. Method: Mice (n = 7-8) were individually placed in a beaker (16 cm in diameter) filled with 20 cm of water maintained at 23.5-24.5 °C. Total swim distance during the 6 min test period was automatically recorded by a camera mounted above the cylinders and stored on a computer equipped with Ethovision (Noldus, Holland). 3c was dissolved in saline (0.9% NaCl) and given subcutaneously 20 min prior to testing in an injection volume of 10 mL/kg. Data analysis: The first minute was omitted from the data before statistical analysis. This is because animals generally swim extensively for the first minute, irrespective of treatment; hence, any true treatment effect only becomes apparent after one minute. Swim distance was analyzed using a one-way analysis of variance (ANOVA) and followed by pairwise comparisons of the predicted means using the Planned Comparisons procedure. To ensure variance homogeneity and normality, a requirement of the ANOVA approach, data were log-transformed before statistical analysis. Differences were considered significant for p < 0.05. Results: The ANOVA revealed a significant main effect of 3c treatment (F2,20 = 10.47; p < 0.001). Planned Comparisons showed that swim distance was significantly increased by 30 mg/kg 3c (p < 0.001).

ASSOCIATED CONTENT

Supporting Information

All general procedures and characterization for all synthesized compounds, copies of ¹H and ¹³C NMR spectra for compounds **1a–g**, **2a–g**, **3a–g**, **7a–f**, **8a–d**, **9a–f**, and **10**, as well as tables containing the full data set from the nAChR-pharmacology. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: (+45) 35336487. E-mail: jesper.kristensen@sund.ku. dk.

Present Address

⁸Medicinal Chemistry Research, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark.

Author Contributions

^{II}F.C. and A.A.J. contributed equally

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Danish Council for Independent Research-Medical Sciences, the Lundbeck Foundation, and the Novo Nordisk Foundation for financial support.

REFERENCES

 (1) (a) Jensen, A. A.; Frolund, B.; Liljefors, T.; Krogsgaard-Larsen, P. Neuronal Nicotinic Acetylcholine Receptors: Structural Revelations, Target Identifications, and Therapeutic Inspirations. J. Med. Chem.
2005, 48, 4705–4745. (b) Arneric, S. P.; Holladay, M.; Williams, M. Neuronal Nicotinic Receptors: A Perspective on Two Decades of Drug Discovery Research. Biol. Pharmacol. 2007, 74, 1092–1101.
(c) Steinlein, O. K. Ion Channel Mutations in Neuronal Diseases: A Genetics Perspective. Chem. Rev. 2012, 112, 6334–6352. (d) Lemoine, D.; Jiang, R.; Taly, A.; Chataigneau, T.; Specht, A.; Grutter, T. Ligand-Gated Ion Channels: New Insights into Neurological Disorders and Ligand Recognition. *Chem. Rev.* **2012**, *112*, 6285–6318.

(2) (a) Coe, J. W.; Brooks, P. R.; Vetelino, M. G.; Wirtz, M. C.; Arnold, E. P.; Huang, J.; Sands, S. B.; Davis, T. I.; Lebel, L. A.; Fox, C. B.; Shrikhande, A.; Heym, J. H.; Schaeffer, E.; Rollema, H.; Lu, Y.; Mansbach, R. S.; Chambers, L. K.; Rovetti, C. C.; Schulz, D. W.; Tingley, F. D., III; O'Neill, B. T. Varenicline: An $\alpha 4\beta 2$ Nicotinic Receptor Partial Agonist for Smoking Cessation. J. Med. Chem. 2005, 48, 3474–3477. (b) Bertrand, D.; Bertrand, S.; Cassar, S.; Gubbins, E.; Li, J.; Gopalakrishnan, M. Positive Allosteric Modulation of the $\alpha 7$ Nicotinic Acetylcholine Receptor: Ligand Interactions with Distinct Binding Sites and Evidence of a Prominent Role of the M2–M3 Segment. Mol. Pharmacol. 2008, 74, 1407–1416.

(3) (a) Dwoskin, L. P.; Crooks, P. A. Competitive Neuronal Nicotinic Receptor Antagonists: A New Direction for Drug Discovery. J. Pharmacol. Exp. Ther. 2001, 298, 395-402. (b) Iturriaga-Vásquez, P.; Pérez, E. G.; Slater, E. Y.; Bermudez, I.; Cassels, B. K. Aporphine Metho Salts as Neuronal Nicotinic Acetylcholine Receptor Blockers. Bioorg. Med. Chem. 2007, 15, 3368-3372. (c) For recent studies on antagonists of *a*7 nAChR, see: Ambrus, J. I.; Halliday, J. I.; Kanizaj, N.; Absalom, N.; Harpsøe, K.; Balle, T.; Chebib, M.; McLeod, M. D. Covalent Attachment of Antagonists to the α 7 Nicotinic Acetylcholine Receptor: Synthesis and Reactivity of Substituted Maleimides. Chem. Commun. 2012, 6699-6701. (d) For recent studies on selective antagonists of $\alpha 4\beta 2$ nAChR, see: Faundez-Parraquez, M.; Farias-Rabelo, N.; Gonzalez-Gutierrez, J. P.; Etcheverry-Berrios, A.; Alzate-Morales, J.; Adasme-Carreño, F.; Varas, R.; Bermudez, I.; Iturriaga-Vásquez, P. Neonicotinic Analogues: Selective Antagonists for $\alpha 4\beta 2$ Nicotinic Acetylcholine Receptors. Bioorg. Med. Chem. 2013, 21, 2687 - 2694

(4) (a) Boekelheide, V.The Erythrina Alkaloids. In The Alkaloids: Chemistry and Physiology; Academic Press: New York, 1960; Vol. 7, pp 201–227. (b) Reimann, E. Synthesis Pathways to Erythrina Alkaloids and Erythrina Type Compounds. In Progress in the Chemistry of Organic Natural Products; Springer: New York, 2007; Vol. 88, pp 1–62.

(5) Erysovine and Erythraline have not yet been characterized on the nAChRs.

(6) Iturriaga-Vásquez, P.; Carbone, A.; García-Beltrán, O.; Livingstone, P. D.; Biggin, P. C.; Cassels, B. K.; Wonnacott, S.; Zapata-Torres, G.; Bermudez, I. Molecular Determinants for Competitive Inhibition of $\alpha 4\beta 2$ Nicotinic Acetylcholine Receptors. *Mol. Pharmacol.* **2010**, *78*, 366–375.

(7) In comparison DH β E has a K_i of 98 nM at the $\alpha 4\beta 2$ nAChR and a K_i of 10.5 μ m at the α 7 subtype.

(8) Decker, M. W.; Anderson, D. J.; Brioni, J. D.; Donnellyroberts, D. L.; Kang, C. H.; Oneill, A. B.; Piattonikaplan, M.; Swanson, S.; Sullivan, J. P. Erysodine, a Competitive Antagonist at Neuronal Nicotinic Acetylcholine Receptors. *Eur. J. Pharmacol.* **1995**, 280, 79–89.

(9) (a) Bergmeier, S. C.; Ismail, K. A.; Arason, K. M.; McKay, S.; Bryant, D. L.; McKay, D. B. Structure–Activity Studies of Ring E Analogues of Methyllycaconitine. Part 2: Synthesis of Antagonists to $\alpha 3\beta 4^*$ Nicotinic Acetylcholine Receptors Through Modifications to the Ester. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3739–3742. and references cited therein. (B) For a recent example at the $\alpha 4\beta 2$ nAChR subtype, see: German, N.; Kim, J.-S.; Jain, A.; Dukat, M.; Pandya, A.; Ma, Y.; Weltzin, M.; Schulte, M. K.; Glennon, R. A. Deconstruction of the $\alpha 4\beta 2$ Nicotinic Acetylcholine Receptor Positive Allosteric Modulator Desformylflustrabromine. *J. Med. Chem.* **2011**, *54*, 7259– 7267.

(10) (a) Hider, R. C.; Walkinshaw, M. D.; Saenger, W. Erythrina Alkaloid Nicotinic Antagonists: Structure–Activity Relationships. *Eur. J. Med. Chem.* **1986**, *21*, 231–234. (b) Megirian, D.; Leary, D. E.; Slater, I. H. The Action of some Derivatives of beta-Erythroidine on Peripheral Neuro-effector Systems. *J. Pharmacol. Exp. Ther.* **1955**, *113*, 212–227. (11) Cabedo, N.; Protais, P.; Cassels, B. K.; Cortes, D. Synthesis and Dopamine Receptor Selectivity of the Benzyltetrahydroisoquinoline, I-(+)-*nor*-Roefractine. *J. Nat. Prod.* **1998**, *61*, 709–712.

(12) For a recent review on the synthesis of *Erythrina*-type compounds, see: Riemann, E. Synthesis Pathways to Erythrina Alkaloids and Erythrina Type Compounds. *Prog. Chem. Org. Nat. Prod.* **2007**, *88*, 1–62.

(13) For recent examples on the stereocontrolled syntheses of *Erythrina*-type products, see: (a) Zhang, F.; Simpkins, N. S.; Blake, A. J. New Approaches for the Synthesis of Erythrinan Alkaloids. *Org. Biomol. Chem.* **2009**, *7*, 1963–1979. (b) He, Y.; Funk, R. L. Total Syntheses of (\pm) - β -Erythroidine and (\pm) -8-oxo- β -Erythroidine by an Intramolecular Diels–Alder Cycloaddition of a 2-Amidoacrolein. *Org. Lett.* **2006**, *8*, 3689–3692. (c) Reimann, E.; Ettmayr, C. An Improved Stereocontrolled Route to *cis*-Erythrinanes by Combined Intramolecular *Strecker* and *Bruylants* Reaction. *Monatsh. Chem.* **2004**, 135, 1143–1155.

(14) Tietze, L. F.; Tölle, N.; Noll, C. Highly Efficient Domino Reaction for the Synthesis of the Erythrina and B-Homoerythrina Alkaloid Skeleton. *Synlett* **2008**, 525–528.

(15) Isobe, K.; Mohri, K.; Tokoro, K.; Fukushima, C.; Higuchi, F.; Taga, J.-I.; Tsuda, Y. Selective Cleavage of Aromatic Rings by Ozonolysis. I. Application to *o*-Dimethoxybenzene Derivatives. *Chem. Pharm. Bull.* **1988**, *36*, 1275–1282.

(16) Šmidrkal, J. Synthesis of Fagaronine. Collect. Czech. Chem. Commun. 1988, 53, 3184–3192.

(17) Bailey, K. R.; Ellis, A. J.; Reiss, R.; Snape, T. J.; Turner, N. J. Template-Based Mnemonic for Monoamine Oxidase (MAO-N) Catalyzed Reactions and its Application Towards the Chemo-Enzymatic Deracemisation of the Alkaloid (\pm)-Crispine A. *Chem. Commun.* 2007, 3640–3642.

(18) (a) Ruchirawat, S.; Chaisupakitsin, M.; Patranuwatana, N.; Cashaw, J. L.; Davis, V. E. A Convenient Synthesis of Simple Tetrahydroisoquinolines. *Synth. Commun.* **1984**, *14*, 1221–1228. (b) For a recent review about the Pictet–Spengler reaction, see: Stöckigt, J.; Antonchick, A. P.; Wu, F.; Waldmann, H. The Pictet–Spengler Reaction in Nature and in Organic Chemistry. *Angew. Chem., Int. Ed.* **2011**, *50*, 8538–8564.

(19) Jensen, A. A.; Mikkelsen, I.; Frølund, B.; Baüner-Osborne, H.; Falch, E.; Krogsgaard-Larsen, P. Carbamoylcholine Homologs: Novel and Potent Agonists at Neuronal Nicotinic Acetylcholine Receptors. *Mol. Pharmacol.* **2003**, *64*, 865–875.

(20) Armishaw, C. J.; Singh, N.; Medina-Franco, J. L.; Clark, R. J.; Scott, K. C. M.; Houghten, R. A.; Jensen, A. A. A Synthetic Combinatorial Strategy for Developing α -Conotoxin Analogs as Potent α 7 Nicotinic Acetylcholine Receptor Antagonists. *J. Biol. Chem.* **2010**, 285, 1809–1821.

(21) Shahsavar, A.; Kastrup, J. S.; Nielsen, E. Ø.; Kristensen, J. L.; Gajhede, M.; Balle, T. Crystal Structure of *Lymnaea stagnalis* AChBP Complexed with the Potent nAChR Antagonist DHBE Suggests a Unique Mode of Antagonism. *PLoS One* **2012**, *7*, e40757.

(22) Quattrocki, E.; Baird, A.; Yurgelun-Todd, D. Biological Aspects of the Link Between Smoking and Depression. *Harvard Rev. Psychiatry* **2000**, *8*, 99–110.

(23) Philip, N. S.; Carpenter, L. L.; Tyrka, A. R.; Price, L. H. Nicotinic Acetylcholine Receptors and Depression: A Review of the Preclinical and Clinical Literature. *Psychopharmacology (Berlin, Ger.)* **2010**, *212*, 1–12.

(24) Andreasen, J. T.; Nielsen, E. O.; Christensen, J. K.; Olsen, G. M.; Peters, D.; Mirza, N. R.; Redrobe, J. P. Subtype-selective Nicotinic Acetylcholine Receptor Agonists Enhance the Responsiveness to Citalopram and Reboxetine in the Mouse Forced Swim Test. *J. Psychopharmacology* **2011**, *25*, 1347–1356.

(25) Andreasen, J. T.; Redrobe, J. P. Nicotine, but not Mecamylamine, Enhances Antidepressant-like Effects of Citalopram and Reboxetine in the Mouse Forced Swim and Tail Suspension Tests. *Behav. Brain Res.* 2009, 197, 150–156. (26) Popik, P.; Kozela, E.; Krawczyk, M. Nicotine and Nicotinic Receptor Antagonists Potentiate the Antidepressant-like Effects of Imipramine and Citalopram. *Br. J. Pharmacol.* **2003**, *139*, 1196–1202.

(27) Caldarone, B. J.; Harrist, A.; Cleary, M. A.; Beech, R. D.; King, S. L.; Picciotto, M. R. High-Affinity Nicotinic Acetylcholine Receptors are Required for Antidepressant Effects of Amitriptyline on Behavior and Hippocampal Cell Proliferation. *Biol. Psychiatry* **2004**, *56*, 657–664.

(28) Andreasen, J. T.; Olsen, G. M.; Wiborg, O.; Redrobe, J. P. Antidepressant-like Effects of Nicotinic Acetylcholine Receptor Antagonists, but not Agonists, in the Mouse Forced Swim and Mouse Tail Suspension Tests. J. Psychopharmacol. 2009, 23, 797–804.

(29) Kristensen, A. S.; Andersen, J.; Jørgensen, T. N.; Sørensen, L.; Eriksen, J.; Loland, C. J.; Strømgaard, K.; Gether, U. SLC6 Neurotransmitter Transporters: Structure, Function, and Regulation. *Pharmacol. Rev.* **2011**, *63*, 585–640.

(30) Andersen, J.; Kristensen, A. S.; Bang-Andersen, B.; Strømgaard, K. Recent advances in the understanding of the interaction of antidepressant drugs with serotonin and norepinephrine transporters. *Chem. Commun.* **2009**, 3677–3692.

(31) Menachery, M. D.; Lavanier, G. L.; Wetherly, M. L.; Guinaudeau, H.; Shamma, M. Simple Isoquinoline Alkaloids. *J. Nat. Prod.* **1986**, *49*, 745–778.

(32) Bembenek, M. E.; Abell, C. W.; Chrisey, L. A.; Rozwadowska, M. D.; Gessner, W.; Brossi, A. Inhibition of Monoamine Oxidases A and B by Simple Isoquinoline Alkaloids: Racemic and Optically Active 1,2,3,4-Tetrahydro-, 3,4-Dihydro-, and Fully Aromatic Isoquinolines. *J. Med. Chem.* **1990**, 33, 147–152.

(33) Shulman, K. I.; Herrmann, N.; Walker, S. E. Current Place of Monoamine Oxidase Inhibitors in the Treatment of Depression. *CNS Drugs* **2013**, *27*, 789–797.

(34) (a) Slattery, D. A.; Cryan, J. F. Using the Rat Forced Swim Test to Assess Antidepressant-like Activity in Rodents. *Nature Protoc.* **2012**, 7 (6), 1009–1014. (b) Can, A.; Dao, D. T.; Arad, M.; Terrillion, C. E.; Piantadosi, S. C.; Gould, T. D. The Mouse Forced Swim Test. *J. Vis. Exp.* **2012**, 29, e3638.

(35) Schneider, W.; Müller, B. Beiträge zur Chemie der Carbinolamine. I Untersuchungen an Py-Tetrahydroisochinolinen. *Justus Liebigs Ann. Chem.* **1958**, *615*, 34–42.

(36) Ahmed-Schofield, R.; Mariano, P. S. A Photochemical Route for Erythrane Ring Construction. J. Org. Chem. **1987**, 52, 1478–1482.

(37) (a) Armishaw, C.; Jensen, A. A.; Balle, T.; Clark, R. J.; Harpsøe, K.; Skonberg, C.; Liljefors, T.; Strømgaard, K. Rational Design of α -Conotoxin Analogues Targeting α 7 Nicotinic Acetylcholine Receptors: Improved Antagonistic Activity by Incoporation of Proline Derivatives. J. Biol. Chem. 2009, 284, 9498–9512. (b) Hansen, C. P.; Jensen, A. A.; Christensen, J. K.; Balle, T.; Liljefors, T.; Frølund, B. Novel acetylcholine and carbamoylcholine analogues: development of a functionally selective alpha4beta2 nicotinic acetylcholine receptor agonist. J. Med. Chem. 2008, 51, 7380–7395.

(38) Sørensen, L.; Andersen, J.; Thomsen, M.; Hansen, S. M.; Zhao, X.; Sandelin, A.; Strømgaard, K.; Kristensen, A. S. Interaction of Antidepressants with the Serotonin and Norepinephrine Transporters: Mutational Studies of the S1 Substrate Binding Pocket. *J. Biol. Chem.* **2012**, *287*, 43694–43707.