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

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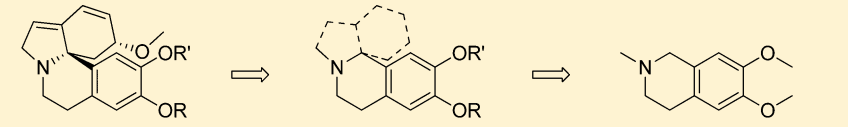

Aromatic Erythrinanes | 21 Ligands | Selective α4β2 antagonist K_i = 0.87 μM and IC_{50} = 12 μM

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 $\alpha4\beta2$ nAChR with more than 300-fold selectivity over $\alpha4\beta4$, $\alpha3\beta4$, and $\alpha7$. 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 (DHβE). DHβE is a member of the *Erythrina* 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 DHβE, have been investigated at the nAChRs.[5] Erysodine was found to be the most potent competitive antagonist of the four at the $\alpha4\beta2$ nAChR, displaying a binding affinity of 50 nM in a [^{3}H]cytisine binding assay and a K_i value of 7.5 μM at the $\alpha7$ nAChR.[6,7] Moreover, erysodine has also been compared with DHβE 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 DHβE.

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

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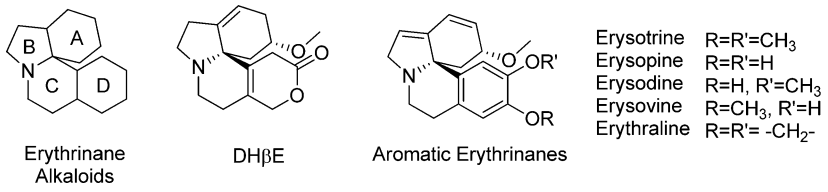


Figure 1. The erythrinane scaffold, DHβE, and structure of aromatic erythrinanes.

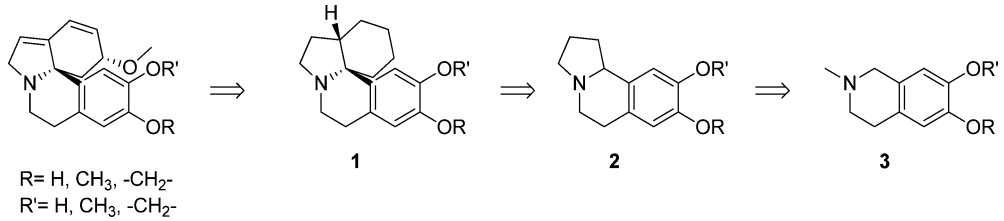
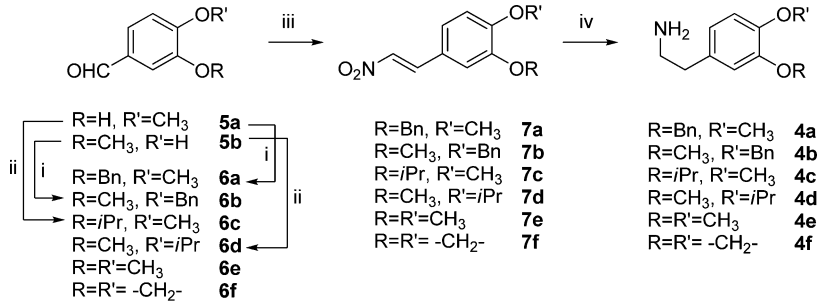


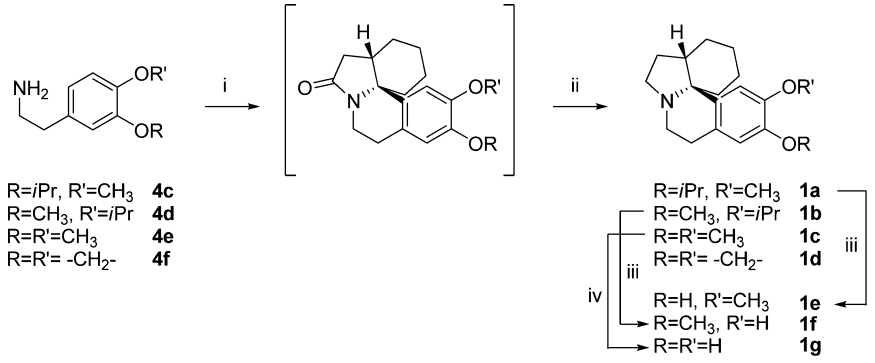
Figure 2. Aromatic erythrinanes and new ligands in which the structural complexity is gradually reduced.

Scheme 1. Synthesis of the Phenethylamines 4a–f[a]



[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) NO_2Me (3.6 equiv), NH_4OAc (3 equiv), AcOH, 120 °C, 49–81%; (iv) $LiAlH_4$ (5 equiv), dry THF, 0 °C to reflux conditions, 84–99%.

Scheme 2. Synthesis of *cis*-Erythrinane Derivatives 1a–g[a]



[a]Reagents and conditions: (i) method A; (a) $AlMe_3$ (2 equiv), ethyl 2-cyclohexanoneacetate (1.05 equiv), $Sc(OTf)_3$ (12.5 mol %), CH_3CN, 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) $LiAlH_4$ (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) BBr_3 (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 A-ring was omitted and the double bonds in the A- and B-ring were saturated, giving the first series of analogues, then the A-

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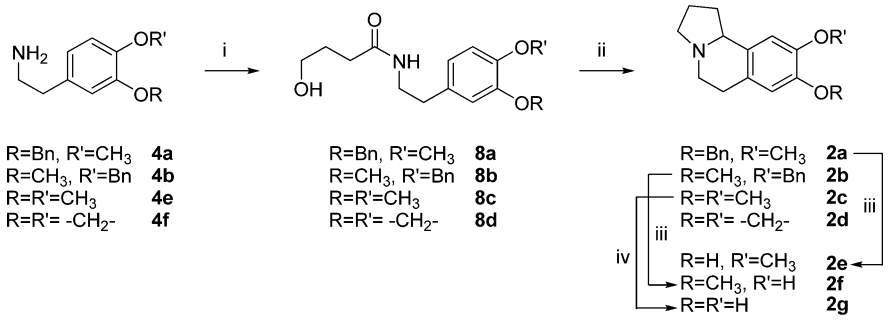
Scheme 3. Synthesis of Tetrahydroisoquinoline Derivatives 2a–g[a]



[a]Reagents and conditions: (i) γ-butyrolactone (1.1 equiv), PhMe, reflux conditions, 46–72%; (ii) (a) $POCl_3$ (10 equiv), PhMe, reflux conditions, (b) $NaBH_4$ (2 equiv), EtOH–AcOH (10:1), 0 °C to rt, 42–69%; (iii) H_2, Pd/C (10 mol %), MeOH, rt, 72–75%; (iv) BBr_3 (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

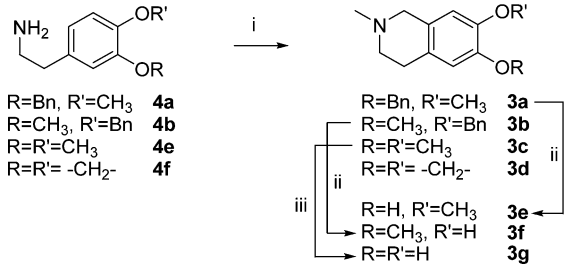
First, the synthesis of the required substituted phenethylamines **4a–f**, common intermediates for the preparation of all the designed ligands, is outlined in Scheme 1. Benzyl protection of the hydroxyl group of commercially available isovanillin **5a** and vanillin **5b** furnished aldehydes **6a** 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 $LiAlH_4$ 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 2-cyclohexanoneacetate, forming successively three new bonds in one process. The resulting crude intermediates were reduced with an excess of $LiAlH_4$, 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 $LiAlH_4$ of the crude products. The deisopropylation of compounds **1a** and **1b** were performed in $AcOH/H_2SO_4$ at reflux,[16] leading to the desired *cis*-erythrinane derivatives **1e** and **1f**. The treatment of **1c** with 3 equivalents of BBr_3 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 $POCl_3$. Subsequent reduction of the intermediate iminium salts with $NaBH_4$ gave isoquinoline substrates **2a–d** with 42–69% yields. Debenzylation of **2a** and **2b** using H_2/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 BBr_3 afforded dihydroxyisoquinoline **3g** as its hydrobromic salt in 69% yield (Scheme 4).

Scheme 4. Synthesis of Tetrahydroisoquinolines 3a–g[a]



[a]Reagents and conditions: (i) HCOH 37% aq (5 equiv), HCO_2H, 100 °C, 58–99%; (ii) H_2, Pd/C (10 mol %), MeOH, rt, 83–89%; (iii) BBr_3 (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 $POCl_3$. Subsequent reduction of the iminium salts with $NaBH_4$ led to **9a–f** in 78–88% yield over three steps (Scheme 5).

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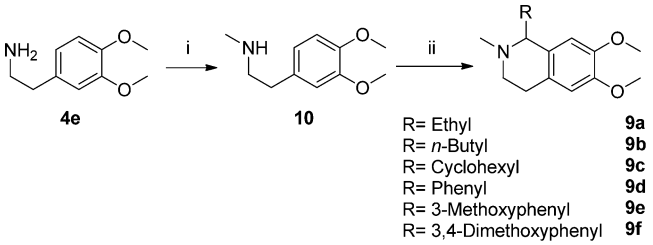
Scheme 5. Synthesis of 1-Substituted Tetrahydroisoquinolines 9a–f[a]



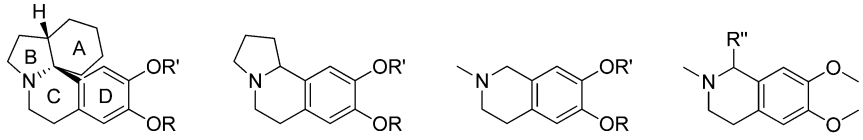
[a]Reagents and conditions: (i) (a) $(Boc)_2O$ (1.1 equiv), DCM, rt, (b) $LiAlH_4$ (1.5 equiv), dry THF, 0 °C to reflux conditions, 83% over 2 steps; (ii) (a) RCOCl (1.1 equiv), TEA (1.1 equiv), dry DCM, 0 °C to rt, (b) $POCl_3$ (10 equiv), dry PhMe, reflux conditions, (c) $NaBH_4$ (5 equiv), MeOH, 0 °C to rt, 78–88% over 3 steps.

RESULTS AND DISCUSSION

The ligands were characterized in a [^{3}H]epibatidine binding assay using membranes from HEK293 cells stably expressing the rat heteromeric nAChR subtypes $\alpha4\beta2$, $\alpha3\beta4$, and $\alpha4\beta4$ and in a [^{3}H]MLA binding assay using membranes from tsA201 transiently expressing the $\alpha7/5\text{-HT}_{3A}$ chimera (composed of the amino-terminal domain of the rat $\alpha7$ 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 $\alpha4\beta2$ nAChR and at a HEK293 cell line stably expressing the rat $\alpha3\beta4$ nAChR in the FLIPR Membrane Potential Blue assay was determined as previously described.[20] (*S*)-Nicotine (EC_{70}–EC_{80} for the respective receptors, i.e., 1 μM for $\alpha4\beta2$ and 3 μM for $\alpha3\beta4$) was used as agonist in the antagonist experiments.

All 15 compounds in the series displaced [^{3}H]epibatidine binding from the rat $\alpha4\beta2$ nAChR in a concentration-dependent manner, exhibiting K_i values at the receptor ranging from 0.55 to ~100 μM. In contrast, the vast majority of the compounds exhibited negligible binding affinity to the rat $\alpha4\beta4$ and $\alpha3\beta4$ nAChRs and to the $\alpha7/5\text{-HT}_{3A}$ chimera (Table 1). Overall, this SAR was also reflected in the inhibitory potencies

Table 1. Chemical Stuctures of the Synthesized Compounds and Their Pharmacological Properties at nAChRs[a]



			binding K_i (μM)				functional IC_{50} (μM)	
compd	R	R′	$\alpha4\beta2$	$\alpha4\beta4$	$\alpha3\beta4$	$\alpha7/5\text{-HT}_{3A}$	$\alpha4\beta2$	$\alpha3\beta4$
1c	CH_3	CH_3	~100	~100	~100	~30	31	~50[b]
1d	$-CH_2-$		7.3	~50	~30	18	9.2	~50[b]
1e	H	CH_3	8.5	~50	~100	20	2.3	~50[b]
1f	CH_3	H	18	23	~30	5.5	2.9	33
1g	H	H	1.5	19	~30	7.3	0.58	~50[b]
2c	CH_3	CH_3	5.5	~100	~300	>500	31	~100[b]
2d	$-CH_2-$		0.55	21	~100	~100	3.3	~100[b]
2e	H	CH_3	~100	~100	~300	~500	~50[b]	~300[b]
2f	CH_3	H	3.4	~100	~100	~300	23	~300[b]
2g	H	H	11	~50	~100	~300	~100[b]	~1000[b]
3c	CH_3	CH_3	0.87	~300	~300	>500	12	~100[b]
3d	$-CH_2-$		1.7	~50	~300	~500	2.6	~300[b]
3e	H	CH_3	10	~100	~300	>500	33	~1000[b]
3f	CH_3	H	1.7	~100	~300	>500	4.4	>1000
3g	H	H	6.8	~100	~100	>500	16	>1000

		binding K_i (μM)				functional IC_{50} (μM)	
	R″	$\alpha4\beta2$	$\alpha4\beta4$	$\alpha3\beta4$	$\alpha7/5\text{-HT}_{3A}$	$\alpha4\beta2$	$\alpha3\beta4$
9a	ethyl	~300				>300	
9b	*n*-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 $\alpha4\beta2$, $\alpha4\beta4$, and $\alpha3\beta4$ nAChR subtypes were obtained in a [^{3}H]epibatidine binding assay and the K_i values for the compounds at the $\alpha7/5\text{-HT}_{3A}$ chimera were determined in a [^{3}H]MLA binding assay. The functional IC_{50} values for the compounds at the mouse $\alpha4\beta2$ nAChR and the rat $\alpha3\beta4$ nAChR were obtained in the FLIPR Membrane Potential Blue assay. (*S*)-Nicotine (EC_{70}–EC_{80} for the respective receptors, i.e., 1 μM for $\alpha4\beta2$ and 3 μM for $\alpha3\beta4$) 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. [b]Because a complete concentration–inhibition curve could not be obtained for the compound in the concentration ranges tested, the IC_{50} value given is an estimate.

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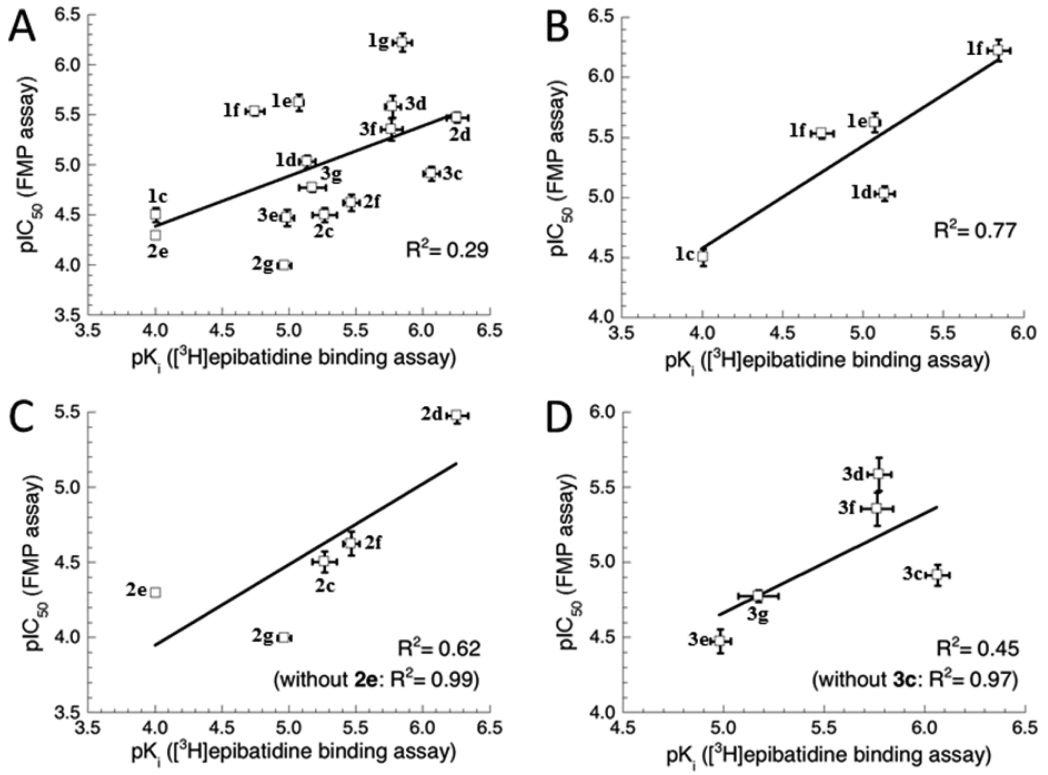

Figure 3. Correlation between binding and functional data for compound series **1**−**3** at the $\alpha4\beta2$ nAChR. In (A), data from all three series are shown together, whereas (B−D) show them individually.

on mouse $\alpha4\beta2$ nAChR and rat $\alpha3\beta4$ signaling in the FMP Blue assay (Table 1). The overall correlation between binding affinities and functional inhibitory potencies of the 15 compounds at the $\alpha4\beta2$ 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 $\alpha7$, 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 $\alpha7$ of compound **1e** of 20 μM is in the same range as the 7.5 μM reported for erysodine.[6] In contrast, the binding affinity for erysodine at $\alpha4\beta2$ drops from 50 nM to 8.5 μM for **1e** (Table 1). Deleting the A-ring gives **2e** with negligible affinity for $\alpha7$ and $\alpha4\beta2$ (~500 and ~100 μM, respectively). Removal of the B-ring in **1e** (**3e**) restores affinity for $\alpha4\beta2$ 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 $\alpha4\beta2$. 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 $\alpha4\beta2$ of all compounds in this study (0.55 μM), but this compound also exhibits affinity for $\alpha4\beta4$.

The three dimethoxy analogues comprise one of the most potent antagonists in the entire series (**3c**) where affinity for $\alpha4\beta2$ 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 $\alpha4\beta2$ increases to 5.5 μM 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 $\alpha4\beta2$ of 0.6 μM.[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 $\alpha4\beta2$ as shown in Table 1. With the exception of **9b** and **9d**, the ligands were virtually devoid of affinity for $\alpha4\beta2$. 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 catechol unit when it comes to affinity for $\alpha4\beta2$. This can be

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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 $\alpha4\beta2$, 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 DHβE, with a different binding mode.[21] This structure suggests that the methoxy group on the A-ring of DHβE 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 DHβE 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 DHβE 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 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 [^{3}H]-neurotransmitter uptake inhibition assays, and we found **3c** to have K_i values of 47 and 130 μM 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]

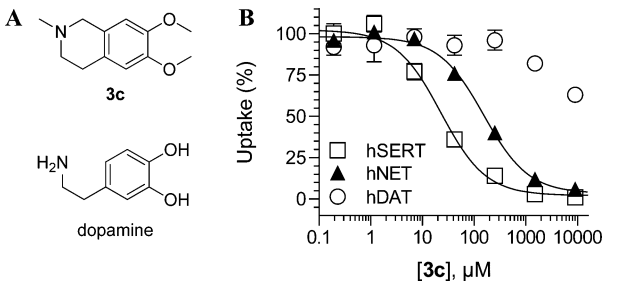

Figure 4. (A) Structure of **3c** and dopamine showing that **3c** is a restricted analogue of dopamine. (B) Dose−response curves from representative experiments of inhibition by **3c** of [^{3}H]neurotransmitter uptake in COS7 cells expressing human SERT, NET, or DAT. Data points represent the mean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

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 μM, 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.

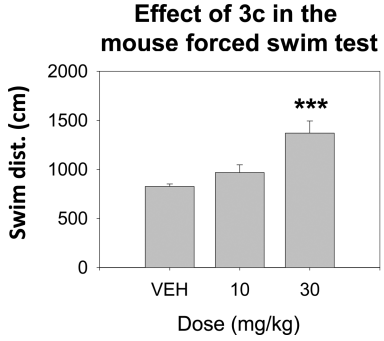

Figure 5. Results from mouse forced swim test of **3c**.

The relatively high dose needed to evoke the antidepressant-like 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 DHβE doses (3 mg/kg). Indeed, this dose of DHβE 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 $\alpha4\beta2$ 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 $\alpha4\beta2$, but **3c** remains an interesting and readily manipulated lead structure.

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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 ^{1}H NMR and LC-MS. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} 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 μm from Fisher Scientific using the solvent system as stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300 (Mercury and Gemini) instruments, using $CDCl_3$ or DMSO-d_6 as solvents and TMS as internal standard. Coupling constants (J values) are given in hertz (Hz). Multiplicities of ^{1}H 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 Na_2SO_4, 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 $POCl_3$ (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 $NaBH_4$ (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 Na_2SO_4, 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 $AlMe_3$ (2 M in toluene, 2 equiv) dropwise, $Sc(OTf)_3$ (12.5 mol %), and ethyl 2-cyclohexanoneacetate (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 $NaHCO_3$ at 0 °C, the mixture was extracted twice with EtOAc and the combined organic layers were washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude was then dissolved in dry THF (5 mL) and slowly added to a suspension of $LiAlH_4$ (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 Na_2SO_4, 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 $NaHSO_3$ (2 M), the mixture was extracted twice with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude was then dissolved in dry THF (5 mL) and slowly added to a suspension of $LiAlH_4$ (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 Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel to provide the pure corresponding *cis*-erythrinane **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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $C_{12}H_{17}NO_2$: 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $C_{11}H_{13}NO_2$: 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $C_{14}H_{19}NO_2$: 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.9, 145.8, 132.1, 127.4, 108.6, 106.1, 100.8, 63.7, 53.6, 48.8, 30.8,

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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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $[C_{20}H_{30}NO_2]^+$ 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $[C_{20}H_{30}NO_2]^+$ 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $[C_{17}H_{22}NO_2]^+$ 272.1651; found 272.1649.

1.3. In Vitro and in Vivo Pharmacology. *nAChR Binding Assays.* The binding properties of the compounds at rat $\alpha4\beta2$, $\alpha4\beta4$, and $\alpha3\beta4$ nAChRs were determined in a [^{3}H]epibatidine binding assay and at the $\alpha7$/5-HT_{3A} chimera in a [^{3}H]MLA binding assay as previously described.[19] IC_{50} 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 $\alpha4\beta2$ and rat $\alpha3\beta4$ 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 Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) containing 0.1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (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 [^{3}H]-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. IC_{50} 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 ^{1}H and ^{13}C 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.

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Notes

The authors declare no competing financial interest.

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