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Novel enantiopure ferrugininoids active as nicotinic agents: Synthesis and radioligand binding studies

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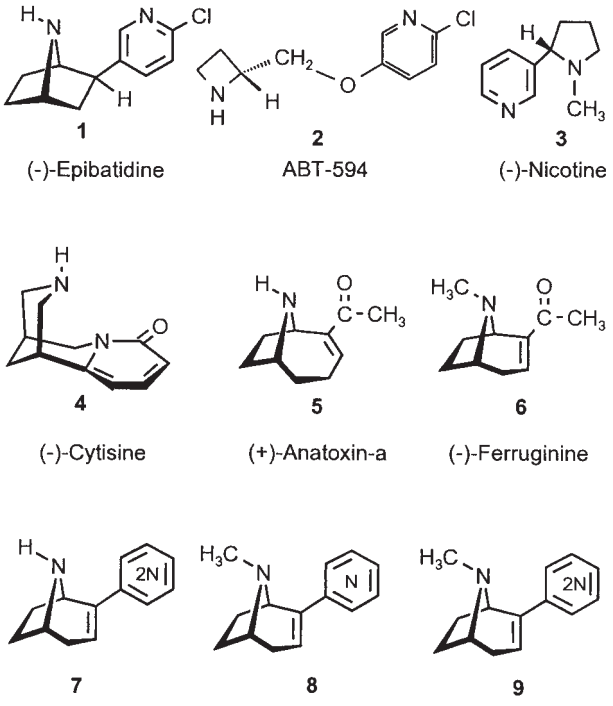
A series of hitherto unknown enantiopure (−)-ferruginine analogues of type **8** and **9** was prepared and tested for the affinity toward the nicotinic acetylcholine receptor (nAChR) subtypes $(\alpha4)_2(\beta2)_3$, $\alpha7^*$, $\alpha3\beta4^*$, and $(\alpha1)_2\beta1\gamma\delta$. The stereoconservative asymmetric syntheses started with (−)-cocainhydrochloride (**10**) from the chiral pool which was transformed into the chiral building blocks (+)-2-tropanone (**11**) and to (−)-anhydroecgonine (**18**). Key steps of the syntheses are novel extensions to existing methodology e.g. a Suzuki Pd(0)-mediated cross-coupling of vinyl triflate (**12**) with the heteroaryl organoboranes **13**–**15** and an inverse type [4+2]-cycloaddition with 1,2,4,5-tetrazine (**21**).The bioisosteric replacement of the 3-acetyl pharmacophoric element of the lead **6** by a 3-pyridyl, 5-chloropyridyl, 5-pyrimidinyl, 2-pyrazinyl, or 4-pyridazinyl moiety resulted in nAChR ligands with K_i-values ranging from 1.1–713 nM toward the $(\alpha4)_2(\beta2)_3$ subtype combined with significant differentiation among the nAChR subtypes when tested in vitro by radioligand binding studies. Generally the ferrugininoids are less potent than the corresponding norferrugininoids. Similar to results of the norferrugininoid series the novel azine substituted ferrugininoids **8** proved to be more potent than the diazine analogues **9**; both exhibited higher affinities compared to the lead **6**. The 5-chloropyridyl-containing variant **8b** [1*R*, 5*S*)-enantiomer] turned out to be the most active nAChR ligand with a 12-fold higher affinity toward the $(\alpha4)_2(\beta2)_3$ subtype than the corresponding (1*S*, 5*R*)-form *ent*-**8b**.

1. Introduction

The discovery of (−)-epibatidine (**1**) (Spande et al. 1992), a trace alkaloid in skin extracts of an Ecuadorean frog, and subsequent introduction of this intriguing toxoid as a nicotinic agonist and analgetic provided the impetus for worldwide efforts to obtain analogues that would retain analgetic activity, while being much less toxic. One such variant, ABT 594, looked promising (Bannon et al. 1998; Daly 2003) especially with regard to analgesia, but the efforts failed because of nicotine-like unfavorable effects. Numerous interesting variants of different natural nicotinic agonists such as (−)-nicotine (**3**), (−)-cytisine (**4**), (+)-anatoxin-a (**5**) or (−)-ferruginine (**6**) are still under investigation and are involved in the study of the role of nAChRs in pain perception (Holladay et al. 1997; Lin and Meyer 1998; Bannon et al. 1998). We have pursued this goal successfully by synthesizing various nAChR ligands by bioisosteric or other modifications of the alkaloidal toxins **1**, and **3**–**6** to gain variants with possibly promising therapeutic utility (Che et al. 2001; Imming et al. 2001; Gündisch et al. 2001; Gündisch et al. 2002; Gohlke et al. 2002; Gohlke et al. 2003).



In continuation of one of our programs aimed at the development of hitherto unknown ferruginine based nAChR ligands, we focused our efforts on the synthesis of novel (−)-ferruginine analogues of type **8** and **9**. These are structurally similar to the (−)-norferruginine series of type **7** published recently from our laboratory (Gündisch et al. 2001). All of these enantiopure ferruginine-type ligands **7**–**9** are characterized by the requisite pharmacophoric elements typical of class C ligands in Schmitt's classification (Schmitt 2000); they comprise both the N-bicycle (a tropane scaffold) and the hydrogen bond acceptor π-system (HBA/π, various azine or diazine rings) within separate non-fused rings; both of the pharmacophoric elements are joined by a pivot bond. The decisive structural difference between ligands of type **7** in comparison with those of type **8** and **9** is, that the nitrogen of the bicycle is methylated. Thus, it was anticipated that as in the case of (−)-nornicotine/(−)-nicotine (Glennon and Dukat 1996) introduction of a N-methyl group could increase affinity to the multifarious nAChRs.

Herein we wish to report the synthesis of the two enantiopure pyridyl bioisosteres **8a** and **8b** of the lead **6** together with the three diazine substituted variants **9a**, **9b,** and **9c**. In addition, the preliminary biological profile of these potential nAChR ligands is described along with the stereodiscrimination of the ligands **8b**/*ent*-**8b** and **9c**/*ent*-**9c**.

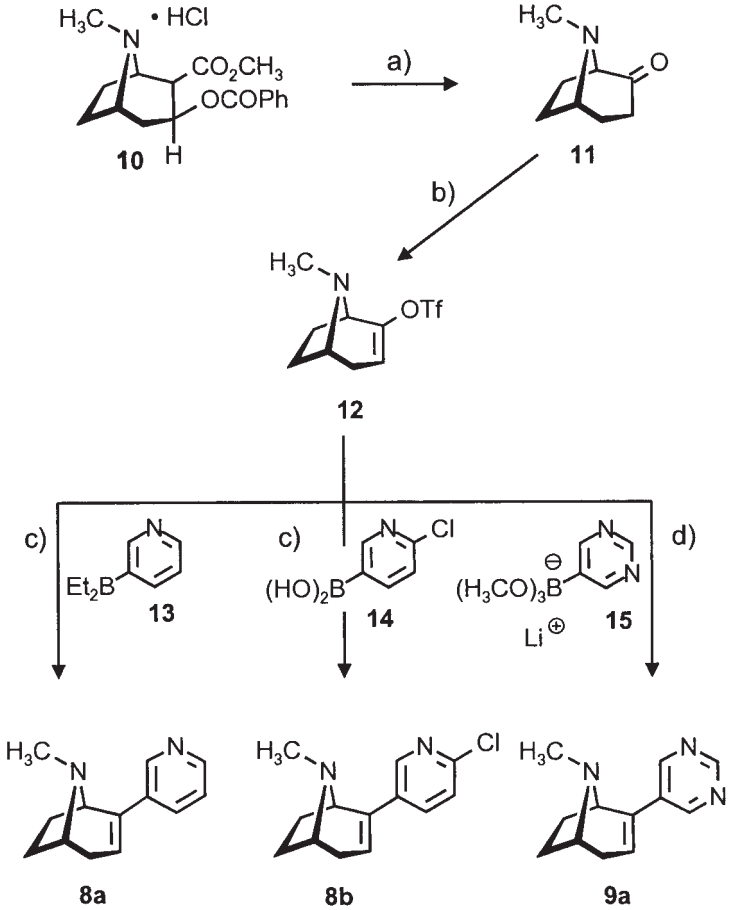
2. Investigations, results and discussion

2.1. Chemistry

The ferrugininoid ligands **8a**, **8b** and **9a** were synthesized according to Scheme 1. Our synthetic strategy towards these azine substituted target compounds, in which the acetyl group of the lead **6** was replaced by a bioisosteric pyridine or pyrimidine scaffold, started with the enantiomerically pure (+)-2-tropanone **11** as chiral building block. This is easily available by conversion of (−)-cocaine hydrochloride (**10**) into the tropanoid ketone **11** in an efficient high-yielding three-step procedure without isolation and purification of intermediates (Zhang et al. 1997). Most promising for the introduction of the requisite azine units into the bulky tropane moiety seemed to be an approach using a Pd(0)-mediated Suzuki-type cross-coupling (Oh-e et al. 1993; Seifert et al. 2003). Thus, the vinyl triflate **12** and the organoboranes **13**–**15** (Seifert et al. 2003) were examined as appropriate starting materials for the synthesis of the enantiopure target ligands **8a**, **8b**, and **9a**.

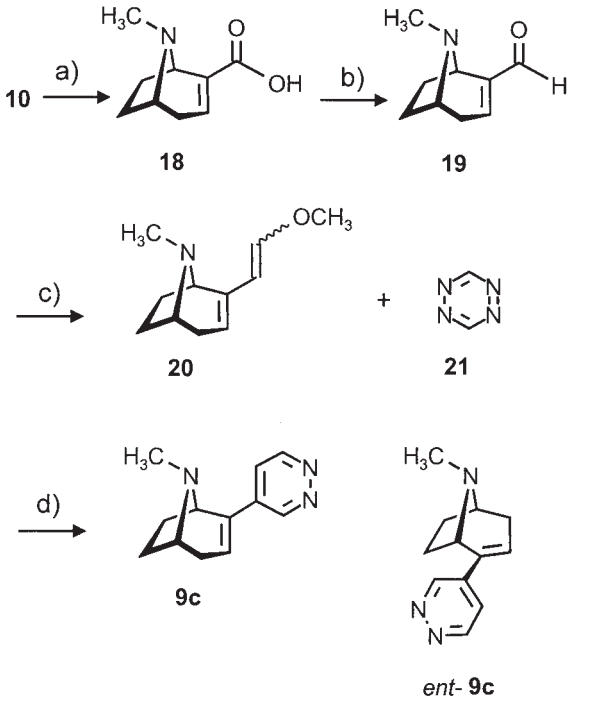
For the synthesis of **8a** (Scheme 1) ketone **11** was converted into its 2-vinyl triflate **12** by base-catalyzed reaction with Comin's *N*-(5-chloro-2-pyridyl)triflimide (Wegge et al. 2000) to provide compound **12** in 96% yield. Then the 3-pyridyl group was introduced into the 2-position of the azabicycle by reacting triflate **12** with 3-diethylboranylpyridine (**13**) (Terashima et al. 1983) in THF using bis(tri-phenylphosphane)palladium(II) chloride as catalyst

Scheme 1



Reagents and conditions: (a) see Zhang et al. (1997); (b) 1. LDA, −85 °C; 2. 2-N(Tf_2)-5-chloro-pyridine, 12 h, 80 °C → RT. 94%; (c) $Pd(PPh_3)_2Cl_2$, THF, 2 M aqueous Na_2CO_3, 18 h, 80 °C 81% and 65%; (d) analogous (c) in THF : Ethanol = 3 : 1, 51%

Scheme 2

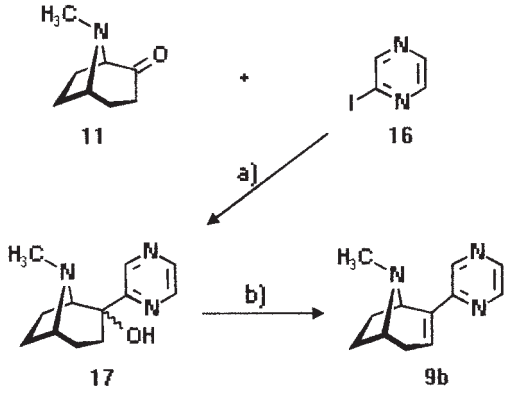


Reagents and conditions: (a) see Zhang et al. (1997). (b) see Baasov et al. (1985); (c) $Ph_3PCH_2OCH_3^+$ Cl^-/KO-tert-Bu, Et_2O, 24 h, −15 °C → RT., 51%. (d) toluene, 20 h, reflux, 81%

and aqueous sodium carbonate as nucleophilic activator. The coupling reaction proceeded with good success to give the ferrugininoid **8a** in 81% yield. A similar approach for introduction of the 2-chloropyridine nucleus into the bulky tropane moiety used the requisite 2-chloropyridyl-5-yl boronic acid (**14**) (Gronowitz et al. 1986) to afford the target species **8b** with 65% yield. Additionally, lithium trimethoxy-5-(5-pyrimidyl)-boronate (**15**) (Haidar et al. 2001) offered an elegant access to the pyrimidine substituted ferrugininoid **9a**. Cross coupling with the vinyl triflate **12** could be achieved under identical conditions as described before yielding the coupling product **9a** with 51% yield.

As illustrated in Scheme 2 the synthesis of the pyrazine-substituted ferrugininoid **9b** was achieved with Burger's methodology (Reed and Burger 1971; Baker et al. 1991).

Scheme 3



Reagents and conditions: (a) 1. BuLi, −35 °C, Et_2O, 2. H_2O, then CH_2Cl_2-extraction, 49%. (b) 1. $SOCl_2$, 0 °C, CH_2Cl_2, 2 h, RT. 2. H_2O, then CH_2Cl_2-extration, 67%

Thus, 2-lithiopyrazine, generated by lithiation of 2-iodopyrazine (**16**) with *n*-butyllithium at 35 °C in diethyl ether, was reacted with the enantiopure ketone **11** affording the tertiary alcohol **17** in 49% yield as a mixture of two diastereomeres in a 7 : 1 ratio. Subsequent treatment of the mixture with thionyl chloride followed by basic work up led to the desired elimination product **9b** with 67% yield.

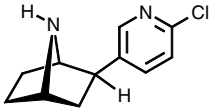
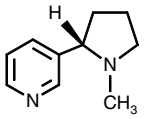
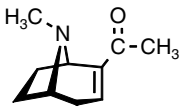
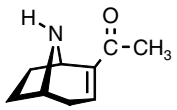
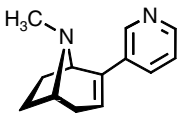
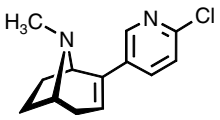
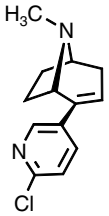
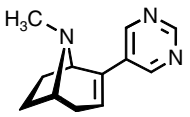
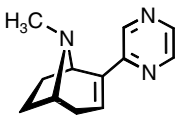
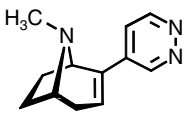
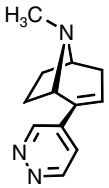
The synthetic route to the pyridazine analogue **9c**, originating from confiscated grade (−)-cocaine hydrochloride (**10**) is outlined in Scheme 3. Treatment of the alkaloidal salt **10** with concentrated hydrochloric acid under reflux afforded the hydrochloride of (−)-anhydroecgonine (**18**) in almost quantitative yield using Trudell's protocol (Zhang et al. 1997).

Our efforts were now directed to the construction of the enantiopure enol ether **20** serving as dienophile in the above mentioned inverse type Diels-Alder reaction. This intermediate was synthesized starting from the carboxylic acid **18** which was transformed into the requisite carbaldehyde **19** according to Sheves' protocol (Baasov and Sheves 1985). This could be converted into the target enol ether **20** in 91% yield using the high-temperature Wittig-reaction with potassium tert.-butoxide as the base in diethyl ether at −15 °C to −20 °C and triphenyl-(methoxymethylene)-phosphoniumchloride as ylide precursor (Stehl et al. 2002a). As determined by ^{1}H NMR (400 MHz, $CDCl_3$) enol ether **20** was isolated as a mixture of *E*- and *Z*-isomers in a ratio E : Z = 70 : 30. With the enol ether **20** in hands, we studied the outcome of the $LUMO_{diene}$/$HOMO_{dienophile}$ controlled Diels-Alder reaction of the tetrazine **21** as electron-deficient diazadiene-system with enol ether **20** as electron-rich dienophile (Scheme 3) (Stehl et al. 2002b). Heating the reactants **20** and **21** in toluene for 20 h under reflux the pyridazine substituted ferrugininoid **9c** could be isolated in good yield (81%) after [4+2] cycloaddition with subsequent expulsion of nitrogen and 1,2-elimination of methanol. With *ent*-**18** as starting material (Stehl et al. 2002b) the enantiomeric ferrugininoid *ent*-**9c** was obtained in satisfying yield according to the protocol described for **9c**.

2.2. In vitro receptor binding

To address the issue of binding selectivity among nAChR subtpyes, affinities of the novel (−)- and (+)-ferruginine variants **8a**, **8b**, and **9a**–**c**, listed in the Table, were measured in four different competition assays and compared with those of (±)-epibatidine (**1**), (−)-nicotine (**3**), (−)-ferruginine (**6**) and (−)-norferruginine (**6a**). To determine the affinities for the $(\alpha4)_2(\beta2)_3$ nAChR subtype a previously described competition assay (Gündisch et al. 1999) was used with (±)-[^{3}H]epibatidine and P2 membrane fraction of Sprague-Dawley rat forebrain. These studies demonstrated that the specific binding of (±)-[^{3}H]epibatidine to crude synaptic membranes of rat forebrain, at concentrations up to 800 pM, is characterized by a single population of binding sites with $K_d = 8 \pm 2$ pM (Gündisch et al. 1999). To characterize binding of each of the ferrugininoids **8** and **9** to the $\alpha7^*$ nAChR subtype, [^{3}H]MLA and membrane fractions isolated from the rat brain were used, [^{3}H]MLA bound to a single population of binding sites exhibited a K_d value of 1.2 0.2 nM (n = 3). The affinity determined was in good agreement with previously published values Davies et al. 1999 ($K_d = 1.86$ nM); Gündisch et al. 2002; Gohlke et al. 2002. [^{3}H]MLA bound to rat brain membranes with regional distribution charac-

Table: Radioligand binding affinities[a] of novel (−)- and (+)-ferruginine variants to $(\alpha4)_2(\beta2)_3$, $\alpha7^*$, $\alpha3\beta4^*$ and $(\alpha1)_2\beta1\gamma\delta$ nAChRs in comparison with (±)-epibatidine, (−)-nicotine, (−)-ferruginine, and (−)-norferruginin

Structure	Compd.	$(\alpha4)2(\beta2)3^{b)}$ (±)-[^{3}H]EB Rat brain K_i (nM)	$\alpha7^{*b)}$ [^{3}H]MLA Rat brain K_i (nM)	$\alpha3\beta4^{*b)}$ (±)-[^{3}H]EB Pig. adrenal gland K_i (nM)	$(\alpha1)2\beta1\gamma\delta$ [^{3}H]EB *Torp. calif.* electroplax K_i (nM)
	(±)-Epibatidine (**1**)	0.008 ± 0.001	4 ± 0.5 [^{125}I] α-BTX	0.022 ± 0.0015	4.0 ± 0.3 7.5 ± 0.5 [^{125}I] α-BTX
	(−)-Nicotine (**3**)	0.838 ± 0.132	127 ± 5 [^{125}I]α-BTX	73 ± 2	1000 ± 0.1 [^{125}I] α-BTX
	(−)-Ferruginine (**6**)	120 ± 2	330 ± 23	1,455 ± 319	>50,000
	(−)-Nor-ferruginine (**6a**)	94 ± 5	>100,000	2,300 (n = 1)	10,805 (n = 1)
	**8a**	3.4 ± 1.4	53.3 ± 9.3	44 ± 5.7	4,390 ± 50
	**8b**	1.1 ± 0.2	56.6 ± 3.1	20.7 ± 2	1,361 (n = 1)
	*ent*-**8b**	13.2 ± 3.7	371 ± 31.8	156.5 ± 3.5	1,430 ± 24
	**9a**	12.6 ± 0.07	500 (n = 1)	234 ± 16.9	15,269 (n = 1)
	**9b**	713 ± 49	10,000 (n = 1)	6,444 ± 50	>50,000 (n = 1)
	**9c**	29.7 ± 0.8	1,300 (n = 1)	1,559 ± 297	>50,000 (n = 1)
	*ent*-**9c**	29.5 ± 4.7	2,225 ± 353	305 ± 148	>50,000 (n = 1)

[a] Values represent mean ± SEM obtained from n independent experiments where n = 3–5
[b] Naturally expressed nAChRs.

teristic of α-BTX-sensitive, putative α7* subunit-containing nAChRs (Davies et al. 1999; Lukas et al. 1999). To estimate the affinity for nAChRs containing α3 and β4 subunits an assay using (±)-[^{3}H]epibatidine and a membrane fraction from pig adrenal glands was developed. This assay was based on a previous study, which showed that (±)-[^{3}H]epibatidine, in addition to its high affinity for $(\alpha4)_2(\beta2)_3$ nAChRs in rat brain, bound to cells stably expressing receptors of the α3β4* subtype (Stauderman et al. 1998; Xiao et al. 1998). Further studies (Criado et al. 1997; Wenger et al. 1997) suggested that the adrenal glands were rich in nAChR subtypes. Binding assays with (±)-[^{3}H]epibatidine using pig adrenal gland membranes demonstrated a single population of binding sites (data not shown) with a K_d value of 50 ± 7 pM (n = 5) comparable to a previous study using rat adrenal glands (Mukhin et al. 2000). To determine the affinity for $(\alpha1)_2\beta\gamma\delta$ nAChR (muscle type) a radioligand binding assay using (±)-[^{3}H]epibatidine and total membrane fractions of *Torpedo californica* electric organ was established.

(±)-[^{3}H]epibatidine binds with high affinity (K_d value of 2.0 ± 0.3 nM) to $(\alpha1)_2\beta\chi\delta$ nAChR. In a previous assay with ^{125}I-α-bungarotoxin as radioligand (±)-epibatidine was found to exhibit a K_i value of 7.5 nM (Mukhin et al. 2000). In contrast to ^{125}I-α-bungarotoxin, (±)-[^{3}H]epibatidine showed low nonspecific binding, and did not exceed 10% of total binding. This is an agreement with radioligand binding assays using (±)-[^{3}H]epibatidine for $(\alpha4)_2(\beta2)_3$ and α3β4* nAChR subtypes. With the exception of (±)-epibatidine which binds with affinity [K_i value of 7.5 nM, (Mukhin et al. 2000); K_i = 4.0 0.5, Table] to muscle type nAChR, all other compounds present low (micromolar range) or no affinity for $(\alpha1)_2\beta\gamma\delta$ nAChR.

As shown in the Table the above characterized competition assays yielded K_i values of 0.008 nM for (±)-epibatidine (**1**) and 0.84 nM for (−)-nicotine (**3**) for the $(\alpha4)_2(\beta2)_3$ subtype. These results are consistant with recently reported *in vitro* measurements of the alkaloids (Gündisch et al. 1999; Mukhin et al. 2000). Compared to (−)-nicotine (**3**) the ferruginines **6** and **6a** exhibited a ca. 130-fold lower affinity (K_i = 120 and 94 nM, resp.) for the $(\alpha4)_2(\beta2)_3$ subtype and approximately a ca. 2.6-fold lower affinity for the α7* subtype (**6** : K_i = 330 nM , Gündisch et al. 2001).

The bioisosteric replacement of the 2-acetyl moiety as structural part of the lead alkaloid **6** by a 3-pyridyl, 5-chloropyridyl, 5-pyrimidinyl, 2-pyrazinyl or 4-pyridazinyl pharmacophoric element led to ferrugininoids **8** and **9** [structurally close to (−)-ferruginine (**6**)] which were identified as potent nicotinic ligands and which differentially activated specific subtypes of nAChRs. This could be demonstrated by studies of the *in vitro* affinity for $(\alpha4)_2(\beta2)_3$, α7*, α3β4*, and $(\alpha1)_2\beta1\gamma\delta$ nAChR subtypes by the above mentioned radioligand competition assays. Thus, the azine and diazine analogues **8** and **9** can be considered as pharmacologically attractive bioisosteres of the lead **6** with a profound effect of the heteroaryl moiety on the binding potency and selectivity toward the nAChRs under investigation. Generally the azine substituted bioisosteres **8** exhibited higher affinities than the diazines **9**. Similar to results of the anatoxin-a series (Gohlke et al. 2002; Sharpless et al. 2002) the novel ferrugininoids **8** and **9** proved to be more potent than the lead **6**. The 5-chloropyridyl – containing modification **8b** turned out to be the most active nAChR ligand in the ferrugininoid series. Like (−)-nicotine (**3**) (K_i = 0.84 nM) this ligand interacts potently (K_i = 1.1 nM) with the $(\alpha4)_2(\beta2)_3$ subtype, ca. 100-fold more intensely compared to the lead **6**, and differentiates, similarly to **3**, among the nAChR subtypes investigated. The relative affinities of ligand **8b** for the four subtypes are $(\alpha4)_2(\beta2)_3 : \alpha7^* : \alpha3\beta4^* : (\alpha1)_2\beta1\gamma\delta$ = 1 : 50 : 18 : 1215.

That means, the novel ferrugininoid **8b** binds with e.g. 18-fold lower affinity to the ganglionic α3β4* subtype [that probably accounts for toxicity e.g. of (−)-nicotine (**3**)] than to the $(\alpha4)_2(\beta2)_3$ subtype.

Probing the issue of enantioselectivity which is important for refining the concept of the nicotinic pharmacophore we examined whether ferrugininoids of type **8** or **9** have the same stereochemical bias to nicotinic receptors as e.g. observed with (*S*)-nicotine (**3**). This possesses a 14-fold higher affinity than (*R*)-nicotine *ent*-(**3**) e.g. toward the $(\alpha4)_2(\beta2)_3$ nAChR subtype. In contrast, this stereodiscrimination is not observed for epibatidine (**1**) that interacts with nAChRs in a nonstereoselective manner (Abreo et al. 1996; Schmitt, 2000; Tønder and Olesen, 2001). Interestingly, ligand **8b** resembles (−)-nicotine (**3**) with respect to the enantioselectivity. The Table reveals that the (1*R*,5*S*)-enantiomer **8b** exhibits a ca. 12-fold higher affinity e.g. toward the $(\alpha4)_2(\beta2)_3$ nAChR subtype than the corresponding (1*S,* 5*R*)-form *ent*-**8b**. This stereodiscrimination is more or less also true in regard to the different nAChR subtypes under investigation.

To examine the bioisosteric potential of diazines also in the field of ferruginine-type structures we investigated the effect of three diazine moieties on binding affinity. Generally, when an additional nitrogen atom is incorporated into the heteroaromatic HBA/π pharmacophoric element a deleterious effect with regard to the affinity is observed, a well known fact in view of diazine substituted variants of epibatidine (**1**) (Seerden et al. 1998; Che et al. 2001) and anatoxin-a (**5**) (Gohlke et al. 2002; Sharpless et al. 2002). Among the diazine substituted ferrugininoids **9** the pyrimidine-containing bioisostere **9a** turned out to be the most active ligand, however, as expected (Gohlke et al. 2002; Gohlke et al. 2003) being approximately 10-fold less active toward the nAChR subtypes under investigation than the chloropyridyl-substituted ligand **8b**.

The affinity profile of the novel ferrugininoids **9a**–**c** demonstrates that the three isomeric diazine heterocycles are suitable bioisosteres to the acetyl moiety of the lead compound **6**; however, a change from a 1,2- to a 1,3- or 1,4-diazine results in nAChR ligands of different affinities and selectivities. The trend regarding the effect of the diazine moiety on binding affinities is similar to that in the anatoxinoid series (Gohlke et al. 2002) with an order of potency 5-pyrimidinyl > 4-pyridazinyl > 2-pyrazinyl (cf. **9a**, **9b**, **9c**). Surprisingly, the stereodiscrimination e.g. toward the $(\alpha4)_2(\beta2)_3$ nAChR subtype found for the chloropyridine substituted ferrugininoids **8b**/*ent*-**8b** is not observed for the pyridazine substituted analogs **9c**/*ent* **9c**. These enantiomeric ligands interact with the $(\alpha4)_2(\beta2)_3$ nAChR subtype in a nonstereoselective manner; toward the α3β4* subtype however ligand *ent*-**9c** exhibited a ca. 5-fold higher affinity. Concerning the effect of the azabicyclic moiety with respect to *N*-methylation in tropane analogs, the trends are contrary to those found for nicotine/nornicotine [difference in affinity of a factor 2.3 : 25.1, (Abreo et al. 1996)] and more similar to those found for (−)-cytisine/(−)-caulophyline (Imming et al. 2001). In this case there is a difference in affinity of a factor 56 between the two ligands. Generally the ferrugininoids of the diazine series are ca. 3-fold less potent compared to the corresponding norferrugininoids (Gündisch et al. 2001).

2.3. Conclusion

Bioisosterism once again proved to be a successful approach in medicinal chemistry for the rational modification e.g. of the lead (−)-ferruginine (**6**) into ligands with higher affinities and more or less improved selectivities. Here we investigated the bioisosteric potential of two azines and three diazines replacing the 3-acetyl HBA/π pharmacophoric element of (−)-ferruginine (**6**) by a 3-pyridyl, 5-chloropyridyl, 5-pyrimidinyl, 2-pyrazinyl, and 4-pyridazinyl moiety. Utilizing the tropane-based vinyl triflate **12** and (−)-anhydroecgonine (**18**) as versatile chiral building blocks, the enantiopure bioisosteres **8a, b** and **9a**–**c** of the lead **6** are easily accessible. The Pd(0)-catalysed Suzuki cross-coupling and the [4+2] cycloaddition with inverse electron demand constitute the key reactions. Studies of the *in vitro* affinity toward four important nAChR subtypes by radioligand binding assays demonstrated that the novel ferrugininoids **8** and **9** can be considered as pharmacologically attractive bioisosteres of the lead **6** with different effects on the binding affinity and selectivity dependent on the heterocyclic HBA/π pharmacophoric element of the nAChR ligands.

3. Experimental

For general procedures and *in vitro* binding studies, see Gohlke et al. (2002).

3.1. Chemistry

3.1.1. (1R, 5S)-Trop-2-ene-2-trifluoromethylsulfonate (**12**)

To a solution of dried diisopropylamine (607 mg, 6.00 mmol) in dry THF (15 mL) was added dropwise within 20 min at −90 °C a solution of *n*-butyllithium (3.6 mL, 5.8 mmol, 1.6 M) in hexane via syringe. After the mixture was stirred for 40 min at −80 °C a solution of ketone **11** (716 mg, 5.14 mmol) in dry THF (5 mL) was added dropwise within 25 min at −85 °C. The mixture was stirred at −85 °C for 2.5 h, then a solution of 2-[*N*,*N*-bis(trifluoro-methansulfonyl)-amino]-5-chloropyridine (2.14 g, 5.45 mmol) in dry THF (5 mL) was added under argon. The mixture was stirred at −80 °C for 5 h, and then allowed to warm to room temperature within 12 h. The solvent was removed from the reaction mixture *in vacuo*, and the residue was purified by flash chromatography on silica gel (column 5 × 25 cm, eluent ethyl acetate) to provide **12** as an orange-yellow oil (1.31 g, 94%), $R_f = 0.33$ (ethyl acetate) $[\alpha]_D^{20} = -28.9$ (c = 0.2 in CH_3OH); IR (film): ν (cm^{-1}) = 2951, 1716, 1673, 1591; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.52–1.57 (m, 1 H, 6-H), 1.78–1.88 (dd, J = 18.0 Hz, J = 4.2 Hz,1 H, 6-H), 2.08–2.15 (m, 2 H, 7-H), 2.15–2.17 (m, 1 H, 4-H), 2.44 (s, 3 H, NCH_3), 2.60–2.65 (m, 1 H, 4-H), 3.25–3.27 (m,1 H, 5-H), 3.33 (m, 1 H, 1-H),5.58–5.59 (m, 1 H, 3-H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 28.9, 30.1, 34.2, 34.5, 55.8, 61.3, 112.8, 118.4 (q, $^1J_{CF} = 318.6$ Hz), 149.8; MS (70 eV), m/z (%): 271 (9, M^+), 138 (100); Exact mass calcd for $C_9H_{12}F_3NO_3S$: 271.0490, found 271.0482.

3.1.2. (1R,5S)-8-Methyl-2-(pyridin-3′-yl)-8-azabicyclo[3.2.1]oct-2-ene (**8a**)

To a solution of bis(triphenylphosphane)palladium(II) chloride (8 mg, 0.01 mmol) and the organoborane **13** (220 mg, 1.4 mmol) in THF (5 mL) an aqueous solution of sodium carbonate (2M, 2mL) was added and the mixture heated to 80 °C. Then a solution of triflate **12** (270 mg, 1.0 mmol) in THF (5 mL) was added dropwise and the mixture heated at 80 °C for 18 h. Water (20 mL) was added and the mixture extracted with dichloromethane (4 × 30 mL). The combined organic phases were dried with Na_2SO_4, filtered and the solvent evaporated in vacuo. The residue was purified by column chromatography on silica gel (column 2 × 10 cm, CH_2Cl_2 : CH_3OH = 3 : 1) to yield compound **8a** (163 mg, 81%) as a pale yellow oil. $R_f = 0.31$ (CH_2Cl_2 : CH_3OH = 3 : 1). $[\alpha]_D^{21} = -48.0$ (c = 0.10, CH_3OH); IR (film): ν (cm^{-1}) = 2943, 1567, 1444, 1413; UV ($CHCl_3$): λ_{max} (lgε) = 301 nm (2.45), 266 (3.01); ^1H NMR (500 MHz, $CDCl_3$) δ = 1.56–1.57 (m, 1 H, 6-H), 1.75–1.82 (dd, J = 18.6 Hz, J = 4.4 Hz, 1 H, 6-H), 1.88–1.91 (m, 1 H, 7-H), 2.15–2.17 (m, 2 H, 4-H and 7-H), 2.39 (s, 3 H, NCH_3), 2.58–2.62 (d, broad, J = 18.8 Hz, 1 H, 4-H), 3.23–3.26 (m, 1 H, 5-H), 3.59–3.60 (m, 1 H, 1-H),5.85- 5.86 (m, 1 H, 3-H), 7.17–7.18 (m, 1 H, 5′-H), 7.51–7.53 (m, 1 H, 4′-H), 8.39–8.40 (m, 1 H, 6′-H) 8.51–8.52 (m,1 H, 2′-H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 30.2, 31.3, 34.2, 36.2, 57.2, 61.5, 121.4, 123.1, 132.2, 135.8, 137.9, 146.7, 148.0; MS (70 eV), m/z (%): 200 (75, M^+), 171 (100). Exact mass calcd. for $C_{13}H_{16}N_2$: 200.1313; found 200.1321.

3.1.3. (1R,5S)-2-(2′-Chloropyridin-5′-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene (**8b**) *and (ent-***8b**)

According to the same protocol as described for the synthesis of ligand **8a** 152 mg (65%) of compound **8b** were obtained as a yellow oil from 220 mg (1.4 mmol) of the organoborane **14** and 270 mg (1.0 mmol) of triflate **12**. Column chromatography on silica gel (column 2 × 10 cm, eluent CH_2Cl_2 : CH_3OH = 9 : 1); $R_f = 0.45$; $[\alpha]_D^{20} = -44.3$ (c = 0.1 in CH_3OH); IR (film): ν (cm^{-1}) = 3045, 2941, 2878, 1634, 1580; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.50–1.60 (m, 1 H, 6-H), 1.81–1.83 (dd, J = 18.8 Hz, J = 4.4 Hz, 1 H, 6-H), 1.86–1.89 (m, 1 H, 7-H), 2.15–2.25 (m, 2 H, 4-H and 7-H), 2.41 (s, 3 H, NCH_3), 2.55–2.65 (m, 1 H, 4-H), 3.26–3.40 (m, 1 H, 1-H), 3.56–3.57 (m, 1 H, 5-H), 5.87–5.88 (m, 1 H, 3-H), 7.18–7.21 (m, 1 H, 3′-H), 7.49–7.51 (dd, J = 8.2 Hz, J = 2.5 Hz, 1 H, 4′-H), 8.26–8.27 (d, J = 2.7 Hz, 1 H, 6′-H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 30.0, 31.4, 34.1, 36.4, 57.2, 61.5, 122.2, 123.8, 134.6, 135.1, 136.9, 146.4, 149.7; MS (70 eV), m/z (%) = 234 (100, M^+). Exact mass calcd. for $C_{13}H_{15}ClN_2$: 234.0923, found 234.0903.

Ligand *ent-***8b** was prepared by the same procedure from triflate *ent-***12** (704 mg, 2.60 mmol) and organoborane **14** (5.72 mg, 3.63 mmol) to yield 517 mg (85%) of *ent-***8b** as a yellow oil with $[\alpha]_D^{20} = +43.4$ (c = 0.31 in CH_3OH). Further analytical data of *ent-***8b** correspond to those of the enantiomeric **8b**.

3.1.4. (1R,5S)-8-Methyl-2-(pyrimidin-5′-yl)-8-azabicyclo[3.2.1]oct-2-ene (**9a**)

According to the same protocol as described for the synthesis of ligand **8a** 103 mg (51%) of compound **9a** were obtained as a pale yellow oil from 260 mg (1 mmol) of triflate **12** and 530 mg (3.0 mmol) of the organoborane salt **15** resolved in THF : ethanol = 3 : 1 (5 mL). $R_f = 0.22$ (CH_2Cl_2 : CH_3OH = 3 : 1). $[\alpha]_D^{20} = -23.6$ (c = 0.13 in $CHCl_3$). IR (film): ν (cm^{-1}) = 2955, 2368, 1660, 1580; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.60–1.68 (m, 1 H, 6-H), 1.89–1.95 (2m, 2 H, 6-H and 7-H), 2.25–2.28 (m, 2 H, 7-H and 4-H), 2.47 (s, 3 H, NCH_3), 2.65–2.72 (m, 1 H, 4-H), 3.36–3.38 (m, 1 H, 4-H), 3.70–3.71 (m, 1 H, 1-H), 5.98–5.99 (m, 1 H, 3-H), 8.63 (s, 2 H, 4′-H and 6′-H), 9.03 (s, 1 H, 2′-H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 29.7, 31.5, 34.0, 36.3, 57.4, 61.2, 123.5, 132.9, 135.0, 153.2 (2C), 157.3; MS (70 eV), m/z (%): 201 (77, M^+), 172 (100). Exact mass calcd. for $C_{12}H_{15}N_3$: 201.1265; found: 201.1263

3.1.5. (1R,5S)-8-Methyl-2-(pyrazin-2′-yl)-8-azabicyclo[3.2.1]octan-2-ol (**17**)*, mixture of diastereomers*

BuLi (5 mL of a 1.6 M solution in hexane, 9.7 mmol) was added dropwise at −35 °C to a solution of 2-iodopyrazine (1.00 g, 4.85 mmol) in dry diethyl ether (25 mL), at −35 °C. The solution was stirred for 2 h before adding dropwise a solution of ketone **11** (0.69 g, 4.85 mmol) in dry diethyl ether (10 mL) within 45 min, at −35 °C. The brownish suspension was allowed to warm to RT and stirred for 2.5 h at ambient temperature. Water (10 mL) was added, and the mixture was stirred for 15 min before separating the organic phase and washing with water (2 × 5 mL). The combined aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL) and the organic phase dried with Na_2SO_4 (5 g), filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel (column 3 × 25 cm, CH_2Cl_2 : CH_3OH = 9 : 1) to give compound **17** (500 mg, 49%) as yellow oil, a mixture of two diastereomeres in a 7 : 1 ratio as calculated from the ^1H NMR spectral data (relative intensity of the integrals of the N-CH_3 protons). IR (film): ν (cm^{-1}) = 3377, 3053, 2796, 1650; MS (70 eV), m/z (%): 219 (88, M^+), 82 (100). Exact mass calcd. for $C_{12}H_{17}N_3O$: 219.1371; found 219.1366.

The mixture was utilized for the elimination process without separation and further characterization.

3.1.6. (1R,5S)-8-Methyl-2-(pyrazin-2′-yl)-8-azabicyclo[3.2.1]oct-2-ene (**9b**)

To a solution of the diastereomeric mixture of the alcohols **17** (490 mg, 2.23 mmol) in CH_2Cl_2 (25 mL) was added dropwise at 0 °C a solution of thionyl chloride (600 mg, 5.05 mmol) in CH_2Cl_2 (5 mL). The solution was warmed to RT and stirred for 2 h at this temperature. Water (15 mL) was added and the mixture stirred for 15 min. The aqueous phase was brought to pH = 8 by adding dropwise a saturated aqueous K_2CO_3-solution. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with Na_2SO_4 (5 g), filtered and the solvent evaporated *in vacuo*. The residue was chromatographed on silica gel (column 2 × 15 cm, CH_2Cl_2 : CH_3OH = 3 : 1) to give ligand **9b** (300 mg, 67%) as a yellow oil. $R_f = 0.44$; $[\alpha]_D^{20} = -48$ (c = 0.1 in CH_3OH); UV (CH_3OH): λ_{max} (lgε) = 243 nm (3.19), 289 (4.01); IR (film): ν (cm^{-1}) = 2963, 2548, 1642; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.83–1.85 (m, 1 H, 6-H), 2.13–2.15 (m, 1 H, 7-H), 2.30–2.35 (dd, J = 19.8 Hz, J = 4.4 Hz, 1 H, 6-H), 2.50–2.70 (2m, 2 H, 6-H and 7-H), 2.77 (s, 3 H, NCH_3), 3.05–3.14 (m, broad, 1 H, 4-H), 3.86–3.88 (m, 1 H, 5-H), 4.80–4.82 (m, 1 H, 1-H), 6.54–6.55 (m, 1 H, 3-H), 8.41 (dd, J = 2.5 Hz, J = 1.6 Hz, 1 H, 6′-H), 8.44 (dd, J = 2.6 Hz, J = 1.6 Hz, 1 H, 5′-H), 8.76–8.77 (d, J = 1.6 Hz, 1 H, 3′-H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 28.4, 31.6, 32.9, 34.5, 58.9, 60.0, 124.3, 135.7, 141.3, 143.3,

143.4, 144.3, 149.5; MS (70 eV), m/z (%): 201 (100, M^+). Exact mass calcd. for $C_{12}H_{15}N_3$: 201.1266, found 201.1265.

3.1.7. E- and Z-(1R,5S)-2-(2′-Methoxyethenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene, (E)- and (Z)-(**20**)

Potassium *tert*.-butanolate (2.37 g, 21.1 mmol) was added over a period of 0.5 h via a side-arm addition funnel under an argon atmosphere to a suspension of (methoxymethyl)-triphenylphosphonium chloride (7.23 g, 21.1 mmol) in anhydrous diethyl ether (60 mL) at −15 °C. The reaction mixture was stirred at −15 °C for 2 h, then a solution of aldehyde **19** (1.7 g, 11.32 mmol) in anhydrous diethyl ether (20 mL) was added over a period of 30 min at this temperature. Stirring at RT was continued for 24 h. The reaction mixture was hydrolyzed with water (50 mL) at RT, and the organic and aqueous phases were separated. The aqueous phase was extracted with diethyl ether (4 × 70 mL) and petroleum ether (40–60 °C, 2 × 70 mL), and the combined organic layers were dried with magnesium sulfate (15 g). Cooling of the filtrate to −30 °C for 24 h resulted in crystallization of triphenylphosphine oxide, which was separated by filtration. Concentration in vacuo at ca. 25 °C yielded an oil which was purified by "Kugelrohrdestillation" (80 °C, 0.1 Torr). Yield 867 mg (51%), colorless oil of a mixture of *E*- and *Z*-**20** in the ratio 10 : 6 as estimated from the integrals of the signals of the OCH_3-protons δ = 3.55 and 3.48, respectively in the raw ^{1}H NMR spectrum of **20**. Because of instability the stereoisomeric enol ethers (*E*)- and (*Z*)-**20** were used for the following [4+2] cycloaddition reaction without further characterization.

3.1.8. (1R,5S)-8-Methyl-2-(pyridazinyl-4′-yl)-8-azabicyclo[3.2.1]-oct-2-ene (**9c**) *and ent-*(**9c**)

To a solution of the enol ether **20** (360 mg, 2.0 mmol) of raw material in dried toluene (10 mL) was added dropwise a solution of the tetrazine **21** (250 mg, 3.0 mmol) in dried toluene (10 mL). The solution was heated under Ar at reflux for 20 h until the red colour of the mixture turned yellow. The mixture was cooled to RT and after evaporation of the solvent in vacuo the residue was purified by flash CC (column 20 × 3 cm, eluting with $CH_2Cl_2 : CH_3OH = 9 : 1$) to provide **9c** as a pale yellow oil (326 mg, 81%); $R_f = 0.14$ ($CH_2Cl_2 : CH_3OH = 9 : 1$). $[\alpha]_D^{20} = -41.8°$ (c = 0.1 in $CHCl_3$); IR (film): ν (cm^{-1}) = 3045, 2946, 1737, 1653, 1551; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (dd, J = 1.1 Hz, J = 2.4 Hz, 1 H, 3′-H), 9.01 (dd, J = 5.5 Hz, J = 1.1 Hz, 1 H, 6′-H), 7.25 (dd, J = 2.4 Hz, J = 5.5 Hz, 1 H, 5′-H), 6.24–6.25 (m, 1 H, 3-H), 3.61–3.62 (m, 1 H, 1-H), 3.26–3.29 (m, 1 H, 5-H), 2.60–2.68 (m, 1 H, 4-H), 2.37 (s, 3 H, NCH_3), 2.18–2.20 (m, 2 H, 4-H and 7-H), 1.84–1.89 (m, 2 H, 6-H and 7-H), 1.57–1.59 (m, 1 H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.1, 148.3, 137.3 136.1, 126.6, 120.7, 60.5, 57,2 36.4, 34.3, 31.8, 30.2. MS (70 eV), m/z (%): 201 (100, M^+). Exact mass calcd. for $C_{12}H_{15}N_3$: 201.1266, found 201.1265. Ligand *ent*-**9c** was prepared by the same procedure from enol ether *ent*-**19c** (360 mg, 2 mmol). Yield 298 mg (74%), $[\alpha]_D^{20} = +41.6$ (c = 0.1 in $CHCl_3$). Further analytical data of *ent*-**9c** correspond to those of the enantiomeric **9c**.

3.2. In vitro binding studies

Membrane preparation, binding assays, and data analysis see Gohlke et al. (2002); Gündisch et al. (2001) and (2002).

3.2.1. $(\alpha 1)_2\beta\gamma\delta$ nAChR

Frozen *in vitro* binding studies for *Torpedo californica* electric organ was purchased from Marinus Inc. (Long Beach, CA, U.S.A.). Total membrane fraction from *Torpedo californica* electric organ were obtained according the procedure of Mukhin et al. 2000. Assays were carried out in Hepessalt solution (Gündisch et al. 1999) at 22 °C. Each assay was performed in duplicates. Non specific binding was determined in the presence of 300 μM (−)-nicotine. Membranes were incubated for 90 min in 0.5 ml HSS containing 0.5 nM (±)-[^{3}H]epibatidine and different concentrations of test compounds. The reaction was terminated by vacuum filtration through Whatman GF/B glass fiber filters, pre-soaked in 1 % poly(ethylenimine) using a Brandel 48 – channel cell harvester. The radioactivity was measured using a liquid scintillation counter (Tri-Carb 2100 TR; Packard, Dreieich, Germany). Competition binding data were analyzed using nonlinear regression methods. K_i values were calculated by the Cheng-Prusoff equation (K_i = independent experiments preformed on the same membrane preparations that were used for the competition assays. $IC_{50}/(1 + F/K_d)$, where F is the used radioligand concentration) based on the measured IC_{50} values and $K_d = 2$ nM for binding of (±)-[^{3}H]epibatidine. The K_d values were obtained from five independent experiments preformed on the same membrane preparations that were used for the competition assays.

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