Pyrazole Derivatives as Partial Agonists for the Nicotinic Acid Receptor

T. van Herk,† J. Brussee,† A. M. C. H. van den Nieuwendijk,† P. A. M. van der Klein,† A. P. IJzerman,*,† C. Stannek,‡ A. Burmeister,‡ and A. Lorenzen‡

Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, P.O. Box 9502, 2300 RA Leiden, The Netherlands, and Institute of Pharmacology, Im Neuenheimer Feld 366, University of Heidelberg, D-69120 Heidelberg, Germany

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Nicotinic acid as a hypolipidemic agent appears unique due to its potential to increase HDL cholesterol levels to a greater extent than other drugs. However, it has some side effects, among which severe skin flushing is the most frequent and often limits patients' compliance. In a search for novel agonists for the recently identified and cloned G protein-coupled nicotinic acid receptor, we synthesized a series of substituted pyrazole-3-carboxylic acids that proved to have substantial affinity for this receptor. The affinities were measured by inhibition of [^{3}H]nicotinic acid binding to rat spleen membranes. Potencies and intrinsic activities relative to nicotinic acid were determined by their effects on [^{35}S]GTPγS binding to rat adipocyte and spleen membranes. Interestingly, most compounds were partial agonists. In particular, 2-diazabicyclo-[3,3,0^{4,8}]octa-3,8-diene-3-carboxylic acid (**4c**) and 5-propylpyrazole-3-carboxylic acid (**4f**) proved active with K_i values of approximately 0.15 μM and EC_{50} values of approximately 6 μM, while their intrinsic activity was only ∼50% when compared to nicotinic acid. Even slightly more active was 5-butylpyrazole-3-carboxylic acid (**4g**) with a K_i value of 0.072 μM, an EC_{50} value of 4.12 μM, and a relative intrinsic activity of 75%. Of the aralkyl derivatives, **4q** (5-(3-chlorobenzyl)pyrazole-3-carboxylic acid) was the most active with a relatively low intrinsic activity of 39%. Partial agonism of the pyrazole derivatives was confirmed by inhibition of G protein activation in response to nicotinic acid by these compounds. The pyrazoles both inhibited the maximum effect elicited by 100 μM nicotinic acid and concentration dependently shifted nicotinic acid concentration−response curves to the right, pointing to a competive mechanism of action.

Introduction

Nicotinic acid is one of the drugs used in hyperlipidemia. It inhibits the formation of nonesterified fatty acids from adipose tissue. As a consequence the hepatic synthesis of triglycerides, the secretion of VLDL, and its conversion into LDL are reduced. Interestingly, nicotinic acid increases serum HDL concentrations by approximately 25% at a standard daily dose of 1−2 g. The latter characteristic is very beneficial, and not seen with any of the other standard regimens, including the use of statins.[1]

The target for nicotinic acid in the adipocyte is a G protein-coupled receptor. It has been characterized pharmacologically in rat adipocytes and spleen and mouse macrophages.[2,3] Nicotinic acid is a full agonist and has submicromolar affinity for this receptor, as has acipimox, a close analogue that is also used clinically (see Figure 1 for both structures). The receptor is coupled to the enzyme adenylate cyclase through $G_{i/o}$ proteins; upon activation of the receptor adipocyte cAMP levels are reduced. These findings have yielded facile screening methods in the form of receptor binding assays with [^{3}H]nicotinic acid as the radioligand to determine the affinity of novel chemical entities, and [^{35}S]GTPγS binding assays to assess potency and intrinsic activity of such compounds. These methods proved also instrumental in the cloning of the nicotinic receptor that was reported very recently. It appears that two receptor subtypes may exist with high and low affinity for nicotinic acid, respectively.[4]

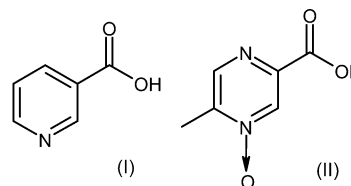

Figure 1. Structures of nicotinic acid (I) and acipimox (II).

The favorable characteristics of nicotinic acid can be accompanied by disturbing side effects, including flushing of the skin, an effect that is judged unbearable by 10% of patients. Apparently, the actions of nicotinic acid are not tissue selective. Tissue selectivity may potentially be achieved with partial agonists. Partial agonists can behave as full agonists in tissues with high receptor density and/or efficient coupling to G proteins and effector enzymes, whereas in other tissues such compounds may have reduced efficacy or may even be "silent". We have adopted that reasoning for the design of partial agonists for the adenosine A_1 receptor, and were successful in synthesizing compounds with full antilipolytic activity on rat adipose tissue, whereas their cardiovascular effects were largely reduced.[5,6]

In analogy, we established a program for the design of partial agonists for the nicotinic receptor as well. We

* Corresponding author. Tel: (+31) 71 5274651. Fax: (+31) 71 5274565. E-mail: ijzerman@lacdr.leidenuniv.nl.
† Leiden/Amsterdam Center for Drug Research.
‡ University of Heidelberg.

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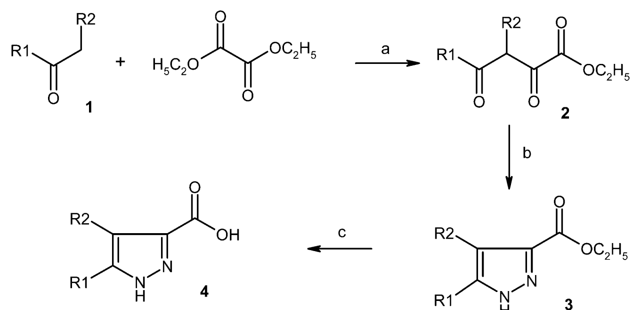
Scheme 1. Synthetic Route for Substituted Pyrazole-3-carboxylic Acids[a]



[a] (a) Na/ETOH, 18 h, rt; (b) H_2NNH_2/HAc, 8 h, reflux; (c) NaOH/dioxane, 18 h, rt.

were intrigued by a series of substituted pyrazole derivatives, reported by Seki et al. two decades ago.[7] These authors noticed substantial in vivo hypolipidemic activity for some of their compounds when administered to rats. We resynthesized a number of their derivatives (**4e**−**h** in the present study), synthesized other analogues as well, demonstrated their interaction with the nicotinic acid receptor, and developed structure−activity relationships. Most compounds tested appeared to be partial agonists for this receptor.

Results and Discussion

Chemistry. The synthetic route for the preparation of 5-substituted-1H-pyrazole-3-carboxylic acids involved a three-step synthesis and is depicted in Scheme 1. The first step for compounds **4e**−**s** consisted of a Claisen condensation of the appropiate alkyl-methyl ketone **1** (R2 = H) with diethyl oxalate by the use of sodium ethoxide in absolute ethanol. This afforded the corresponding α,γ-diketo esters **2**.[7,8] Alkyl-methyl ketones that were not commercially available (**1n**−**p**) were prepared by reaction of methyllithium with the corresponding carboxylic acid.[9,10] The cyclic compounds **2b**−**d** were prepared in the same way starting from butyrolactone,[11] cyclopentanone, and cyclohexanone,[12] respectively. The second step involved the addition of hydrazine hydrate in acetic acid to the α,γ-diketoesters **2** to form the pyrazole-3-carboxylic acid ethyl esters **3**.[8,13] In most cases, yields were high, except for the benzyl-substituted derivatives **3m**−**q**. In the latter case, enol formation (step b in Scheme 1) also occurred at the benzylic carbon position of the corresponding ketones, which rendered yields of the desired intermediates less than optimal. Hydrolysis of the ethyl esters with 0.25 M NaOH in H_2O/dioxane and acidification of the reaction mixture to pH 2 afforded the substituted pyrazole carboxylic acids **4b**−**s**.[8]

Biological Evaluation. Table 1 displays radioligand binding data for all the synthesized 5-substituted-1H-pyrazole-3-carboxylic acids. For reasons of comparison, data for nicotinic acid and potassium pyrazole-3-carboxylate (**4a**) were also included. [^{3}H]nicotinic acid (20 nM) was used as the radioligand. This compound binds with reasonably high affinity to rat spleen membranes (K_D = 23 nM) with an acceptable level of nonspecific binding (<30% of total binding at K_D).[2] It has similar binding characteristics on epididymal adipocyte membranes (the target tissue in hyperlipidemia), but due to higher yields per animal we preferred the spleen membrane preparation. All compounds with the exception of **4b** fully displaced [^{3}H]nicotinic acid from the receptor. The unsubstituted pyrazole-3-carboxylate **4a** was 18-fold less active in this respect than nicotinic acid, suggesting that the pyrazole pharmacophore is somewhat less well accommodated by the receptor than the pyridine ring system in nicotinic acid. For substitution on R1 alkyl, aryl and aralkyl groups were chosen. An alkoxy group linking positions R1 and R2 (as in **4b**) was not tolerated at all. Even at a concentration of 100 μM, this compound displayed less than 50% displacement of the radioligand. Smaller alkyl groups were introduced in **4c**−**g**, i.e., propyl, isopropyl, and butyl, either free or linked to R2. The (free) butyl substituent on R1 (**4g**) led to the highest affinity of the whole series, being only 2-fold less active than nicotinic acid. An extended alkyl chain as in **4h** was quite unfavorable for receptor affinity. Next (substituted) phenyl groups were introduced on R1 (**4i**−**l**). This modification yielded compounds with poor receptor affinity. Even the most active with a 4-methyl group on the phenyl ring (**4l**) was more than 500 times less active than **4g**. Going from phenyl (**4i**) to benzyl (**4m**), phenylethyl (**4r**) and phenylpropyl (**4s**), affinities improved with an optimum for benzyl (1.25 μM) and phenylethyl (1.57 μM). With **4m** as a lead, we synthesized a few more derivatives (**4n**−**q**) based on a substituent decision tree suggested by Topliss.[14] The 3-chlorobenzyl derivative (**4q**) was slightly more active than the parent compound.

The preferential coupling of the nicotinic acid receptor to $G_{i/o}$ proteins rendered the setup feasible of a [^{35}S]-GTPγS binding assay.[2] In this assay, the binding of the stable and radiolabeled GTP analogue [^{35}S]GTPγS is increased by the concomitant presence of receptor agonists. Minute quantities of protein usually suffice in such assays, which, in this case, allowed the use of both

Table 1. Inhibition of [^{3}H]Nicotinic Acid Binding to Rat Spleen Membranes

compd	R1	R2	K_i (μM, 95% CI)	
nicotinic acid			0.033	(0.028−0.040)
4a	H	H	0.594	(0.522−0.675)
4b	-CH_2CH_2O-		>100	
4c	-C_3H_6-		0.156	(0.133−0.184)
4d	-C_4H_8-		3.54	(2.21−5.67)
4e	*i*-C_3H_7	H	0.683	(0.500−0.931)
4f	C_3H_7	H	0.143	(0.121−0.169)
4g	C_4H_9	H	0.072	(0.060−0.085)
4h	$C_{11}H_{23}$	H	21.4	(15.6−29.2)
4i	C_6H_5	H	101	(81.7−125)
4j	3-Cl−C_6H_4	H	63.7	(39.7−102)
4k	4-Cl−C_6H_4	H	107	(91.8−126)
4l	4-CH_3−C_6H_4	H	37.9	(13.2−109)
4m	C_6H_5−CH_2	H	1.25	(1.15−1.37)
4n	4-Cl−C_6H_4 −CH_2	H	3.61	(2.48−5.27)
4o	4-CH_3−C_6H_4 −CH_2	H	20.3	(13.9−29.6)
4p	4-OCH_3−C_6H_4 −CH_2	H	66.0	(25.2−173.0)
4q	3-Cl−C_6H_4−CH_2	H	0.504	(0.450−0.566)
4r	C_6H_5−C_2H_4	H	1.57	(1.29−1.92)
4s	C_6H_5−C_3H_6	H	6.30	(5.51−7.20)

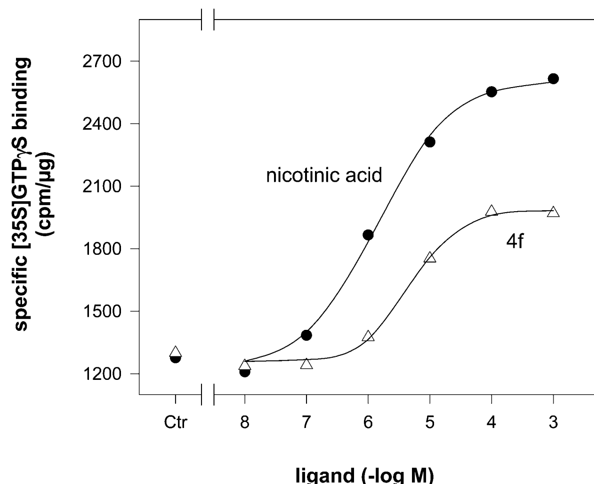


Figure 2. G protein activation by nicotinic acid and compound **4f** in rat adipocyte membranes. [^{35}S]GTPγS binding was performed as described in Experimental Section with 1 μg of membrane protein for 90 min at 25°C. Data from one representative experiment are shown. All experiments ($n = 3$) were performed in triplicate. (Ctr = control)

Table 2. Potencies (EC_{50} Values) and Relative Intrinsic Activities (RIA) in Stimulation of [^{35}S]GTPγS Binding to Rat Adipocyte and Spleen Membranes

comp	adipocytes EC_{50} (μM)		adipocytes RIA (%)	spleen EC_{50} (μM)		spleen RIA (%)
NA	1.70	(0.94−3.07)	100	0.74	(0.57−0.96)	100
4a	16.0	(12.5−20.5)	78.5 ± 3.4	21.5	(15.6−29.8)	85.3 ± 5.5
4c	6.44	(3.31−12.5)	51.7 ± 4.5	6.97	(4.31−11.3)	55.9 ± 6.2
4d	85.1	(70.6−103)	29.2 ± 2.7	64.6	(25.4−165)	47.2 ± 4.7
4e	13.7	(8.16−22.9)	49.2 ± 6.9	21.8	(13.6−34.8)	67.7 ± 10.0
4f	6.10	(4.78−7.79)	53.5 ± 2.2	5.09	(3.93−6.58)	69.9 ± 5.7
4g	4.12	(3.03−5.60)	75.7 ± 0.6	2.26	(1.00−5.13)	80.7 ± 5.6
4m	70.7	(35.0−143)	52.4 ± 7.5	86.9	(68.2−111)	50.3 ± 4.3
4q	46.6	(24.6−88.3)	39.4 ± 2.3	n.d.	n.d.	

rat spleen and rat adipocyte membranes. We selected the compounds with the highest affinities from Table 1 and tested them with respect to potency (EC_{50} values) and intrinsic activity relative to nicotinic acid (100%). Interestingly, all these compounds proved to be partial agonists in these assays. In Figure 2 the concentration−response curves for nicotinic acid and **4f** are graphically represented, showing the reduced maximal effect of the latter compound. Intrinsic activities varied between 29 and 85% for the pyrazole derivatives (Table 2). The intrinsic activities did not grossly differ between adipocyte and spleen membranes, which is also evident from Figure 3 showing a close correlation between the intrinsic activities in these two preparations. Compounds **4d**−**f** were somewhat more efficacious in spleen.

EC_{50} values are reported in Table 2 and ranged from 1.7 to 85.1 and 0.74 to 86.9 μM in the epididymal adipocyte and spleen preparation, respectively. A strong correlation ($r = 0.97$) was found between ligand potencies determined in adipocyte and spleen membranes (Figure 4, upper panel). In these activation assays EC_{50} values for nicotinic acid were approximately 1 μM, substantially higher than the K_i/K_D values (approximately 30 nM) observed in the binding assays. The experimental conditions in both assays are very different, however. The inclusion of sodium ions and GDP in the GTPγS binding assay, essential for a sufficient and robust "read-out", is known to reduce agonist affinity.

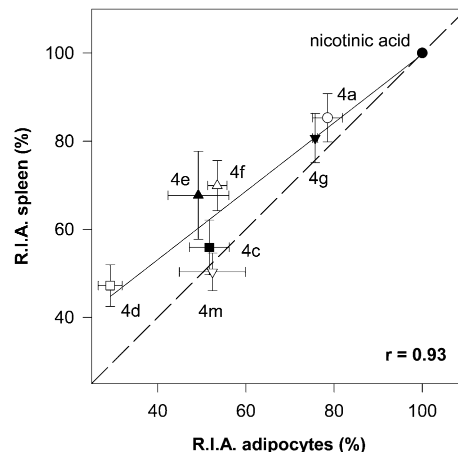
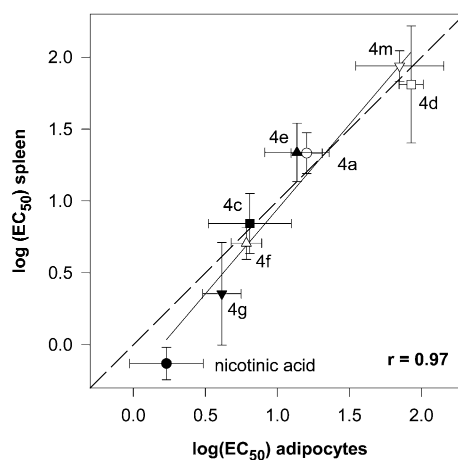


Figure 3. Comparison of intrinsic activities at rat adipocyte and spleen membranes. Relative intrinsic activities (RIA) of pyrazole compounds were determined in G protein activation studies and are relative to the maximum effect induced by nicotinic acid (100%). Dashed line indicates 1:1 stoichiometry.



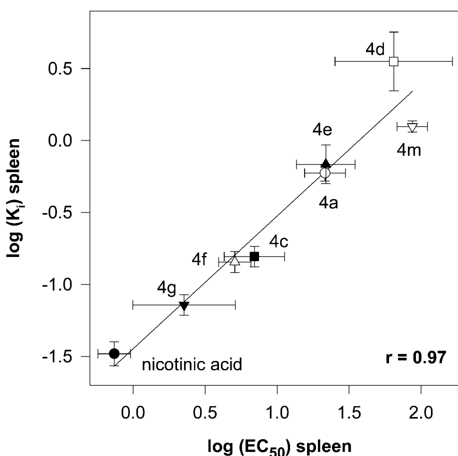


Figure 4. Comparison of affinities and potencies of nicotinic acid receptor ligands. Since receptor occupancy depends on the log concentration of ligands, both axes are log scale. The upper panel shows the correlation between potencies to activate G proteins at adipocyte and spleen membranes (dashed line indicates 1:1 stoichiometry). The lower panel shows the correlation between potencies in G protein activation and binding affinities in spleen membranes.

Correlations between K_i and EC_{50} values from the spleen preparation were also highly significant ($r = 0.97$; Figure 4, lower panel). The slopes of the two lines were

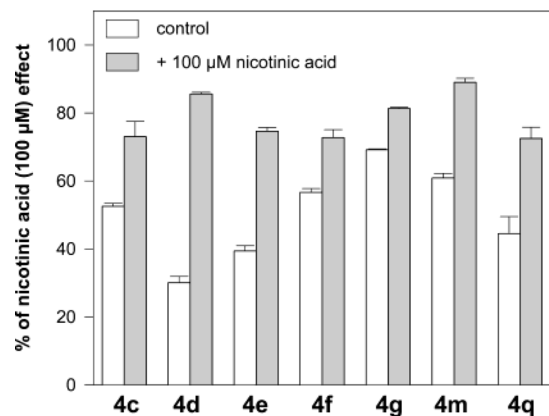


Figure 5. Stimulation of basal [^{35}S]GTPγS binding and inhibition of nicotinic acid-stimulated [^{35}S]GTPγS binding by pyrazole derivatives. [^{35}S]GTPγS binding was determined with 1 μg of adipocyte membrane protein in absence or presence of 100 μM nicotinic acid. Pyrazole derivatives (1 mM) stimulate basal [^{35}S]GTPγS binding (open columns) and inhibit the stimulatory effect of nicotinic acid (grey columns). Unstimulated binding of [^{35}S]GTPγS was 1191 $\pm$ 86 cpm/μg, binding in the presence of 100 μM nicotinic acid was 2634 $\pm$ 178 cpm. All experiments ($n = 3$) were performed in triplicate.

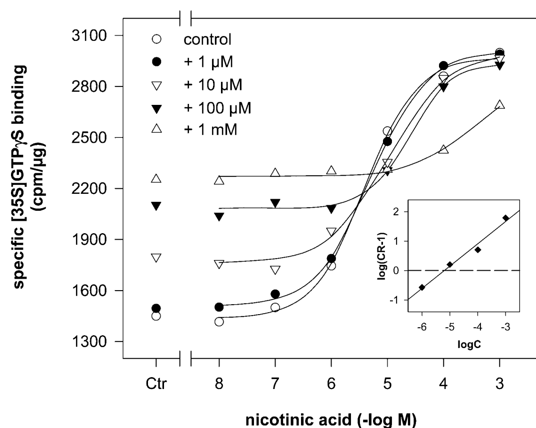


Figure 6. Inhibition by pyrazole **4f** of G protein activation by nicotinic acid. Concentration–effect curves for nicotinic acid in the absence or presence of increasing concentrations of **4f** are shown. [^{35}S]GTPγS binding to 1 μg of adipocyte membrane protein was performed as described in Experimental Section. The inset shows the Schild plot of the data. One out of four experiments, performed in triplicate, is shown.

close to unity, 1.18 (upper panel) and 0.92 (lower panel), respectively, suggestive of identical receptors and a common mechanism of action in all assays. Compounds **4c** and **4f** combined relatively high potency with substantially reduced intrinsic activity. Such materials may serve as lead structures for the development of antagonists of nicotinic acid receptors, which have not been described to date. They are, however, urgently required for the characterization of this receptor and may also be of therapeutic relevance, e.g., in the treatment of obesity.

To confirm that pyrazoles were indeed partial agonists at the nicotinic acid receptor, we investigated their effects on nicotinic acid-induced G protein activation in adipocyte membranes. [^{35}S]GTPγS binding was measured in the presence of 1 mM of the compounds under study, with or without the addition of 100 μM nicotinic acid (Figure 5). The pyrazoles increased [^{35}S]GTPγS binding in the absence of nicotinic acid to less than the maximum stimulation observed in the presence of 100 μM of this full agonist. When [^{35}S]GTPγS binding was measured in the presence of 1 mM of the pyrazoles and a maximally stimulating concentration (100 μM) of nicotinic acid under control conditions, it was observed that the pyrazoles inhibited guanine nucleotide binding. This inhibition of the effects of nicotinic acid, although indicative only, demonstrates that the pyrazoles investigated also possess antagonist activities, which is an intrinsic characteristic of partial agonists.

Therefore, this antagonistic effect was further investigated in more detail for compound **4f**. Concentration–response curves of G protein activation in adipocyte membranes for nicotinic acid in the absence or presence of increasing concentrations (1 μM to 1 mM) of pyrazole **4f** are shown in Figure 6. Nicotinic acid concentration-dependently stimulated [^{35}S]GTPγS binding. With the addition of increasing concentrations of **4f**, control binding in the absence of nicotinic acid increased, and the concentration–response curves for nicotinic acid were shifted to the right, yielding higher EC_{50} values for this full agonist. Evaluation of the experimental data by Schild plot analysis revealed competitive antagonism by pyrazole **4f** with a K_B value of 6.33 (4.36–9.19, $n = 4$) μM for inhibition of nicotinic acid effects, which is in good agreement with the EC_{50} value determined for **4f** (6.10 μM).

Little information seems to be available on the medicinal chemistry of nicotinic acid and related heterocyclic compounds, even for the pyrazole compounds in the present study. We have found previously that imidazole derivatives were inactive, and furan-3-carboxylic acid has only low potency at nicotinic acid receptors.[2] 5-Methyl- and 5-ethylpyrazole-3-carboxylic acid have been described as both hypoglycemic and hypocholesteremic compounds as assayed in in vivo animal models.[15,16] Another compound based on an isoxazole core rather than a pyrazole template, 3-methylisoxazole-5-carboxylic acid, also proved effective as an antilipolytic agent.[17] Substitution of the carboxylic group for a tetrazole function in nicotinic acid and derivatives appeared allowed, i.e., such compounds retained substantial antilipolytic activity.[18]

Conclusion

We synthesized a series of pyrazole derivatives with substantial affinity for the nicotinic acid receptor as present on rat epididymal adipocytes and spleen tissue. Some of the compounds had affinities comparable to nicotinic acid itself. Interestingly, the pyrazole derivatives were all partial agonists compared to nicotinic acid as identified in [^{35}S]GTPγS binding assays. Partial agonists generally induce tissue selectivity, which may be relevant in this particular case, since nicotinic acid, although very useful as a lipid-lowering agent, displays serious side effects occurring distantly from the target tissue. This series of compounds may provide useful tools for a more detailed functional characterization of the nicotinic acid receptor, which was recently cloned. Future medicinal chemistry efforts aiming at both partial agonists with even higher affinity and antagonists appear worthwhile.

Experimental Section

Chemicals and Solvents. Guanosine 5′(-γ-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS); 1250 Ci/mmol was obtained from

NEN Life Science Products (Rodgau, Germany). [5,6-^{3}H]-nicotinic acid was purchased from Biotrend (Cologne, Germany). 4-Methoxyphenylacetone, 3-chlorophenylacetic acid, 4-tolylacetic acid, 4-chloroacetophenone, 4-chlorophenylacetic acid, *p*-methylacetophenone, and methyllithium were purchased from Acros Organics ('s Hertogenbosch, The Netherlands). Nicotinic acid, 2-hexanone, hydrazine monohydrate, and bovine serum albumin (fraction V) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Isopropylmethyl ketone was purchased from Fluka Chemica (Zwijndrecht, The Netherlands). Diethyloxalate, cyclopentanone, and Silica gel 60 (0.040−0.063 mm) were purchased from Merck-Schuchardt (Hohenbrunn, Germany). Cyclohexanone was purchased from Avocado (Karlsruhe, Germany). 3-[(3-Cholamidopropyl)-dimethylammonio]propanesulfonate (CHAPS) and adenosine deaminase were purchased from Roche Biochemicals (Almere, The Netherlands). Acipimox was a generous gift from Pharmacia-Upjohn (Kalamazoo, MI). All other materials were from standard sources and of the highest purity commercially available.

Instruments and Analysis. ^{1}H NMR spectra were measured at 200 MHz with a Bruker AC 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ^{13}C NMR spectra were measured at 50 MHz. Chemical shifts for ^{1}H and ^{13}C are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard, and coupling constants are given in Hertz. Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. Combustion analyses of new target compounds were performed by the analytical department of the Gorlaeus Laboratories, Leiden University (The Netherlands), and are within ±0.4% of theoretical values unless otherwise specified.

Synthesis. Compounds **2b**−**s** were prepared according to literature procedures.[8,11,12,19,20]

Oxo-(2-oxo-tetrahydrofuran-3-yl)-acetic Acid Ethylester (2b).[11] Prepared from butyrolacton. ^{1}H NMR (CDCl$_3$) δ 1.39 (t, J = 7.31 and 6.58, 3H, CH$_3$ ester), 3.30 (t, J = 8.04 and 7.31, 2H, CH$_2$), 4.37 (q, J = 7.31 and 6.57 CH$_2$ ester), 4.51 (t, J = 7.31 and 7.30, 2H, CH$_2$O).

(2-Oxo-cyclopentyl)-glycoxylic Acid Ethyl Ester, Sodium Salt (2c).[12] Prepared from cyclopentanone. ^{1}H NMR (MeOD) δ 1.31 (t, J = 7.31, 3H, CH$_3$ ester), 1.82 (q, J = 7.31 and 8.04, 2H, CH$_2$), 2.19 (t, J = 7.31 and 8.04, 2H, CH$_2$), 2.05 (t, J = 6.58 and 7.31, 2H, CH$_2$), 4.23 (q, J = 7.31, 6.58, 2H, CH$_2$ ester).

(2-Oxo-cyclohexyl)-glycoxylic Acid Ethyl Ester (2d).[12,19] Prepared from cyclohexanone. ^{1}H NMR (CDCl$_3$) δ 1.38 (t, J = 7.31, CH$_3$ ester), 1.72 and 2.46 (2× m, combination of 4× CH$_2$), 3.76 (t, J = 7.31, 1H, CH, ketoform), 4.34 (q, J = 7.31 and 6.58, 2H (CH$_2$ ester).

5-Methyl-2,4-dioxo-hexanoic Acid Ethylester, Sodium Salt (2e).[8,20] ^{1}H NMR (MeOD) δ 1.04 (d, J = 6.58, 6H, 2× CH$_3$ isopropyl), 1.31 (t, J = 7.31 and 6.58, 3H, CH$_3$ ester), 3.06 (m, J = 7.31 and 6.58, 1H, CH isopropyl), 4.19 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester), 5.14 and 5.73 (2× s, 1H (cis/trans)).

2,4-Dioxoheptanoic Acid Ethyl Ester, Enolic Form (2f). ^{1}H NMR (CDCl$_3$) δ 0.96 (t, 3H, J = 7.3 Hz, CH$_3$), 1.38 (t, 3H, J = 7.3 Hz, CH$_3$ ester), 1.70 (m, 2H, CH$_2$), 2.47 (t, 2H, J = 7.3 Hz, CH$_2$C=O), 4.36 (q, 2H, J = 7.3 Hz, OCH$_2$), 6.37 (s, 1H, =CH), 12−13 (broad s, 1H, =COH).

2,4-Dioxo-octanoic Acid Ethylester, Enolic Form (2g). ^{1}H NMR: (CDCl$_3$) δ 0.94 (t, J = 7.31, 3H, CH$_3$ butyl), 1.25 (m, 2H, CH$_2$), 1.38 (t, J = 7.31 and 6.58, 3H, CH$_3$ ester), 1.65 (quintet, J = 6.58 and 8.04, 2H, CH$_2$), 2.50 (t, J = 7.31 and 8.04, 2H, CH$_2$), 4.36 (q, J = 7.31, 2H, CH$_2$ ester), 6.37 (s, 1H, CH).

2,4-Dioxopentadecanoic Acid Ethyl Ester, Sodium Salt (2h). ^{1}H NMR (MeOD) δ 0.90 (t, 3H, J = 7.3 Hz, CH$_3$), 1.29 (m, 21H, CH$_2$ and CH$_3$ ester), 1.57 (m, 2H, CH$_2$), 2.58 (t, 2H, J = 7.3 Hz, CH$_2$C=O), 4.18 (q, 2H, J = 7.3 Hz, OCH$_2$), 5.71 (s, 1H, =CH).

4-(4-Chloro-phenyl)-2,4-dioxo-4-phenyl-butyric Acid Ethylester, Sodium Salt (2k). ^{1}H NMR (MeOD) δ 1.28 (m, 3H, CH$_3$ ester), 4.27 (m, 2H, CH$_2$ ester), 5.74 and 6.43 (2× s, CH, cis/trans isomers), 7.38 (d, J = 8.41, 2H, arom.), 7.78 (d, J = 8.40, 2H, arom.).

4-(4-Methyl-phenyl)-2,4-dioxo-butyric Acid Ethylester, Sodium Salt (2l). ^{1}H NMR: (MeOD) δ 1.33 (t, J = 7.31, 3H, CH$_3$ ester), 2.36 (s, 3H, CH$_3$ arom.), 4.24 (t, J = 7.31 and 6.58, 2H, CH$_2$ ester), 6.50 (s, 1H, CH), 7.19 (d, J = 8.04, 2H, arom.), 7.71 (d, J = 8.04, 2H, arom.).

2,4-Dioxo-5-phenyl-pentanoic Acid Ethyl Ester, Enolic Form (2m). ^{1}H NMR (CDCl$_3$) δ 1.36 (t, 3H, J = 7.3 Hz, CH$_3$), 3.79 (s, 2H, CH$_2$), 4.35 (q, 2H, J = 7.3, OCH$_2$), 6.38 (s, 1H, =CH), 7.29 (m, 5H, arom.), 10.60 (broad s, 1H, OH).

5-(3-Chloro-phenyl)-2,4-dioxo-pentanoic Acid Ethylester, Enolic Form (2n). ^{1}H NMR: (CDCl$_3$) δ 1.36 (t, J = 7.31, 3H, CH$_3$ ester), 3.75 (s, 2H, CH$_2$ benzylic), 4.34 (q, J = 7.31, 2H, CH$_2$ ester), 6.36 (s, 1H, CH), 7.13 (m, 1H, arom.), 7.30 (m, 3H, arom.).

5-(4-Chloro-phenyl)-2,4-dioxo-pentanoic Acid Ethylester (2o). ^{1}H NMR: (CDCl$_3$) δ 1.35 (t, J = 7.3, 3H, CH$_3$ ester), 3.72 (s, 2H, CH$_2$ benzylic), 4.33 (q, J = 7.31, 2H, CH$_2$ ester), 6.35 (s, 1H, CH), 7.36 (d, 2H, arom.), 7.76 (d, 2H, arom.).

5-(4-Methylphenyl)-2,4-dioxo-pentanoic Acid Ethylester (2p). ^{1}H NMR: (CDCl$_3$) δ 1.01 (t, J = 7.31, 3H, CH$_3$ ester), 2.34 (s, 3H, CH$_3$ ring), 3.73 (s, 2H, CH$_2$ benzylic), 4.32 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester), 6.36 (s, 1H, CH), 7.17 (m, 4H, arom.).

5-(4-Methoxy-phenyl)-2,4-dioxo-pentanoic Acid Ethylester (2q). ^{1}H NMR: (CDCl$_3$) δ 1.35 (t, J = 6.57 and 7.31, 3H, CH$_3$ ester), 3.71 (s, 2H, CH$_2$ benzylic), 3.02 (s, 3H, OCH$_3$), 4.32 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester), 6.35 (s, 1H, CH), 6.88 (d, J = 8.78, 2H, arom.), 7.11 (d, J = 8.78, 2H, arom.).

2,4-Dioxo-6-phenyl-hexanoic Acid Ethyl Ester, Sodium Salt (2r). ^{1}H NMR (MeOD) δ 1.08 (t, 3H, J = 7.3 Hz, CH$_3$), 2.86 (m, 4H, 2× CH$_2$), 4.20 (q, 2H, J = 7.3, OCH$_2$), 7.16 (m, 5H, arom.).

2,4-Dioxo-7-phenyl-heptanoic Acid Ethyl Ester (2s). ^{1}H NMR (CDCl$_3$) δ 1.38 (t, 3H, J = 7.3 Hz, CH$_3$), 2.03 (m, 2H, CH$_2$), 2.51 (t, 2H, J = 7.3 Hz, CH$_2$), 2.67 (t, 2H, J = 7.3 Hz, CH$_2$), 4.35 (q, 2H, J = 7.3, OCH$_2$), 6.34 (s, 1H, =CH), 7.24 (m, 5H, arom.).

1,2-Diaza-7-oxa-bicyclo[3,3,0^{4,8}]octa-3,8-diene-3-carboxylic Acid Ethylester (3b). 5.0 g (26.85 mmol) **2b** was dissolved in 10 mL of acetic acid at 0 °C. A total of 1.48 g (29.56 mmol) of hydrazine hydrate was slowly added. The mixture was heated to reflux for 8 h. After cooling of the sample, the solid matter was filtered and dried in vacuo, resulting in 4.9 g (26.90 mmol, ~100%) of white crystals.

^{1}H NMR (CDCl$_3$) δ 1.42 (t, J = 6.58 and 7.31, 3H, CH$_3$ ester), 3.01 (t, J = 7.31 and 6.58, 2H, CH$_2$−O), 4.26 (t, J = 7.31 and 6.58, 2H, CH$_2$), 4.44 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester).

1,2-Diaza-bicyclo[3,3,0^{4,8}]octa-3,8-diene-3-carboxylic Acid Ethylester (3c)[19]. 1.07 g (21.34 mmol) of hydrazine hydrate was slowly added to a cooled suspension of 4.00 g (19.40 mmol) **2c** in 10 mL of acetic acid. The mixture was heated to reflux for 8 h, poured into ice-H$_2$O, neutralized with NaHCO$_3$, and extracted with dichloromethane (3 × 100 mL) and ethyl acetate (1 × 100 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated. Yield 62%.

^{1}H NMR (CDCl$_3$) δ 1.37 (t, J = 7.31 and 6.58, 3H, CH$_3$ ester), 2.47 (m, 2H, CH$_2$), 2.77 (m, 4H, 2× CH$_2$), 4.35 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester).

4,5,6,7-Tetrahydro-1H-indazole-3-carboxylic Acid Ethylester (3d). Prepared as described for **3c**. Yield 93%. ^{1}H NMR (CDCl$_3$) δ 1.38 (t, J = 7.31, 3H CH$_3$ ester), 1.81 (m, 4H, 2× CH$_2$), 2.73 (m, 4H, 2× CH$_2$), 4.37 (q, J = 7.31, 6.58, 2H, CH$_2$ ester).

5-Isopropyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3e). Prepared as described for **3c**. Yield 89%. ^{1}H NMR (CDCl$_3$) δ 1.29 (d, J = 6.58, 6H, 2x CH$_3$ isopropyl), 1.36 (t, J = 6.58 and 7.31, 3H, CH$_3$ ester), 3.03 (septet, J = 7.31 and 6.58, 1H, CH isopropyl), 4.37 (q, J = 7.31 and 6.58, 2H, CH$_2$ ester), 6.62 (s, 1H, CH pyrazole).

5-Propyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3f). Prepared as described for **3b**. Yield 76%. ^{1}H NMR (CDCl$_3$) δ 0.97 (t, 3H, J = 7.3 Hz, CH$_3$), 1.39 (t, 3H, J = 7.3 Hz, CH$_3$

ester), 1.69 (m, 2H, CH_2), 2.70 (t, 2H, $J = 7.3$ Hz, CH_2), 4.38 (q, 2H, $J = 7.3$ Hz, OCH_2), 6.62 (s, 1H, =CH).

5-Butyl-1H-pyrazole-3-carboxylic Acid Ethylester (3g). Prepared as described for **3c**. Yield 93%. ^1H NMR: ($CDCl_3$) δ 0.90 (t, $J = 7.31$, 3H, CH_3 butyl), 1.33 (m, 2H, CH_2), 1.34 (t, J = 6.58 and 7.31, CH_3 ester), 1.62 (m, 2H, CH_2), 2.69 (t, J = 7.31 and 8.04, 2H, CH_2), 4.35 (q, $J = 7.31$ and 6.58, 2H, CH_2 ester), 6.59 (s, 1H, CH pyrazole).

5-Undecyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3h). Prepared as described for **3b**. Yield 97%. ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, $J = 7.3$ Hz, CH_3), 1.26 (m, 18H, CH_2), 1.39 (t, 3H, $J = 7.3$ Hz, CH_3 ester), 1.65 (m, 2H, CH_2), 2.67 (t, 2H, $J = 7.3$ Hz, CH_2), 4.38 (q, 2H, $J = 7.3$ Hz, OCH_2), 6.62 (s, 1H, =CH).

5-(4-Chlorophenyl)-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3k). Prepared as described for **3b**. Yield 94%. ^1H NMR: ($CDCl_3$) δ 1.42 (t, $J = 6.94$ and 7.31, 3H, CH_3 ester), 4.42 (q, J = 7.31 and 6.94, 2H, CH_2 ester), 7.11 (s, 1H, pyrazole), 7.41 (d, $J = 8.77$, 2H, arom.), 7.72 (d, $J = 8.78$, 2H, arom.).

5-(4-Methylphenyl)-1H-pyrazole-3-carboxylic Acid Ethylester (3l). Prepared as described for **3b**. Yield 90%. ^1H NMR: ($CDCl_3$) δ 1.40 (t, $J = 6.58$ and 7.31, 3H, CH_3 ester), 2.39 (s, 3H, CH_3), 4.40 (q, $J = 7.31$, 2H, CH_2 ester), 7.07 (s, 1H, CH pyrazole), 7.25 (d, $J = 8.77$, 2H, arom.), 7.61 (d, J = 8.04, 2H, arom.).

5-Benzyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3m). Prepared as described for **3b**. Yield 79%. ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, $J = 7.3$ Hz, CH_3), 4.05 (s, 2H, CH_2), 4.36 (q, 2H, $J = 7.3$, OCH_2), 6.59 (s, 1H, =CH), 7.28 (m, 5H, arom.), 11.00 (broad s, 1H, NH).

5-(3-Chlorobenzyl)-1H-pyrazole-3-carboxylic Acid Ethylester (3n). Prepared as described for **3c**. Purification by silica gel column chromatography, eluens: ethyl acetate/petroleum ether = 2:3. Yield 42%. ^1H NMR: ($CDCl_3$) δ 1.33 (t, $J = 7.31$ and 6.58, 3H, CH_3 ester), 4.04 (s, 2H, CH_2 benzylic), 4.34 (q, $J = 7.31$ and 6.57, 2H, CH_2 ester), 6.56 (s, 1H, CH pyrazole), 7.11 (m, 1H, arom.), 7.20 (m, 3H, arom.).

5-(4-Chlorobenzyl)-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3o). Prepared as described for **3c**. Purification by silica gel column chromatography, eluens: ethyl acetate/petroleum ether = 2:1. Yield 31%. ^1H NMR: ($CDCl_3$) δ 1.36 (t, $J = 7.31$, 3H, CH_3 ester), 4.02 (s, 2H, CH_2 benzyl), 4.35 (q, $J = 7.31$ and 6.58, 2H, CH_2 ester), 6.56 (s, 1H, pyrazole), 7.15 (d, $J = 8.78$, 2H, arom.), 7.27 (d, $J = 8.77$, 2H, arom.).

5-(4-Methylbenzyl)-1H-pyrazole-3-carboxylic Acid Ethylester (3p). Prepared as described for **3c**. Purification by silica gel column chromatography, eluens: ethyl acetate/petroleum ether = 2:3. Yield 35%. ^1H NMR: ($CDCl_3$) δ 1.36 (t, $J = 7.31$, 3H, CH_3 ester), 2.33 (s, 3H, CH_3 ring), 4.00 (s, 2H, CH_2 benzylic), 4.35 (q, $J = 7.31$, 2H, CH_2 ester), 6.59 (s, 1H, CH pyrazole), 7.12 (s, 4H arom.), 10.73 (s, broad, 1H, NH).

5-(4-Methoxybenzyl)-1H-pyrazole-3-carboxylic Acid Ethylester (3q). Prepared as described for **3c**. Purification by silica gel column chromatography, eluens: ethyl acetate/petroleum ether = 2:1, followed by crystallization from ethyl acetate/petroleum ether. Yield 12%. ^1H NMR: ($CDCl_3$) δ 1.35 (t, $J = 7.30$ and 6.58, 3H, CH_3 ester), 3.79 (s, 3H, O−CH_3), 3.98 (s, 2H, CH_2 benzylic), 4.35 (q, $J = 7.31$, 2H, CH_2 ester), 6.57 (s, 1H, CH pyrazole), 6.84 (d, $J = 8.77$, 2H arom.), 7.14 (d, $J = 8.77$, 2H, arom.), 10.90 (s, broad, 1H, NH).

5-(2-Phenyl)ethyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3r). Prepared as described for **3b**. Yield 56%. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, $J = 7.3$ Hz, CH_3), 2.99 (m, 4H, 2× CH_2), 4.37 (q, 2H, $J = 7.3$, OCH_2), 6.60 (s, 1H, =CH), 7.19 (m, 5H, arom.).

5-(3-Phenyl)propyl-1H-pyrazole-3-carboxylic Acid Ethyl Ester (3s). Prepared as described for **3b**. Yield 73%. ^1H NMR ($CDCl_3$) δ 1.41 (t, 3H, $J = 7.3$ Hz, CH_3), 1.99 (m, 2H, CH_2), 2.70 (m, 4H, 2× CH_2), 4.37 (q, 2H, $J = 7.3$, OCH_2), 6.63 (s, 1H, =CH), 7.21 (m, 5H, arom.).

1,2-Diaza-7-oxa-bicyclo[3,3,0^{4,8}]octa-3,8-diene-3-carboxylic Acid (4b). 1 g (5.49 mmol) of **3b** was dissolved in 22 mL of 1,4-dioxane. 22 mL of 0.5 M NaOH was added, and the mixture was allowed to stand overnight at room temperature. The mixture was acidified with conc HCl and concentrated in vacuo. The precipitate was collected. Yield 64%; mp 216−217 °C. ^1H NMR (MeOD) δ 3.00 (t, $J = 5.85$ and 6.57, 2H CH_2), 3.79 (t, $J = 6.58$ and 5.85, 2H, CH_2−O). ^{13}C NMR (MeOD) δ 25.6 (CH_2), 62.0 (CH_2−O), 107.2 (C4), 136.9 (C3), 157.9 (C5), 160.0 (carbonyl). Anal. ($C_6H_6N_2O_3$ H_2O) C, H, N.

1,2-Diaza-bicyclo[3,3,0^{4,8}]octa-3,8-diene-3-carboxylic Acid (4c)[19]. Prepared as described for **4b**. Yield 42%; mp 270 °C (dec). ^1H NMR (MeOD) δ 2.52 (q, $J = 6.58$ and 8.04, 2H, CH_2), 2.75 (m, 4H, 2× CH_2). ^{13}C NMR (DMSO) δ 23.4, 23.9 and 30.0 (3× CH_2), 128.4 and 129.7 (C3 and C4), 159.3 (C5), 161.7 (carbonyl). Anal. ($C_7H_8N_2O_2$ 0.1 H_2O) C, H, N.

4,5,6,7-Tetrahydro-1H-indazole-3-carboxylic Acid (4d). Prepared as described for **4b**. Yield 53%; mp 247−248 °C. ^1H NMR ($CDCl_3$) δ 1.89 (m, 4H, 2× CH_2), 2.81 (m, 4H, 2× CH_2). ^{13}C NMR (DMSO) δ 21.2, 21.5, 22.0, and 22.5 (4× CH_2), 119.4 (C4), 135.2 (C3), 144.8 (C5), 161.9 (carbonyl). Anal. ($C_8H_{10}N_2O_2$ 1.9 H_2O) C, H, N.

5-Isopropyl-1H-pyrazole-3-carboxylic Acid (4e).[8] Prepared as described for **4b**. Yield 91%; mp 150−152 °C (Lit. 153−154°C). ^1H NMR ($CDCl_3$) δ 1.44 (d, $J = 7.31$, 6H, 2x CH_3 isopropyl), 3.28 (septet, $J = 6.58$ and 7.31, 1H, CH isopropyl), 6.88 (s, 1H, CH pyrazole). ^{13}C NMR ($CDCl_3$) δ 21.7 (2× CH_3 isopropyl), 25.9 (CH isopropyl), 106.1 (CH pyrazole), 138,9 (C3), 155.4 (C5), 160.0 (carbonyl).

5-Propyl-1H-pyrazole-3-carboxylic Acid (4f).[8] Prepared as described for **4b**. Yield 42%; mp 186−188 °C (Lit. 189−190 °C). ^1H NMR (MeOD) δ 0.96 (t, 3H, $J = 7.3$ Hz, CH_3), 1.69 (m, 2H, CH_2), 2.64 (t, 2H, J = 7.3 Hz, CH_2), 6.56 (s, 1H, CH pyrazole). ^{13}C NMR (MeOD) δ 13.9 (CH_3 propyl), 23.6 (CH_2), 28.7 (CH_2), 107.2 (CH pyrazole), 143.3 (C3), 148.6 (C5), 165.0 (carbonyl).

5-Butyl-1H-pyrazole-3-carboxylic Acid (4g).[8] Prepared as described for **4b**. Recrystallization from methanol. Yield 20%; mp 165−167 °C (Lit. 167−169 °C). ^1H NMR: (MeOD) δ 0.95 (t, $J = 7.31$, 3H, CH_3 butyl), 1.37 (sextet, $J = 7.31$, 8.04, and 6.58, 2H, CH_2), 1.64 (quintet, $J = 6.57$, 8.05 and 7.31, 2H, CH_2), 2.67 (t, J = 7.31 and 8.05, 2H, CH_2), 6.56 (s, 1H, CH pyrazole). ^{13}C NMR: (MeOD) δ 14.1 (CH_3 butyl), 23.1, 26.3, and 32.4 (3× CH_2), 107.1 (CH pyrazole), 143.5 (C3), 148.6 (C5), 165.2 (carbonyl).

5-Undecyl-1H-pyrazole-3-carboxylic Acid (4h).[8] Prepared as described for **4b**. Yield 87%; mp 152−154 °C (Lit. 151−153 °C). ^1H NMR (MeOD) δ 0.89 (t, 3H, $J = 7.3$ Hz, CH_3), 1.29 (m, 18H, CH_2), 1.66 (m, 2H, CH_2), 2.67 (t, 2H, J = 7.3 Hz, CH_2), 6.66 (s, 1H, CH pyrazole). ^{13}C NMR (MeOD) δ 14.5 (CH_3), 23.7, 26.7, 30.2, 30.3, 30.6, 30.7, 33.0, 107.1 (CH pyrazole), 143.3 (C3), 148.8 (C5), 164.9 (carbonyl).

5-Phenyl-1H-pyrazole-3-carboxylic Acid (4i).[21] Prepared as described for **4b**. Yield 81%; mp 230−232 °C (Lit. 232−234 °C). ^1H NMR: (MeOD) δ 7.19 (s, 1H, CH pyrazole), 7.39 (m, 3H, arom.), 7.82 (m, 2H, arom.).

5-(3-Chlorophenyl)-1H-pyrazole-3-carboxylic Acid (4j). Prepared as described for **4b**. Yield 64%; mp 238−240 °C. ^1H NMR: (MeOD) δ 7.29 (s, 1H, CH pyrazole), 7.45 (m, 2H, arom.), 7.80 (m, 1H, arom.), 7.90 (s, 1H, arom.). Anal. ($C_{10}H_7N_2O_2Cl$ 0.7 H_2O) C, H, N.

5-(4-Chlorophenyl)-1H-pyrazole-3-carboxylic Acid (4k). Prepared as described for **4b**. Yield 89%; mp 250−251 °C. ^1H NMR: (MeOD) δ 7.16 (s, 1H, CH pyrazole), 7.44 (d, $J = 8.78$, 2H, arom.), 7.77 (d, $J = 8.77$, 2H, arom.). ^{13}C NMR (DMSO) δ 105.7 (C4), 127.1 (2C arom.), 129.0 (2C, arom.), 130.4 (ipso), 132.7 (ipso, C−Cl), 139.4 (C3), 147.3 (C5), 161.6 (carbonyl). Anal. ($C_{10}H_7N_2O_2Cl$ H_2O) C, H, N.

5-(4-Methylphenyl)-1H-pyrazole-3-carboxylic Acid (4l). Prepared as described for **4b**. Yield 34%; mp 246−248 °C. ^1H NMR: ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 7.06 (s, 1H, CH pyrazole), 7.26 (d, $J = 8.05$, 2H, arom.), 7.63 (d, $J = 8.04$, 2H, arom.). ^{13}C NMR: (MeOD) δ 21.3 (CH_3), 105.9 (C4), 126.6 (2C, arom.), 128.6 (ipso), 130.6 (2C, arom.), 139.8 (ipso, **C**−CH_3), 142.8 (C3), 149.0 (C5), 164.3 (carbonyl). Anal. ($C_{11}H_{10}N_2O_2$ 0.9H_2O) C, H, N.

5-Benzyl-1H-pyrazole-3-carboxylic Acid (4m). Prepared as described for **4b**. Yield 33%; mp 216−217 °C. ^1H NMR

(MeOD) δ 4.02 (s, 2H, CH_2), 6.55 (s, 1H, CH pyrazole), 7.23 (m, 5H, arom.). ^{13}C NMR (MeOD) δ 33.1 (CH_2), 108.2 (C4), 127.7, 129.6, 129.7, 139.7, 142.6 (C3), 148.6 (C5), 164.2 (carbonyl). Anal. ($C_{11}H_{10}N_2O_2$ 0.95 HCl) C, H, N.

5-(4-Chlorobenzyl)-1H-pyrazole-3-carboxylic Acid (4n). Prepared as described for **4b**. Yield 81%; mp 224−225 °C. ^1H NMR: (MeOD) δ 4.01 (s, 2H, CH_2 benzylic), 6.56 (s, 1H, CH pyrazole), 7.22 (d, $J = 8.78$, 2H, arom.), 7.31 (d, $J = 8.04$, 2H, arom.).^{13}C NMR (DMSO) δ 31.7 (CH_2 benzylic), 107.3 (CH pyrazole), 128.7 (2C, arom.), 130.7 (2C, arom.), 131.4 and 138.4 (2× ipso), 140.9 (C3), 146.8 (C5), 162.8 (carbonyl). Anal. ($C_{11}H_9N_2O_2Cl$) C, H, N.

5-(4-Methylbenzyl)-1H-pyrazole-3-carboxylic Acid (4o). Prepared as described for **4b**. Yield 67%; mp 227−228 °C. ^1H NMR: (MeOD) δ 2.29 (s, 3H, CH_3 ring), 3.93 (s, 2H, CH_2 benzylic), 6.50 (s, 1H, CH pyrazole), 7.10 (s, 4H, arom.). ^{13}C NMR: (DMSO) δ 20.6 (CH_3 ring), 31.6 (CH_2 benzylic), 106.8 (CH pyrazole), 128.4 (2C, arom.), 129.1 (2C, arom.), 135.3 (ipso), 136.1 (ipso, **C**−CH_3), 140.6 (C3), 146.9 (C5), 162.5 (carbonyl). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

5-(4-Methoxybenzyl)-1H-pyrazole-3-carboxylic Acid (4p). Prepared as described for **4b**. Yield 64%; mp 229−230 °C. ^1H NMR: (MeOD) δ 3.76 (s, 3H, O−CH_3), 3.94 (s, 2H, CH_2 benzylic), 6.50 (s, 1H, CH pyrazole), 6.85 (d, $J = 8.77$, 2H, arom.), 7.14 (d, $J = 8.78$, 2H, arom.). ^{13}C NMR: (DMSO) δ 31.2 (CH_2 benzylic), 55.1 (OCH_3), 106.8 (CH pyrazole), 114.0 (2C, arom.), 129.6 (2C, arom.), 131.1 (ipso), 140.8 (C3), 147.2 (C5), 157.9 (ipso, **C**−OCH_3), 162.6 (carbonyl). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

5-(3-Chlorobenzyl)-1H-pyrazole-3-carboxylic Acid (4q). Prepared as described for **4b**. Yield 86%; mp 221−222 °C. ^1H NMR: (MeOD) δ 4.02 (s, 2H, CH_2 benzylic), 6.57 (s, 1H, CH pyrazole), 7.24 (m, 4H, arom.). ^{13}C NMR: (DMSO) δ 32.9 (CH_2 benzylic), 108.3 (CH pyrazole), 127.7, 128.0, 129.6, and 131.1 (4× arom.), 135.4 (ipso, C−Cl), 141.2 (ipso), 142.3 (C3), 148.1 (C5), 164.3 (carbonyl). Anal. ($C_{11}H_9N_2O_2Cl$) C, H, N.

5-(2-Phenyl)ethyl-1H-pyrazole-3-carboxylic Acid (4r). Prepared as described for **4b**. Yield 33%; mp 210−212 °C. ^1H NMR (MeOD) δ 2.96 (s, 4H, 2 x CH_2), 6.53 (s, 1H, CH pyrazole), 7.21 (m, 5H, arom.). ^{13}C NMR (MeOD) δ 28.9 (CH_2), 36.5 (CH_2), 107.5 (CH pyrazole), 127.2, 129.4, 142.1, 142.9 (C3), 148.3 (C5), 164.8 (carbonyl). Anal. ($C_{12}H_{12}N_2O_2$) C, N; H: calcd, 5.59; found, 6.04.

5-(3-Phenyl)propyl-1H-pyrazole-3-carboxylic Acid (4s). Prepared as described for **4b**. Yield 30%; mp 165−166 °C. ^1H NMR (MeOD) δ 1.97 (m, 2H, CH_2), 2.67 (m, 4H, 2× CH_2), 6.59 (s, 1H, =CH), 7.17 (m, 5H, arom.). ^{13}C NMR (MeOD) δ 26.3 (CH_2), 32.1 (CH_2), 36.1 (CH_2), 107.3 (CH pyrazole), 126.9, 129.4, 142.9, 143.2, 148.7, 164.9 (carbonyl). Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

Biological Assays. [^{3}H]Nicotinic Acid Binding. Equilibrium binding of [^{3}H]nicotinic acid to rat spleen membranes was performed with 75 μg of membrane protein per tube in a total volume of 250 μL in 50 mM Tris-HCl, pH 7.4, containing 1 mM $MgCl_2$, 200 U/mL penicillin G, 200 μg/mL streptomycin, and 0.02% CHAPS. Binding experiments were conducted in the presence of 20 nM radioligand for 3.5 h at 25 °C. Nonspecific binding was determined by addition of 100 μM acipimox. Separation of membrane-bound from free radioligand was done by filtration of the samples through nitrocellulose filters washing twice with 4 mL of 50 mM Tris-HCl (pH 7.4) containing 0.02% CHAPS.

[^{35}S]GTPγS Binding. G protein activation in rat membrane preparations was assessed as stimulation of [^{35}S]GTPγS binding as described previously.[2] Briefly, samples (100 μL) contained 50 000 cpm (0.2 nM) [^{35}S]GTPγS, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM NaCl, 10 μM GDP, 0.5 U/mL adenosine deaminase, 0.005% CHAPS, and 0.5% bovine serum albumin. Incubations with 1 to 1.5 μg of membrane protein were done for 90 min at 25 °C and were terminated by filtration over GF/B glass fiber filters (Whatman) followed by two 4-mL washes with ice-cold buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.02% CHAPS).

Data Analysis. K_i values from [^{3}H]nicotinic acid binding and EC_{50} values for stimulation of [^{35}S]GTPγS binding were calculated as described previously.[2] EC_{50}, K_i and K_B values are given as geometric means with 95% confidence limits from at least 3 experiments. Relative intrinsic activities (RIAs) are given as arithmetic means ± SEM with reference to the maximum stimulation induced by nicotinic acid as a full agonist with its intrinsic activity assumed as 100%.

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