

Supporting Information

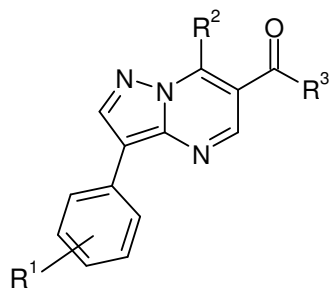
Novel 3,6,7-substituted pyrazolopyrimidines as positive allosteric modulators for the hydroxy-carboxylic acid receptor 2 (GPR109A).

Clara C. Blad, Jacobus P.D. van Veldhoven, Corné Klopman, Dieter R. Wolfram, Johannes Brussee, J. Robert Lane, Adriaan P. IJzerman

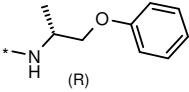
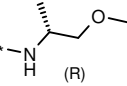
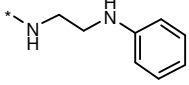
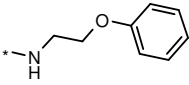
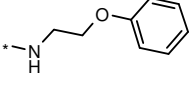
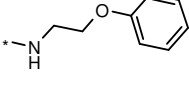
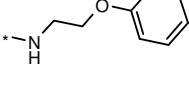
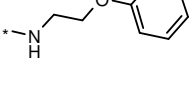
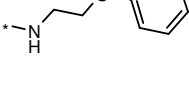
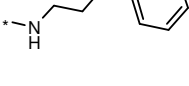

Table of contents

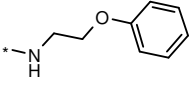
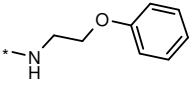
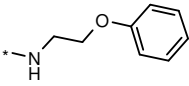
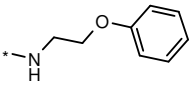
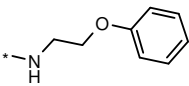
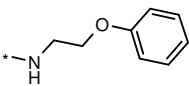
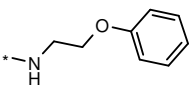
Table S1	S2
Modulation of [3H]-nicotinic acid binding	S5
Figure S1	S5
Correlation between potentiation of nicotinic acid and allosteric agonism	S6
Figure S2	S7
Modulation of the intrinsic efficacy of nicotinic acid	S7
Pyrazolopyrimidines as allosteric agonists	S8
Table S2	S8
Pyrazolopyrimidines as allosteric enhancers of 3-hydroxybutyrate	S9
Table S3	S9
Experimental Section – Chemistry	S9
Experimental Section – Pharmacology	S20
References	S21

Table S1. Effects on [³H]-nicotinic acid binding of a series of 3-(aryl)-7-methyl-*N*-(substituted)pyrazolo[1,5-*a*]pyrimidine-6-carboxamides (measured at 10 μM).



Cpd	R ¹	R ²	R ³	% [³ H]-nicotinic acid binding (±SEM)	
				in equilibrium ^a	after 30 min dissociation ^b
20	4- <i>i</i> Pr	Me		96 (7.8)	91 (5.9)
21	4- <i>i</i> Pr	Me		108 (10.5)	114 (10.9)
22	4- <i>i</i> Pr	Me		111 (5.0)	99 (7.7)
23	4- <i>i</i> Pr	Me		101 (6.8)	112 (9.6)
24	4- <i>i</i> Pr	Me		149 (6.1)	133 (2.3)

25	4- <i>i</i> Pr	Me		109 (5.9)	92 (6.2)
26	4- <i>i</i> Pr	Me		69 (4.0)	118 (15.7)
27	4- <i>i</i> Pr	Me		107 (7.6)	115 (8.1)
28	4- <i>i</i> Pr	H		102 (3.4)	108 (18.7)
29	4- <i>i</i> Pr	Et		143 (3.2)	152 (1.4)
30	4- <i>i</i> Pr	Pr		148 (9.0)	136 (3.8)
32	4-Me	Me		114 (12.1)	130 (2.4)
33	3-Me	Me		117 (9.0)	145 (11.3)
34	2-Me	Me		111 (4.2)	113 (9.1)
35	4-Et	Me		147 (4.4)	148 (23.4)
36	4- <i>t</i> Bu	Me		142 (12.0)	147 (11.5)

37	4-OMe	Me		126 (6.4)	144 (14.3)
38	4-OiPr	Me		143 (6.7)	162 (8.2)
39	4-Ph	Me		106 (3.5)	100 (2.0)
40	4-Cl	Me		108 (0.6)	96 (2.6)
41	3-Cl	Me		101 (4.4)	102 (5.3)
42	3,4-diCl	Me		96 (5.3)	101 (2.6)
43	4-CF ₃	Me		119 (4.5)	103 (1.7)

a) the percentage of [³H]-nicotinic acid specifically bound to HEK293-HCA₂ membranes at equilibrium in the presence of 10 μM of the test compound (100% is without test compound).

b) the percentage of [³H]-nicotinic acid still bound to HEK293-HCA₂ membranes after 30 minutes of dissociation at 15 °C induced by 10 μM of nicotinic acid in the presence of 10 μM of the test compound (100% is without test compound - values above 100% indicate a slower dissociation rate in the presence of the test compound). Values are means (± SEM) of 3 independent experiments performed in duplicate.

Modulation of [³H]-nicotinic acid binding

In Figure S1-A the effect of 10 μ M **24** on the full dissociation curve of the radioligand is shown. The data indicate that in the presence of **24** the dissociation rate is unchanged ($t_{1/2} = 30$ min for both curves) but that a plateau is reached at 40% specific binding in the presence of the modulator. The control curve reached a plateau at 10%.

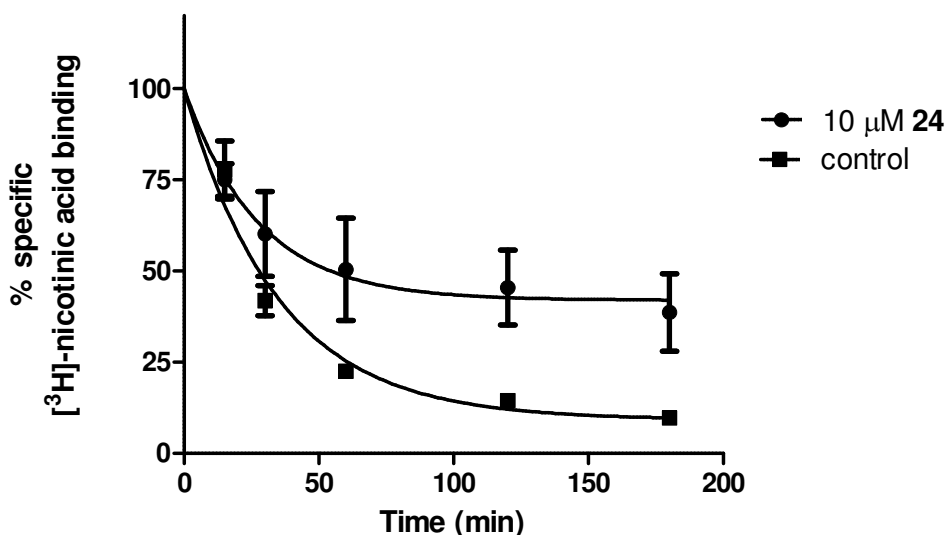
For the compounds that markedly increased the binding of [³H]-nicotinic acid in equilibrium assays (Table S1) we attempted to determine an EC_{50} of this effect. However, at 10 μ M none of the curves had reached a plateau, and the solubility of the compounds did not permit an increase above this concentration. The curves for three selected compounds, **24**, **29** and **38**, are shown in Figure S1-B.

Figure S1.

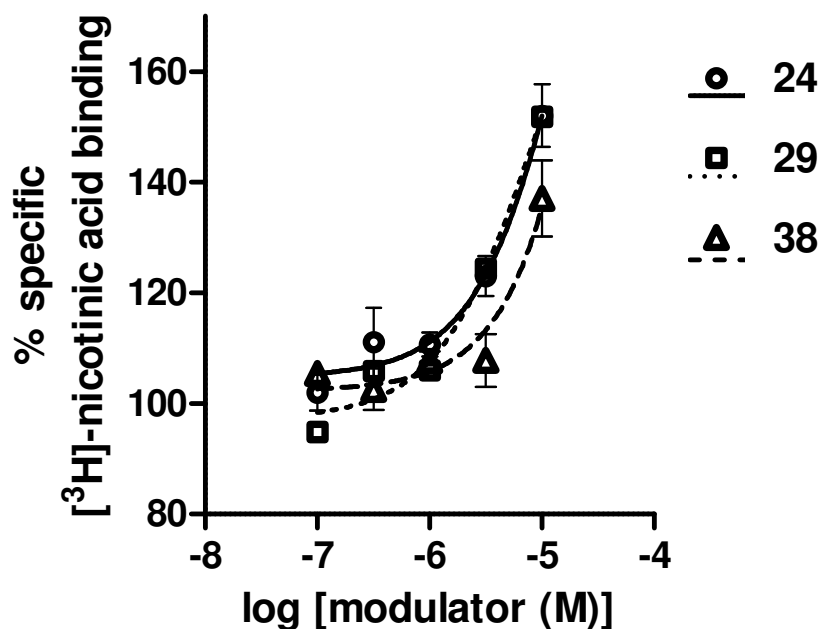
A. Dissociation curves with and without 10 μ M **24** at 15 °C. After 3 hours association of 20 nM [³H]-nicotinic acid to HEK-HCA₂ membranes, dissociation was started by addition of 10 μ M nicotinic acid. The data points are means \pm SEM for 3 independent experiments performed in duplicate.

B. Binding curves (equilibrium) of compounds **24**, **29** and **38**. The assays were performed using 20 nM [³H]-nicotinic acid and 50 μ g HEK-HCA₂ membranes per assay point, and incubation was 1h at 25 °C. The data points are means \pm SEM of 3 independent experiments performed in duplicate.

A



B

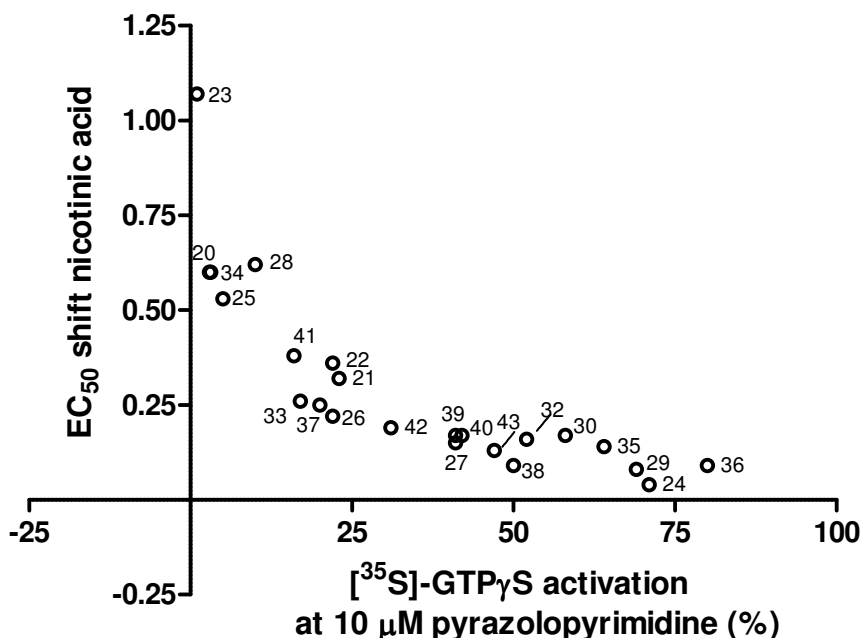


Correlation between potentiation of nicotinic acid and allosteric agonism

To investigate any correlation between the agonistic and modulating effects of the pyrazolopyrimidines, the $[^{35}\text{S}]\text{-GTP}\gamma\text{S}$ binding activation and the potency shift of nicotinic acid, both at $10\ \mu\text{M}$, were plotted against each other (Figure S2), yielding a nonlinear correlation. The plot suggests that the ability of the pyrazolopyrimidine agonists to stabilize active receptor conformations contributes to, or even determines, the modulator strength of the compounds.

The compounds at the bottom right of the curve are the most potent modulators and also the most active agonists. More to the left are compounds that are not potent or efficacious agonists, but still fairly active as modulators. However, it appears that a 3-fold or greater shift in nicotinic acid potency is always coupled to some agonist activity in this series. Closest to the origin of the graph is a cluster of compounds, **41**, **33**, **37** and **26**. Replacing the 4-isopropyl by a 3-methyl (**33**), 3-chloro (**41**) or 4-methoxy (**37**) appears to decrease the agonist action significantly while preserving appreciable modulator strength. Removal of the phenyl from the 6-position substituent (R^3) followed by methylation (**26**) seems to have a similar effect.

Figure S2. Correlation between potency enhancement of nicotinic acid and agonistic activity of the pyrazolopyrimidines on HCA₂.



Modulation of the intrinsic efficacy of nicotinic acid

As described above, many of the active pyrazolopyrimidines are allosteric agonists for HCA₂. In the concentration-effect curves of nicotinic acid with the modulator, this was observed as an increase in the basal receptor activation level. At the same time, many of the same compounds also increased the maximal [³⁵S]-GTP_γS binding. In most cases, nicotinic acid still caused an increase in [³⁵S]-GTP_γS binding of 100%, identical to the range of the curve in absence of a modulator and typical of a full agonist. However, there were a few cases where the allosteric modulator changed the intrinsic efficacy of nicotinic acid. Thus, in the presence of compounds **24**, **32**, **36**, and, to a lesser extent, **26** and **29**, nicotinic acid appeared to be a partial agonist (Table 1). For **36** and **29** this may be due to their high agonist activity and the apparent maximum in E_{max} at approximately 150%, but for the other derivatives this postulated E_{max} ceiling was not reached. For example, in the presence of 10 μM **24**, the basal receptor activation was on average 71% and the E_{max} was 109%, which means nicotinic acid only caused a 38% increase in [³⁵S]-GTP_γS binding (see Table 1 and Figure 2). At 3 μM **24**, nicotinic acid still behaved as a partial agonist with the concentration-effect curve ranging from 51% to 107%, on average.

The lowest concentration of 1 μM **24** had a negligible effect on the intrinsic efficacy of nicotinic acid: here, the basal [^{35}S]-GTP γS binding was 30% and the maximum was 120%.

Compounds **20** and **23** had the opposite effect and made nicotinic acid behave as a superagonist. The effect was highly similar for the two allosteric ligands: in the presence of 10 μM modulator, the basal [^{35}S]-GTP γS binding was unchanged, whereas the E_{max} was increased to approximately 130% (see Table 1 and 2). Thus, the modulators increased the intrinsic efficacy of nicotinic acid by 30%. These enhancers were not tested at lower concentrations.

Pyrazolopyrimidines as allosteric agonists

Further characterization of the most active allosteric agonists in concentration-effect curves (in absence of nicotinic acid) revealed that **24**, **35** and **36** have a similar potency to nicotinic acid on HCA $_2$ (Table S2). The other compounds all seem to have a 2- to 3-fold lower potency, although the difference is not significant. All pyrazolopyrimidines are partial agonists; the most efficacious derivative, **24**, has an intrinsic efficacy close to 90%. None of the pyrazolopyrimidines increased [^{35}S]-GTP γS binding in non-transfected HEK cells (data not shown).

Table S2. Agonism of the pyrazolopyrimidines in [^{35}S]-GTP γS assays on HEK-HCA $_2$ membranes. The values are means (SEM) of 3 independent experiments performed in duplicate.

	EC$_{50}$ (μM) (SEM)	% E$_{\text{max}}$ (SEM)
nicotinic acid	4.7 (0.5)	100
24	3.0 (0.6)	87 (8)
29	9.0 (6.8)	75 (3)
30	9.2 (6.7)	70 (2)
32	9.7 (6.5)	65 (6)
35	3.8 (1.3)	72 (5)
36	3.0 (0.6)	70 (9)
38	9.9 (6.4)	64 (6)

Allosteric enhancement of the endogenous ligand 3-hydroxybutyrate.

Table S3. Potency and intrinsic efficacy (basal activation by modulator minus E_{\max}) of 3-hydroxybutyrate (3-OHB) in the presence and absence of 10 μ M pyrazolopyrimidine. The data is from [35 S]-GTP γ S binding assays performed on HEK-HCA $_2$ membranes. See also Figure 2. The values are means (SEM) of 3 independent experiments performed in duplicate.

	EC$_{50}$ (mM) (SEM)	% efficacy (SEM)
3-OHB alone	3.82 (2.04)	29 (3)
3-OHB+ 24	0.54 (0.14)	39 (6)
3-OHB+ 27	0.62 (0.05)	46 (4)
3-OHB+ 29	0.51 (0.15)	36 (6)
3-OHB+ 38	0.55 (0.16)	51 (8)
3-OHB+ 42	2.58 (1.40)	69 (10)

Experimental Section - Chemistry

All reagents used were obtained from commercial sources and all solvents were of analytical grade. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 (^1H NMR, 400 MHz; ^{13}C NMR, 100 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, ar = aromatic protons, ph = phenyl protons. The purity was confirmed >95% by HPLC and High Resolution Mass Spectroscopy. HPLC was performed on a Gilson 306 system (detection at 254 nm) equipped with an analytical C18 column (4.6 mm x 12.5 cm, 5 μ m) in combination with a gradient of mixture A: 1 MeCN / 9 H $_2$ O, B: 9 MeCN / 1 H $_2$ O. High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electro spray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 $^\circ\text{C}$) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.2842) as a “lock mass”. Reactions were routinely monitored on TLC using Merck silica gel F $_{254}$ plates. Microwave reactions were performed in an Emrys Optimizer (Biotage AB, formerly Personal Chemistry). Wattage was automatically adjusted to maintain the desired temperature. The yields of all products were not optimized. The final products (Tables 1, 2 and 3) were purified by column chromatography and/or by recrystallization.

General procedure for the synthesis of ethyl 2-(ethoxymethylene)-3-oxoates (**3-6**)¹

A solution of the diketone (76.83 mmol), triethylorthoformiate (23.2 g, 153.66 mmol) and acetic anhydride (7.9 g, 76.83 mmol) was refluxed overnight. The products were purified by vacuum distillation.

Ethyl 2-(ethoxymethylene)-3-oxobutanoate (**3**)

Starting from ethyl acetoacetate (10.2 g, 76.83 mmol) The residue after vacuum distillation at 40 °C yielded 10.0 g (70%) of a cis and trans mixture of the desired product **3** as a brown oil. ¹H NMR (DMSO, 400 MHz): δ 7.83 (s, 1H), 7.73 (s, 1H), 4.25 (t, J = 6.8 Hz, 4H), 4.13(t, J = 6.8 Hz, 4H), 2.26 (s, 3H), 2.19 (s, 3H), 1.25 (t, J = 7.2 Hz, 6H) 1.20 (t, J = 6.8 Hz, 6H).

Ethyl 2-(ethoxymethylene)-3-oxopentanoate (**5**)

Starting from ethyl 3-oxopentanoate (2.0 g, 13.87 mmol). The residue after vacuum distillation at 40 °C yielded 1.4 g (50%) of a cis and trans mixture of the desired product **5** as a brown oil. ¹H NMR (DMSO, 400 MHz): δ 7.83 (s, 1H), 7.70 (s, 1H), 4.24 (q, J = 7.2 Hz, 4H), 4.17-4.09 (m, 4H), 2.64-2.55 (m, 4H), 1.27-1.15 (m, 12H), 0.97 (t, J = 7.2 Hz, 6H).

Ethyl 2-(ethoxymethylene)-3-oxohexanoate (**6**)

Starting from ethyl 3-oxohexanoate (2.0 g, 12.64 mmol). The residue after vacuum distillation at 40 °C yielded 1.0 g (37%) of a cis and trans mixture of the desired product **6** as a brown oil. ¹H NMR (DMSO, 300 MHz): δ 7.84 (s, 1H), 7.68 (s, 1H), 4.26-4.19 (m, 4H), 4.14-4.06 (m, 4H), 2.59-2.50 (m, 4H), 1.54-1.46 (m, 4H), 1.26-1.16 (m, 12H), 0.88-0.82 (m, 6H).

General procedure for the synthesis of the ethyl pyrazolopyrimidine carboxylates (**7, 8 and 11-13**)

Procedure as described by Springer *et al.*² A solution of the substituted-pyrazole-amines **1** and **2** (1 eq.) and the diketones **3-6** (1 eq.) in ethanol was refluxed for 3 hours. The reaction mixture was concentrated and purified by column chromatography (DCM) or crystallized overnight from the reaction mixture.

Ethyl 3-(4-isopropylphenyl)-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate (**7**)

Starting from **1** (1.5 g, 7.5 mmol) and **3** (1.39 g, 7.45 mmol) in 80 mL of ethanol. The product was obtained by crystallization yielding 2.29 g (95%) of **7** as a yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 9.04 (s, 1H), 8.55 (s, 1H), 7.97 (d, J = 8Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 4.48 (q, J = 6.8 Hz, 2H), 3.26 (s, 3H), 2.98 (septet, J = 7.2 Hz, 1H), 1.48 (t, J = 6.8 Hz, 3H), 1.32 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 164.8, 151.6, 149.6, 147.5, 144.5, 128.8, 126.9, 126.5, 112.0, 110.7, 61.6, 33.9, 24.0, 15.0, 14.3. HRMS calcd. for [C₁₉H₂₁N₃O₂+H] 324.17065, found 324.17067.

Ethyl 7-hydroxy-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (**8**)

Starting from **1** (5.0 g, 24.8 mmol) and the commercially available diethyl ethoxymethylenemalonate (**4**) (5.0 ml, 24.8 mmol). The reaction mixture was allowed to cool to room temperature and the formed precipitate was collected by filtration and rinsed 2 times with water and diethyl ether. Yielding 4.2 g (52%) of **8** as a white solid. ¹H NMR (DMSO, 400 MHz): δ 12.97 (s br, 1H, OH), 8.35 (s, 1H), 8.22 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 2.94 (septet, J = 6.8 Hz, 1H), 1.29 (t, J = 7.2 Hz, 3H), 1.24 (d, J = 6.8 Hz, 6H).

Ethyl 7-ethyl-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (**11**)

Starting from **1** (676 mg, 3.36 mmol) and **5** (672 mg, 3.36 mmol). The reaction mixture was concentrated and purified by column chromatography using DCM to give 1.0 g (96%) of **11** as a yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 9.00 (s, 1H), 8.51 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.45 (q, J = 7.2 Hz, 2H), 3.75 (q, J = 7.2 Hz, 2H), 2.95 (septet, J = 7.2 Hz, 1H), 1.47-1.43 (m, 6H), 1.29 (d, J = 7.2 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 164.6, 156.5, 150.0, 147.5, 144.7, 128.9, 126.9, 126.6, 111.9, 109.9, 61.6, 33.9, 24.0, 22.0, 14.3, 11.4.

Ethyl 3-(4-isopropylphenyl)-7-propylpyrazolo[1,5-a]pyrimidine-6-carboxylate (**12**)

Starting from **1** (938 mg, 4.66 mmol) and **6** (1.0 g, 4.66 mmol). The reaction mixture was concentrated and purified by column chromatography using DCM to give 1.3 g (80%) of **12** as a yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 9.00 (s, 1H), 8.50 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.44 (q, J = 7.2 Hz, 2H), 3.70 (t, J = 7.6 Hz, 2H), 2.95 (septet, J = 6.8 Hz, 1H), 1.92-1.82 (m, 2H), 1.45 (t, J = 7.2 Hz, 3H), 1.29 (d, J = 7.2 Hz, 6H), 1.14 (t, J = 7.2 Hz, 3H).

Ethyl 7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate (**13**)³

Starting from the commercial available 3-aminopyrazole (**2**) (4.0 g, 48.14 mmol) and **3** (10.8 g, 57.76 mmol) in 30 mL of ethanol. The product was crystallized from the reaction mixture, filtered off and rinsed with ethanol to give 6.60 g (67%) of **13** as yellow crystals. ¹H NMR (CDCl₃, 400 MHz): δ 8.94 (s, 1H), 8.22 (d, J = 1.6 Hz, 1H), 6.73 (d, J = 1.6 Hz, 1H), 4.45 (q, J = 6.8 Hz, 2H), 3.21 (s, 3H), 1.45 (t, J = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 164.8, 151.5, 149.8, 148.6, 146.6, 110.6, 98.1, 61.6, 15.0, 14.3.

Ethyl 3-bromo-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate (**14**)

According to an adapted method of Cuni *et al.*⁴ To a solution of ethyl 7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate (**13**) (1.76 g, 8.58 mmol) in DCM (20 mL) at 0°C was added portion wise N-bromosuccinimide (1.51 g, 8.58 mmol) dissolved in 20 mL of DCM. The reaction mixture was stirred for 1.5 hours at 0°C and was allowed to reach room temperature overnight. The reaction mixture was washed 3 times with water, the organic layer dried over MgSO₄ and concentrated *in vacuo* to yield 2.04 g (83%) of a white solid (**14**). ¹H NMR (CDCl₃, 400 MHz): δ 9.00 (s, 1H), 8.22 (s, 1H), 4.46 (q, J = 7.2 Hz, 2H), 3.19 (s, 3H), 1.45 (t, J = 7.2 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz): δ 164.3, 152.0, 150.7, 146.4, 145.2, 111.4, 85.8, 61.9, 14.6, 14.3.

Ethyl 7-chloro-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (9)

Procedure as described by Springer *et al.*² Ethyl 7-hydroxy-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (**8**) (3.5 g, 10.8 mmol) in 20 mL of POCl₃ and 2 mL of N,N-dimethylaniline was refluxed for 3 hours after which according to TLC (DCM) full conversion was reached. The POCl₃ was distilled off and subsequently water was added while cooled on ice and extracted 3 times with diethyl ether. The organic layer was backwashed with an aqueous saturated NaHCO₃ solution, dried over MgSO₄ and concentrated. The crude product was purified by column chromatography (DCM) resulting in 3.4 g (92%) of **9** as a yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.94 (d, J = 2.0 Hz, 1H), 8.57 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 4.47 (q, J = 7.2 Hz, 2H), 2.94 (septet, J = 6.8 Hz, 1H), 1.45 (t, J = 6.8 Hz, 3H), 1.28 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 162.6, 149.3, 148.1, 145.5, 142.3, 128.1, 127.0, 126.8, 113.6, 111.1, 62.3, 34.0, 24.0, 14.3.

Ethyl 3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (10)

Procedure as described by Almansa *et al.*⁵ A mixture of ethyl 7-chloro-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylate (**9**) (350 mg, 1.0 mmol), NaOAc (208 mg, 2.5 mmol) and 5% Pd/C (35 mg) was hydrogenated at 2.5 bar for 1 hour. On TLC no starting material was visible, the reaction mixture was filtered over celite and concentrated under reduced pressure. The residue was partitioned in water and DCM. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The pure **10** (264 g, 84%) was obtained by column chromatography eluting with DCM. ¹H NMR (CDCl₃, 400 MHz): δ 9.27 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 8.52 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 4.44 (q, J = 7.2 Hz, 2H), 2.94 (septet, J = 6.8 Hz, 1H), 1.43 (t, J = 7.2 Hz, 3H), 1.28 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 163.5, 149.0, 147.8, 145.9, 144.9, 138.5, 128.5, 127.0, 126.6, 112.2, 61.9, 34.0, 24.0, 14.4.

General procedure of the hydrolysis of the esters in to the carboxylic acids (15-19)

To a solution of the ethyl pyrazolopyrimidine carboxylate **7**, **10**, **11**, **12** or **14** (5.71 mmol) in a mixture of H₂O (20 mL), MeOH (20 mL) and THF (60 mL) was added lithium hydroxide (11.42 mmol) and stirred overnight at room temperature. The organic solvents were removed *in vacuo* and the aqueous layer was acidified to pH 2 with a 1 M HCl solution. The precipitated product was collected by filtration and dried *in vacuo* or extracted with EtOAc.

3-(4-Isopropylphenyl)-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylic acid (15)

Starting from **7** (1.8 g, 5.71 mmol), the resulting precipitate was collected by filtration to give 1.7 g of **15** (99%) as a yellow solid. ¹H NMR (DMSO-d₆, 400 MHz): δ 13.63 (br, 1H), 8.91 (s, 1H), 8.82 (s, 1H), 8.01 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 3.10 (s, 3H), 2.89 (m, 1H), 1.22 (d, J = 6.8 Hz, 6H). ¹³C NMR (DMSO-d₆, 100 MHz): δ 166.4, 151.9, 150.5, 147.0, 144.8, 144.4, 129.3, 127.0, 126.4, 111.5, 110.8, 33.7, 24.3, 15.2; HRMS calcd. for [C₁₇H₁₇N₃O₂+H] 296.13935, found 296.13942

3-(4-Isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylic acid (16)

Starting from **10** (112 mg, 0.36 mmol) and the product was extracted with EtOAc to yield 98 mg (97%) of the product as a yellow solid. ¹H NMR (CDCl₃ + drop of MeOD): δ 9.34 (d, J = 2.0 Hz, 1H), 9.05 (d, J = 2.4 Hz, 1H), 8.55 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 2.96 (septet, J = 6.8 Hz, 1H), 1.29 (d, J = 6.8 Hz, 6H).

7-Ethyl-3-(4-isopropylphenyl)pyrazolo[1,5-a]pyrimidine-6-carboxylic acid (17)

Starting from **11** (1.0 g, 3.1 mmol), the resulting precipitate was collected by filtration to give 910 mg (95%) of the product as a yellow solid. ¹H NMR (DMSO-d₆, 400 MHz): δ 13.54 (br, 1H), 8.94 (s, 1H), 8.86 (s, 1H), 8.02 (d, J = 8 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 3.64 (q, J = 7.2 Hz, 2H), 2.90 (septet, J = 6.8 Hz, 1H), 1.33 (t, J = 7.2 Hz, 3H), 1.22 (d, J = 6.8 Hz, 6H). ¹³C NMR (DMSO-d₆, 100 MHz): δ 166.1, 156.2, 150.9, 147.1, 145.1, 144.5, 129.3, 127.1, 126.5, 110.8, 33.7, 24.3, 21.8, 11.7.

3-(4-Isopropylphenyl)-7-propylpyrazolo[1,5-a]pyrimidine-6-carboxylic acid (18)

Starting from **12** (1.3 g, 3.69 mmol), the resulting precipitate was collected by filtration to give 1.2 g (99%) of the product as a yellow solid. ¹H NMR (DMSO-d₆, 400 MHz): δ 13.64 (br, 1H), 8.95 (s, 1H), 8.87 (s, 1H), 8.03 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 3.64 (t, J = 7.2 Hz, 2H), 2.92 (septet, J = 6.8 Hz, 1H), 1.81-1.75 (m, 2H), 1.24 (d, J = 6.8 Hz, 6H), 1.02 (t, J = 7.2 Hz, 3H).

3-Bromo-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylic acid (19)

Starting from **14** (2.03 g, 7.18 mmol) and lithium hydroxide (172 mg, 7.20 mmol). The resulting precipitate was collected by filtration to yield 1.62 g (88%) of the product as a white solid. ¹H NMR (DMSO): δ 13.76 (br, 1H), 8.95 (s, 1H), 8.57 (s, 1H), 3.11 (s, 3H)

General procedure for the synthesis of pyrazolo[1,5-a]pyrimidine carboxamides (20-31)

To a solution of the appropriate pyrazolo[1,5-a]pyrimidine-6-carboxylic acid (**15-19**) (1.0 eq.) and the substituted amine (1.2 eq) in DCM (20 mL per mmol) was added EDC * HCl (1.2 eq.) at room temperature. After 4 hours all of the pyrazolo[1,5-a]pyrimidine-6-carboxylic acid was consumed according to TLC (1-2% MeOH/DCM unless otherwise is indicated). The reaction mixture was concentrated and purified by column chromatography.

N-Benzyl-3-(4-isopropylphenyl)-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxamide (20)

Starting from acid **15** (0.51 mmol) and benzylamine, yield 124 mg (63%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.39 (s, 1H), 8.35 (s, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.28-7.20 (m, 7H), 6.85 (t, J = 5.2 Hz, 1H, NH), 4.50 (d, J = 6.0 Hz, 2H), 2.90 (septet, J = 6.8 Hz, 1H), 2.80 (s, 3H), 1.27 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.2, 147.5, 147.1, 146.6, 144.1, 143.7, 137.5, 128.9, 128.8, 127.8, 127.7, 127.0, 126.5, 116.3, 111.6, 44.3, 33.9, 24.1, 14.7. HRMS calcd. for [C₂₄H₂₄N₄O+H]⁺ 385.20229, found 385.20265.

3-(4-Isopropylphenyl)-7-methyl-N-phenethylpyrazolo[1,5-a]pyrimidine-6-carboxamide (21).

Starting from acid **15** (0.51 mmol) and phenethylamine, yield 127 mg (63%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.30 (s, 1H), 8.22 (s, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.29-7.25 (m, 4H), 7.21-7.17 (m, 3H), 6.50 (t, J = 5.2 Hz, 1H, NH), 3.63 (d, J = 6.8 Hz, 2H), 2.96-2.86 (m, 3H), 2.70 (s, 3H), 1.27 (d, J = 7.2 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.1, 147.4, 146.6, 146.6, 144.0, 143.5, 138.4, 128.9, 128.8, 128.7, 126.9, 126.8, 126.5, 116.6, 111.5, 41.2, 35.3, 33.8, 24.0, 14.5. HRMS calcd for [C₂₅H₂₆N₄O+H]⁺ 399.21794, found 399.21713.

3-(4-Isopropylphenyl)-7-methyl-N-(3-phenylpropyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (22).

Starting from acid **15** (0.33 mmol) and 3-phenylpropylamine, yield 67 mg (48%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.43 (s, 1H), 8.37 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.32 (m, 4H), 7.22 (t, J = 8.0 Hz, 3H), 6.45 (t, J = 5.6 Hz, 1H), 3.46 (q, J = 5.6 Hz, 2H), 2.99 (m, 1H), 2.87 (s, 3H), 2.73 (t, J = 8.4 Hz, 2H), 1.99 (m, 2H), 1.33 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.2, 147.4, 146.7, 146.6, 144.0, 143.6, 141.1, 128.9, 128.6, 128.4, 126.9, 126.5, 126.2, 116.7, 111.6, 40.0, 33.9, 33.5, 31.0, 24.1, 14.7. HRMS calcd for [C₂₆H₂₈N₄O+H]⁺ 413.23359, found 413.23348.

3-(4-Isopropylphenyl)-7-methyl-N-(4-phenylbutyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (23).

Starting from acid **15** (0.51 mmol) and 4-phenylbutylamine, yield 123 mg (56%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.41 (s, 1H), 8.40 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 7.27-7.24 (m, 2H), 7.19-7.15 (m, 3H), 6.26 (t, J = 5.6 Hz, 1H, NH), 3.41 (q, J = 6.8 Hz, 2H), 2.94 (septet, J = 6.8 Hz, 3H), 2.86 (s, 3H), 2.65 (t, J = 7.2 Hz, 2H), 1.73-1.60 (m, 4H), 1.28 (d, J = 7.2 Hz, 6H); ¹³C NMR (CDCl₃): δ 165.2, 147.5, 146.8, 146.6, 144.2, 143.7, 141.9, 129.0, 128.5, 128.4, 127.0, 126.6, 126.0, 116.8, 111.7, 40.2, 35.5, 34.0, 29.1, 28.8, 24.1, 14.8. HRMS calcd for [C₂₇H₃₀N₄O+H]⁺ 427.24924, found 427.24879.

3-(4-Isopropylphenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (24).

Starting from acid **15** (0.33 mmol) and 2-phenoxy-ethylamine, yield 90 mg (64%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.48 (s, 1H), 8.14 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 11.2 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 6.95 (t, J = 7.6 Hz, 1H), 6.87 (d, J = 8.0 Hz, 2H), 6.76 (t, J = 5.6 Hz, 1H), 4.22 (t, J = 5.2 Hz, 2H), 3.82 (q, J = 5.2 Hz, 2H), 2.94 (m, 1H), 2.89 (s, 3H), 1.28 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.5, 147.0, 146.7, 144.2, 143.7, 129.7, 128.9, 126.9, 126.5, 121.4, 116.4, 114.4, 111.7, 66.3, 39.8, 33.9, 24.0, 14.8. HRMS calcd. for [C₂₅H₂₆N₄O₂+H]⁺ 415.21285, found 415.21274.

(R)-3-(4-Isopropylphenyl)-7-methyl-N-(1-phenoxypropan-2-yl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (25).

Starting from acid **15** (0.48 mmol) and (*R*)-1-phenoxy-propan-2-amine.⁶ The crude mixture was purified by column chromatography (1/1 Pet ether/EtOAc), yield 148 mg (72%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.92 (s, 1H), 8.49 (s, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.18 – 7.13 (m, 2H), 6.70 – 6.64 (m, 3H), 4.48 (dd, J_{AX} = 5.2 Hz, J_{AB} = 11.2 Hz, 1H, CH₂), 4.23 (dd, J_{BX} = 5.6 Hz, J_{AB} = 11.2 Hz, 1H, CH₂), 3.93 (sextet, J = 6.4 Hz, 1H), 3.15 (s, 3H), 2.93 (septet, J = 6.8 Hz, 1H), 1.32 (d, J = 6.4 Hz, 3H), 1.28 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃): δ 164.7, 152.0, 149.3, 147.6, 146.8, 144.7, 144.4, 129.5, 128.7, 127.0, 126.6, 117.9, 113.3, 112.0, 110.3, 68.3, 47.7, 33.9, 24.1, 18.3, 15.1. HRMS calcd. for [C₂₆H₂₈N₄O₂+H]⁺ 429.22850, found 429.22750.

(R)-3-(4-Isopropylphenyl)-N-(1-methoxypropan-2-yl)-7-methylpyrazolo[1,5-a]pyrimidine-6-carboxamide (26).

Starting from acid **15** (0.80 mmol) and (*R*)-1-methoxypropan-2-amine hydrochloride.⁷ The crude mixture was purified by column chromatography (1/1 Pet ether/EtOAc), yield 155 mg (53%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.47 (s, 1H), 8.40 (s, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 7.9 Hz, 1H), 4.36 – 4.30 (m, 1H), 3.50 (dd, J = 9.5 Hz, 4.0 Hz, 1H), 3.41 (dd, J = 9.5 Hz, 4.5 Hz, 1H), 3.37 (s, 3H), 2.97- 2.90 (m, 1H), 2.88 (s, 3H), 1.30 – 1.28 (m, 9H). ¹³C NMR (CDCl₃): δ 164.7, 147.3, 146.9, 146.5, 144.1, 143.5, 129.0, 126.9, 126.5, 116.9, 111.6, 75.2, 59.1, 45.9, 33.9, 24.1, 17.6, 14.6. HRMS calcd. for [C₂₁H₂₆N₄O₂+H]⁺ 376.21285, found 376.21293.

3-(4-Isopropylphenyl)-7-methyl-N-(2-(phenylamino)ethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (27).

Starting from acid **15** (0.51 mmol) and *N*-phenylethane-1,2-diamine, yield 118 mg (56%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.36 (s, 1H), 8.32 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.13 (dd, J = 7.6 Hz, J = 0.8 Hz, 1H), 6.75 (t, J = 5.6 Hz, 1H, NH), 6.69 (dd, J = 7.2 Hz, 1H), 5.57 (d, J = 7.6 Hz, 2H), 3.57 (q, J = 5.6 Hz, 2H), 3.31 (t, J = 5.6 Hz, 2H), 2.92 (septet, J = 7.2 Hz, 1H), 2.79 (s, 3H), 1.27 (d, J = 7.2 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.9, 147.8, 147.5, 147.0, 146.6, 144.0, 143.8, 129.5, 128.8, 127.0, 126.6, 118.0, 116.3, 112.8, 111.7, 43.7, 39.9, 33.9, 24.1, 14.7. HRMS calcd. for [C₂₅H₂₇N₅O+H]⁺ 414.22884, found 414.22787.

3-(4-Isopropylphenyl)-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (28).

Starting from acid **16** (0.35 mmol) and 2-phenoxy-ethylamine, yield 83 mg (59%) as a yellow solid. ¹H NMR (CDCl₃): δ 9.19 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.48 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.25 (t, J = 7.2 Hz, 2H), 7.07 (s br, 1H, NH), 6.95

(t, J = 7.2 Hz, 1H), 6.87 (d, J = 8.0 Hz, 2H), 4.16 (t, J = 5.2 Hz, 2H), 3.90 (q, J = 5.2 Hz, 2H), 2.93 (septet, J = 7.2 Hz, 1H), 1.27 (d, J = 7.2 Hz, 6H). ¹³C NMR (CDCl₃): δ 163.5, 158.3, 147.8, 147.2, 145.3, 144.6, 136.1, 129.7, 128.6, 127.0, 126.6, 121.5, 115.5, 114.5, 112.1, 66.4, 39.9, 34.0, 24.1. HRMS calcd. for [C₂₄H₂₄N₄O₂+H]⁺ 401.19720, found 401.19676.

7-Ethyl-3-(4-isopropylphenyl)-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (29).

Starting from acid **17** (2.94 mmol) and 2-phenoxy-ethylamine, yield 897 mg (71%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.47 (s, 1H), 8.43 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.31-7.24 (m, 4H), 6.95 (t, J = 7.2 Hz, 1H), 6.86 (d, J = 8.0 Hz, 2H), 6.72 (t, J = 5.6 Hz, 1H, NH), 4.12 (t, J = 5.2 Hz, 2H), 3.82 (q, J = 5.2 Hz, 2H), 3.36 (q, J = 7.6 Hz, 2H), 2.93 (septet, J = 6.8 Hz, 1H), 1.39 (t, J = 7.6 Hz, 3H), 1.28 (d, J = 6.8 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 151.9, 147.4, 147.0, 144.4, 143.8, 129.7, 129.0, 126.9, 126.5, 121.4, 115.7, 114.4, 111.6, 66.3, 39.8, 33.9, 24.1, 22.1, 11.6. HRMS calcd. for [C₂₆H₂₈N₄O₂+H]⁺ 429.22850, found 429.22830.

3-(4-Isopropylphenyl)-N-(2-phenoxyethyl)-7-propylpyrazolo[1,5-a]pyrimidine-6-carboxamide (30).

Starting from acid **18** (3.69 mmol) and 2-phenoxy-ethylamine, yield 970 mg (60%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.51 (s, 1H), 8.44 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.32-7.24 (m, 4H), 6.97 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 7.6 Hz, 2H), 6.59 (t, J = 5.6 Hz, 1H, NH), 4.16 (t, J = 4.8 Hz, 2H), 3.86 (q, J = 5.2 Hz, 2H), 3.38-3.34 (m, 2H), 2.94 (septet, J = 6.8 Hz, 1H), 1.86 (q, J = 7.6 Hz, 2H), 1.28 (d, J = 7.2 Hz, 6H), 1.01 (t, J = 7.2 Hz, 3H). ¹³C NMR (CDCl₃): δ 165.5, 158.3, 150.7, 147.4, 146.9, 144.5, 143.7, 129.7, 129.0, 126.9, 126.6, 121.4, 116.1, 114.4, 111.6, 66.3, 39.8, 33.9, 30.2, 24.0, 20.7, 14.3. HRMS calcd. for [C₂₇H₃₀N₄O₂+H]⁺ 443.24415, found 443.24391.

3-Bromo-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (31)

Starting from **19** (13.3 mmol) and 2-phenoxy-ethylamine (13.3 mmol). The crude mixture was concentrated and purified by column chromatography (DCM/EtOAc 3:1) to give 4.14 g (91%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.55 (s, 1H), 8.17 (s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 6.97 (t, J = 7.2 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 4.18 (t, J = 4.8 Hz, 2H), 3.89 (q, J = 5.6 Hz, 2H), 2.94 (s, 3H). ¹³C NMR (CDCl₃): δ 164.9, 158.2, 147.9, 147.7, 145.9, 144.8, 129.7, 121.5, 117.0, 114.4, 85.5, 66.2, 39.9, 14.4.

General procedure for the preparation of 3-(aryl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamides via Suzuki-coupling (32-43). According to a modified procedure of Berger.⁸

A mixture of **19** (1.0 eq.), the substituted-phenylboronic acid (2.0 eq.), tetrakis(triphenylphosphine)palladium(0) (0.03 eq.) and sodium carbonate (3.0 eq.) in toluene (3.0 mL) and H₂O (0.5 mL) was heated in the microwave for 2 hours at 150 °C. Water was added and the organics were extracted 3x with DCM. The combined organic layer was dried, concentrated and purified by column (1% MeOH/DCM). The crystalline final products were obtained by recrystallization from MeOH.

7-Methyl-N-(2-phenoxyethyl)-3-(*p*-tolyl)pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (**32**).

Starting from **31** (0.20 mmol) and *p*-tolylboronic acid (0.30 mmol), yield 16 mg (21%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.54 (s, 1H), 8.46 (s, 1H), 7.87 (d, J = 7.2 Hz, 2H), 7.31-7.24 (m, 4H), 6.98 (dd, J = 7.2 Hz, 1H), 6.90 (d, J = 8.0 Hz, 2H), 5.56 (s br, 1H, NH), 4.18 (t, J = 4.8 Hz, 2H), 3.88 (q, J = 4.8 Hz, 2H), 2.96 (s, 3H), 2.38 (s, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.3, 146.7, 144.3, 143.9, 136.5, 129.8, 129.6, 128.6, 126.5, 121.6, 116.4, 114.5, 111.8, 66.4, 39.9, 21.4, 14.9. HRMS calcd. for [C₂₃H₂₂N₄O₂+H]⁺ 387.18155, found 387.18208.

7-Methyl-N-(2-phenoxyethyl)-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (**33**).

Starting from **31** (0.40 mmol) and *m*-tolylboronic acid (0.80 mmol), yield 82 mg (53%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.59 (s, 1H), 8.50 (s, 1H), 7.82-7.80 (m, 2H), 7.36-7.28 (m, 3H), 7.11 (d, J = 7.6 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H), 6.92 (d, J = 8.0 Hz, 2H), 6.50 (s, 1H), 4.20 (t, J = 5.2 Hz, 2H), 3.92 (q, J = 5.2 Hz, 2H), 3.00 (s, 3H), 2.44 (s, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.0, 146.8, 144.2, 143.9, 138.4, 131.3, 129.7, 128.7, 127.5, 127.1, 123.6, 121.4, 116.4, 114.4, 111.6, 66.3, 39.8, 29.8, 21.7, 14.8. HRMS calcd. for [C₂₃H₂₂N₄O₂+H]⁺ 387.18155, found 387.18183.

7-Methyl-N-(2-phenoxyethyl)-3-(*o*-tolyl)pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (**34**).

Starting from **31** (0.40 mmol) and *o*-tolylboronic acid (0.80 mmol), yield 37 mg (24%) as a yellow solid. ¹H NMR (CDCl₃): δ 8.54 (s, 1H), 8.29 (s, 1H), 7.49-7.45 (m, 1H), 7.32-7.27 (m, 5H), 6.98 (t, J = 7.2 Hz, 1H), 6.90 (d, J = 8.4 Hz, 2H), 6.51 (s, 1H, NH), 4.19 (t, J = 5.2 Hz, 2H), 3.89 (q, J = 5.2 Hz, 2H), 3.03 (s, 3H), 2.34 (s, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.2, 146.9, 145.9, 144.9, 136.9, 130.8, 130.7, 130.5, 129.8, 127.7, 126.0, 121.5, 116.5, 114.4, 112.3, 66.3, 39.9, 20.9, 14.9. HRMS calcd. for [C₂₃H₂₂N₄O₂+H]⁺ 387.18155, found 387.18194.

3-(4-Ethylphenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (**35**).

Starting from **31** (0.55 mmol) and (4-ethylphenyl)boronic acid (0.87 mmol), yield 16 mg (8%) of the final product as a yellow solid.

¹H NMR (CDCl₃): δ 8.60 (s, 1H), 8.50 (s, 1H), 7.93 (d, J = 7.6 Hz, 2H), 7.30(d, J = 7.6 Hz, 4H), 7.00 (t, J = 6.8 Hz, 1H), 6.92 (d, J = 7.2 Hz, 2H), 6.45 (s br, 1H), 4.21 (s, 2H), 3.94 (s, 2H), 3.02 (s, 3H), 2.70 (q, J = 7.6 Hz, 2H), 1.27 (t, J = 7.6 Hz, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.3, 146.8, 144.3, 143.9, 143.0, 129.8, 128.9, 128.5, 126.6, 121.6, 116.4, 114.5, 111.9, 66.4, 39.9, 28.8, 15.8, 14.9. HRMS calcd. for [C₂₄H₂₄N₄O₂+H]⁺ 401.19668, found 401.19720.

3-(4-(*tert*-Butyl)phenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide
(36)

Starting from **31** (0.40 mmol) and (4-(*tert*-butyl)phenyl)boronic acid (0.80 mmol), yield 54 mg (31%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.53 (s, 1H), 8.45 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 6.97 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 6.64 (t, J = 5.2 Hz, 1H, NH), 4.16 (t, J = 5.2 Hz, 2H), 3.86 (q, J = 5.2 Hz, 2H), 2.94 (s, 3H), 1.36 (s, 9H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 149.8, 147.2, 146.8, 144.4, 143.9, 129.8, 128.6, 126.4, 125.9, 121.5, 116.5, 114.5, 111.8, 66.3, 39.3, 34.7, 31.4, 14.9. HRMS calcd. for [C₂₆H₂₈N₄O₂+H]⁺ 429.22817, found 429.22849.

3-(4-Methoxyphenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide
(37)

Starting from **31** (0.32 mmol), (4-methoxyphenyl)boronic acid (0.48 mmol), yield 53 mg (41%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.51 (s, 1H), 8.41 (s, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.31-7.27 (m, 2H), 7.00-6.96 (m, 3H), 6.90 (d, J = 7.6 Hz, 2H), 6.60 (t, J = 5.2 Hz, 1H, NH), 4.18 (t, J = 5.2 Hz, 2H), 3.89 (q, J = 5.2 Hz, 2H), 3.84 (s, 3H), 2.95 (s, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.5, 158.3, 147.1, 146.5, 143.9, 143.5, 129.7, 127.7, 124.1, 121.5, 116.3, 114.4, 144.3, 111.5, 66.3, 55.4, 39.8, 14.8. HRMS calcd. for [C₂₃H₂₂N₄O₃+H]⁺ 403.17647, found 403.17618.

3-(4-Isopropoxyphenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide
(38)

Starting from **31** (0.55 mmol) and (4-isopropoxyphenyl)boronic acid (0.83 mmol), yield 20 mg (12%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.54 (s, 1H), 8.42 (s, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.32-7.26 (m, 2H), 7.00-6.90 (m, 3H), 6.91 (d, J = 8.0 Hz, 2H), 6.52 (t, J = 6.4 Hz, 1H), 4.58 (septet, J = 6.4 Hz, 1H), 4.19 (t, J = 4.8 Hz, 2H), 3.90 (q, J = 4.8 Hz, 2H), 2.97 (s, 3H), 1.36 (d, J = 6.0 Hz, 6H). ¹³C NMR (CDCl₃): δ 165.5, 158.3, 156.9, 147.2, 146.6, 144.0, 143.6, 129.8, 127.8, 123.9, 121.6, 116.4, 116.3, 114.5, 111.7, 70.1, 66.4, 39.9, 22.2, 14.9. HRMS calcd. for [C₂₅H₂₆N₄O₃+H]⁺ 431.20777, found 431.20752.

3-([1,1'-Biphenyl]-4-yl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide
(39)

Starting from **31** (0.40 mmol), [1,1'-biphenyl]-4-ylboronic acid (0.60 mmol), yield 21 mg (12%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.63 (s, 1H), 8.56 (s, 1H), 8.11 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 7.6 Hz, 2H), 7.37-7.30 (m, 3H), 7.00 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 8.4 Hz, 2H), 6.47 (t, J = 5.2 Hz, 1H NH), 4.21 (t, J = 5.2 Hz, 2H), 3.94 (q, J = 5.2 Hz, 2H), 3.03 (s, 3H). ¹³C NMR (CDCl₃): δ 165.4, 158.3, 147.5, 147.0, 144.0, 142.2, 141.0, 139.5, 130.7, 129.8, 129.0, 127.7, 127.4, 127.1, 126.9, 126.8, 121.6, 116.6, 114.5, 66.5, 40.0, 15.0. HRMS calcd. for [C₂₈H₂₄N₄O₂+H]⁺ 449.19720, found 449.19700.

3-(4-Chlorophenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (40)

Starting from **31** (0.33 mmol), and (4-chlorophenyl)boronic acid (0.66 mmol), yield 82 mg (62%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.62 (s, 1H), 8.50 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 8.0 Hz, 2H), 7.00 (t, J = 7.6 Hz, 1H), 6.92 (d, J = 8.4 Hz, 2H), 6.42 (s, 1H, NH), 4.21 (t, J = 5.2 Hz, 2H), 3.94 (q, J = 5.6 Hz, 2H), 3.03 (s, 3H). ¹³C NMR (CDCl₃): δ 165.2, 158.2, 147.3, 147.0, 144.3, 143.6, 132.2, 130.0, 129.7, 128.9, 127.5, 121.5, 116.6, 114.4, 110.4, 66.3, 39.9, 14.8. HRMS calcd. for [C₂₂H₁₉ClN₄O₂+H]⁺ 407.12693, found 407.12679.

3-(3-Chlorophenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (41)

Starting from **31** (0.40 mmol) and (3-chlorophenyl)boronic acid (0.60 mmol), yield 15 mg (9%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.61 (s, 1H), 8.49 (s, 1H), 8.06 (s, 1H), 7.90 (d, J = 7.6 Hz, 2H), 7.39-7.23 (m, 4H), 6.99 (t, J = 7.2 Hz, 1H), 6.92 (d, J = 8 Hz, 2H), 6.52 (s, 1H, NH), 4.20 (s, 2H), 3.93 (q, J = 4.0 Hz, 2H), 3.03 (s, 3H). ¹³C NMR (CDCl₃): δ 165.2, 158.3, 147.5, 147.3, 144.6, 143.9, 134.8, 133.4, 130.1, 129.8, 126.7, 126.3, 124.4, 121.6, 116.8, 114.5, 110.3, 66.4, 40.0, 15.0. HRMS calcd. for [C₂₂H₁₉ClN₄O₂+H]⁺ 407.12693, found 407.12678.

3-(3,4-Dichlorophenyl)-7-methyl-N-(2-phenoxyethyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (42)

Starting from **31** (0.51 mmol) and (3,4-dichlorophenyl)boronic acid (0.76 mmol), yield 77 mg (45%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.64 (s, 1H), 8.49 (s, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.88 (d, J¹ = 8.4 Hz J² = 1.6 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.32 (t, J = 8.0 Hz, 2H), 7.00 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 8.0 Hz, 2H), 6.46 (s, 1H, NH), 4.21 (t, J = 5.2 Hz, 2H), 3.94 (q, J = 5.2 Hz, 2H), 3.02 (s, 3H). ¹³C NMR (CDCl₃): δ 164.5, 158.4, 149.0, 146.8, 143.9, 132.6, 131.5, 130.9, 129.6, 128.2, 126.9, 125.6, 120.8, 117.7, 114.6, 107.2, 65.9, 39.1, 14.7. HRMS calcd. for [C₂₂H₁₈Cl₂N₄O₂+H]⁺ 441.08796, found 441.08789.

7-Methyl-N-(2-phenoxyethyl)-3-(4-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidine-6-carboxamide (43)

Starting from **31** (0.40 mmol) and (4-(trifluoromethyl)phenyl)boronic acid (0.60 mmol), yield 54 mg (31%) of the final product as a yellow solid. ¹H NMR (CDCl₃): δ 8.62 (s, 1H), 8.53 (s, 1H), 8.14 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.31 (t, J = 8.4 Hz, 2H), 7.00 (t, J = 7.6 Hz, 1H), 6.92 (d, J = 8.4 Hz, 2H), 6.54 (s, 1H, NH), 4.20 (t, J = 5.2 Hz, 2H), 3.92 (q, J = 5.2 Hz, 2H), 3.03 (s, 3H). ¹³C NMR (CDCl₃): δ 165.2, 158.6, 147.4, 147.6, 145.1, 144.1, 135.5, 129.9, 126.5, 125.9, 125.9, 121.8, 117.2, 114.8, 110.4, 66.7, 40.2, 14.9. HRMS calcd. for [C₂₃H₁₉F₃N₄O₂+H]⁺ 441.15329, found 441.15305.

Experimental Section – Pharmacology

[³H]-Nicotinic acid (60 Ci/mmol) was purchased from BioTrend (Koeln, Germany). [³⁵S]-GTPγS (1250 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA).

Cell culture and membrane preparation

Human embryonic kidney (HEK) 293T cells stably expressing human HCA₂ were cultured in DMEM supplemented with 10% newborn bovine serum, 0.4 mg/mL G418, 50 IU/mL penicillin and 50 μg/mL streptomycin. The cells were harvested by scraping in cold PBS, centrifuged at 1000g for 10 min and resuspended in cold 50 mM Tris-HCl buffer, pH 7.4. Then a DIAX 900 electrical homogenizer (Heidolph, Schwabach, Germany) was used for 15 sec to obtain cell lysis. The suspension was centrifuged at 100 000g for 20 min at 4 °C and the supernatant was discarded. The pellet was resuspended in Tris-HCl, and the homogenization and centrifugation steps were repeated. The membranes were resuspended in cold assay buffer (50 mM Tris HCl, 1 mM MgCl₂, pH 7.4) and the protein content was determined using a BCA assay (Thermo Scientific, Waltham, USA). During membrane preparation the suspension was kept on ice at all times. Membrane aliquots were stored at -80 °C until the day of the assay.

[³H]-nicotinic acid binding assays

Competition assays

Membranes of the HEK293T-HCA₂ cell line (50 μg protein per tube) were incubated 1 hour at 25 °C with 20 nM [³H]-nicotinic acid and increasing concentrations of the test compound in assay buffer (50 mM Tris HCl, 1 mM MgCl₂, pH 7.4). The total assay volume was 100 μL. To assess the total binding a control without test compound was included. The non-specific binding was determined in the presence of 10 μM unlabeled nicotinic acid. Final DMSO concentration in all samples was ≤ 0.25%.

The incubation was terminated by filtering over GF/B filters using a 24-sample harvester (Brandel, Gaithersburg, USA). The filters were washed 3 times with 2 mL cold buffer (50 mM Tris HCl, pH 7.4). Filters were transferred to counting vials and counted in a Perkin Elmer LSA Tri-Carb 2900TR counter after 2 h extraction in 3.5 ml Emulsifier Safe liquid scintillation cocktail (Perkin Elmer, Waltham, USA).

Dissociation assays

HEK-HCA₂ membranes (50 µg protein per tube) were incubated 3 h at 15 °C with 20 nM [³H]-nicotinic acid in 50 mM Tris HCl, 1 mM MgCl₂, pH 7.4, in a total volume of 100 µL. Then, 5 µL nicotinic acid (final concentration 10 µM), in absence or presence of test compound (final concentration 10 µM) was added. In the single point screen dissociation was terminated after 30 min at 15 °C, and for full curves dissociation was measured at different time points. Non-specific binding was determined in the presence of 10 µM nicotinic acid, added prior to the membranes. Otherwise the assay was performed as the competition assay.

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