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ARTICLE TYPE

Improved synthesis of 4-/6-substituted 2-carboxy-1*H*-indole-3-propionic acid derivatives and structure-activity relationships as GPR17 agonists†

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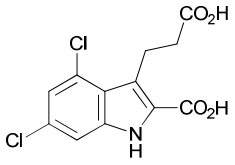
The orphan G protein-coupled receptor GPR17 was shown to be involved in myelin repair and has been proposed as a novel drug target for the treatment of brain and spinal cord injury and for multiple sclerosis. Recently, 3-(2-carboxy-4,6-dichloro-indol-3-yl)propionic acid (MDL29,951, **1a**) was discovered and characterized as a potent synthetic GPR17 agonist. In the present study we substantially optimized the preparation of **1a**, which is carried out via Japp-Klingemann condensation of 3,5-dichloro-phenyldiazonium chloride and deprotonated 2-(ethoxycarbonyl)cyclopentanone yielding phenylhydrazone derivative **5a** followed by Fischer indole (diaza-Cope) rearrangement. A robust synthesis of **1a** (75% yield) was developed to allow upscaling of the procedure. The developed method was applied to the synthesis of a series of 10 derivatives, eight of which represent new compounds. Biological evaluation in calcium mobilization assays using 1321N1-astrocytoma cells recombinantly expressing the human GPR17 provided first insights into their structure-activity relationships. 3-(2-Carboxy-4,6-dibromo-indol-3-yl)propionic acid (**1b**) showed similar potency to **1a** and represents the most potent synthetic GPR17 agonist described to date with an EC_{50} value of 202 nM.

Introduction

The orphan G protein-coupled receptor 17 (GPR17) belongs to the large family of rhodopsin-like class A G protein-coupled receptors (GPCRs). It is coupled to inhibition of adenylate cyclase via G_i proteins resulting in decreased intracellular cAMP levels, and to G_q proteins which activate phospholipase C leading to IP_3-mediated intracellular calcium release.[1,2] GPR17 was recently shown to be involved in myelin repair and has therefore been proposed as a novel drug target for the treatment of multiple sclerosis, brain and spinal cord injury, and neurodegenerative diseases.[2-6] Thus, the development of GPR17 modulators is of high pharmacological relevance. Several compounds have been postulated as physiological agonists of GPR17, including cysteinylleukotrienes (CysLTs) C4 and D4, UDPglucose, UDPgalactose, and UDP.[1] However, several groups, including ours, were unable to confirm the described effects.[2,4,7,8] Recently, Hennen et al. identified 3-(2-carboxy-4,6-dichloro-indol-3-yl)propionic acid (MDL29,951, **1a**, Figure 1) as a synthetic agonist for GPR17 and characterized it broadly in recombinant and native cells.[2] Compound **1a** showed high potency at GPR17 in the nanomolar range; the determined EC_{50} value was found to be dependent on the assay system and the receptor expression level.[2]

The described synthesis of **1a** provides only moderate overall yields. For extended studies of GPR17 using **1a** as a tool compound, and for setting up a high-throughput (HTS) screening assay to identify GPR17 antagonists gram amounts of the agonist are required. Therefore the goal of the present study was to develop a significantly improved synthetic procedure for **1a** by carefully studying and optimizing the critical reaction steps. Furthermore, the new method was to be applied to the preparation of analogs to study their structure-activity relationships (SARs).



1a (MDL29,951)

Figure 1. The first reported synthetic GPR17 agonist[2]

Results and Discussion

Syntheses

The synthetic pathway to indole derivative **1a** and its analogs starts with a Japp–Klingemann reaction of aniline derivatives **2** with 2-(ethoxycarbonyl)cyclopentanone (**4**) to obtain the corresponding phenylhydrazone derivatives **5**. Subsequent heating in the presence of a strong acid (Fischer indole synthesis, a special type of the (diaza)-Cope rearrangement) leads to ring closure producing the indole *mono*–ethyl esters **6**, which can be saponified to yield the desired products **1** (Scheme 1).[2,9] The

published procedures for the preparation of **1a** suffer from low yields.[2,9] Therefore, we systematically studied the low-yielding Fischer indole cyclization of dichlorophenylhydrazone derivative **5a** using four different protocols (Scheme 2). At first we applied the procedure published by Salituro *et. al*.[9] using concentrated sulfuric acid in refluxing ethanol, which in our hands resulted in a low yield of 10.5 % (Scheme 2).[2] By applying polyphosphoric acid in refluxing toluene, or in acetic acid, respectively, formation of the expected product was not observed at all. Finally we applied *para*–toluenesulfonic acid in refluxing ethanol,[9] and again only a low yield of **6a** was obtained (9%, Scheme 2).

Since we suspected that the free carboxylate function of **5a** might be involved in side-reactions we decided to convert it to an ester group prior to Fischer indole cyclization. This simple intermediate reaction step, combined with optimized cyclization reaction conditions led to a dramatically increased yield of indole **6a**. Thus, *mono*-ester **5a** was converted to its *di*–ethyl ester **8a**, which was subsequently cyclized to the indole derivative **9a** according to the following conditions (Scheme 3): compound **8a** was added to a *p*–toluenesulfonic acid (*p*–TSA) solution in toluene, which had previously been dried by means of a Dean-Stark apparatus in refluxing toluene for ca. 1 h (for more details see the experimental part). The solution was heated for 5 h under the same conditions. The ester functions of indole **9a** were subsequently hydrolyzed. With these modifications, an overall yield of the GPR17 agonist **1a** of 75% was obtained starting from the corresponding aniline derivative (3,5-dichloroaniline), as compared to only 10.5% in our previous study,[2] or 40% reported by Salituro et al.,[9] a yield that had not been reproducible by three different experienced chemists in our laboratory using the previously reported method.

The complete optimized synthetic procedure for indole derivative **1a** as well as its analogs **1b-j** is depicted in Scheme 3. In the first step, the substituted anilines (**2**) are diazotized by adding sodium nitrite in the presence of hydrogen chloride at low temperature (0–5°C) to yield diazonium salts **3**, followed by the addition of the 2-(ethoxycarbonyl)cyclopentanone anion (**4'**). The resulting mixture is treated with ice and stirred at 40°C until the phenylhydrazine *mono*–ethyl ester **5** is formed (the reaction is monitored by TLC). Carboxylic acids **5** are then refluxed in ethanol at 100°C in the presence of sulfuric acid to obtain the *di*–ethyl esters of phenylhydrazones **8**, which are subsequently cyclized to the corresponding indole *di*–ethyl ester derivatives **9**. Finally, compounds **9** are saponified to yield the desired indoles **1** containing two carboxylic acid functions (Scheme 3). This improved method was applied to the synthesis of 10 analogous indole derivatives, of which eight were new, not previously described compounds (**1b,c,e,f-j**, Table 1). Besides 4,6-di-substituted indole derivatives (**1a-e**) which were obtained starting from symmetrically 3,5-disubstituted anilines, 4- and 6-monosubstituted derivatives (**1g-j**) were prepared starting from *meta*-mono-substituted anilines. Symmetrically substituted aniline derivatives produced a single product (**1a-f**, Table 1), while *meta*-mono-substituted anilines gave two isomers: 4- and 6-substituted indole derivatives (**1g-j**, Table 1). The separation of the two formed isomers was achieved on the *di*–ester stage by silica gel column chromatography of indoles **9g** / **9h**, and **9i** / **9j**, respectively, using 20% ethyl acetate in cyclohexane as an eluent. All final products were analyzed by ^{1}H- and ^{13}C-NMR spectroscopy, elemental analysis and high-performance liquid chromatography (HPLC) coupled to electrospray ionization (ESI) mass spectrometry (MS). Purity as determined by HPLC-ESI-MS was in all cases greater than 95%.

Biological evaluation

The compounds were investigated for their potential to induce calcium mobilization in 1321N1 astrocytoma cells stably transfected with the human GPR17 using the calcium-chelating fluorescent dye Oregon Green®.

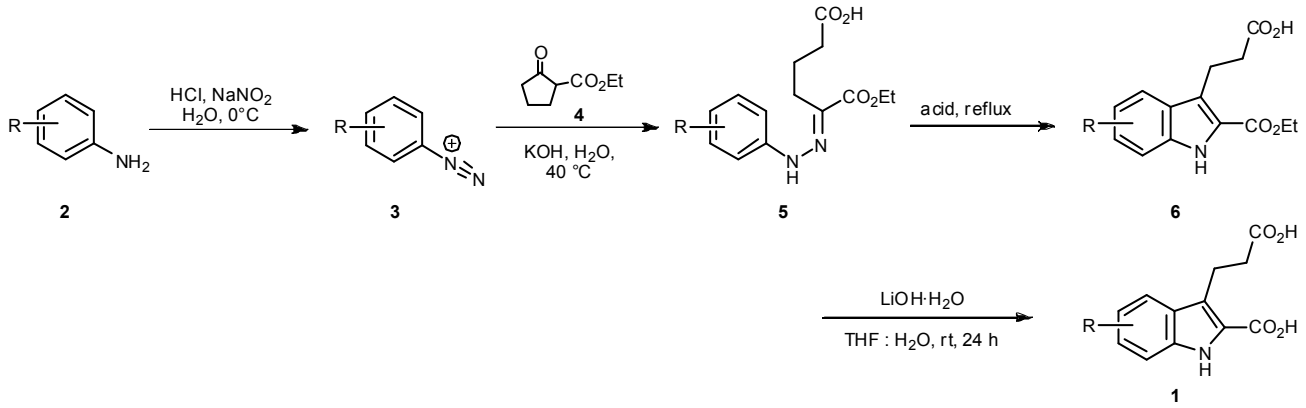
Structure-activity relationships

GPR17 has been shown to be coupled to different G proteins,[1,2] including G_q, which mediates the release of inositol trisphosphate (IP_3). IP_3 binds to ryanodine receptors on the endoplasmic reticulum and triggers the opening of calcium channels resulting in an increase in intracellular calcium levels. In 1321N1 astrocytoma cells recombinantly expressing moderate levels of the human GPR17 an EC_{50} value of 330 nM was determined for **1a** in calcium mobilization assays (see Table 1). The Hill slope of the curve for **1a** as well as for all other investigated indole derivatives was not significantly different from 1.

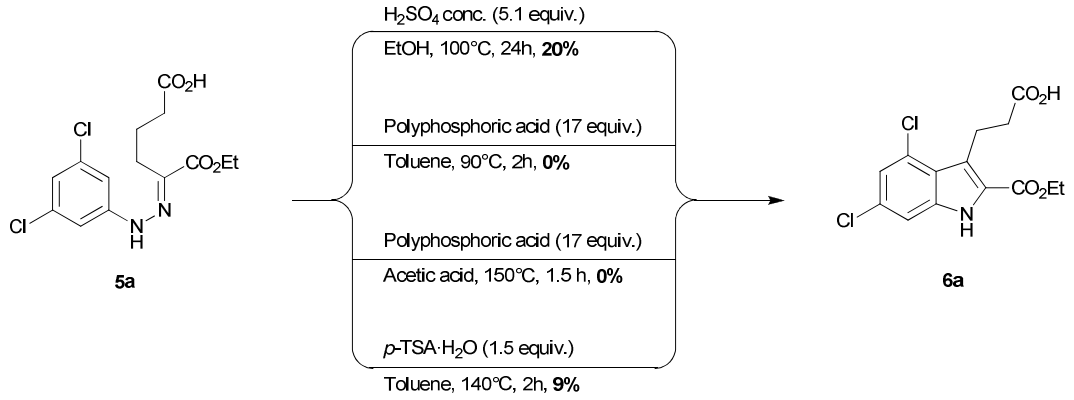
In the present study initial SARs were obtained for a set of 10 compounds. Different *tri*– and *tetra*–substituted indole derivatives were investigated (see Table 1). Analogs of the 4,6-dichloro-substituted indole derivative **1a**, in which the chlorine atoms were replaced by bromine or iodine atoms, or by methoxy or trifluoromethoxy groups showed the following rank order of potency: dibromo (**1b**) > dichloro (**1a**) > diiodo (**1c**) > di-(trifluoromethoxy) (**1e**) >> dimethoxy (**1d**). These results indicate that the size of the substituents is important for receptor interaction. The dibromo-substituted indole showed a somewhat higher potency (EC_{50} = 0.202 μM) than the dichloro-substituted lead structure **1a**, although the difference was not statistically significant. However, larger substituents were less well tolerated and led to reduced potency. Besides sterical effects, electronic effects are also important: high electronegativity of the 4,6-substituents appears to be required for potency (compare e.g. **1d** and **1e**).

Introducing a small substituent (fluoro) in position 5 of lead structure **1a** significantly decreased potency by >150-fold (compare **1a** with **1f**, Table 1). This means that a substituent in position 5, at least one with a high electronegativity, is hardly tolerated by the receptor. Finally, derivatives substituted either in position 4 or 6 of the indole moiety with a halogen atom (Br, I) were investigated. It was found that a bulky halogen atom in the 6-position was very important for high potency. The larger iodine atom was superior to a bromine atom in that position (compare **1h** and **1j**). Contrary, indole derivatives **1g** and **1i**, which were only substituted in the 4- but not in the 6-position, were only weak agonists at GPR17. Based on a series of 10 indole derivatives it can be recognized that their SARs as GPR17 agonists are steep, and small modifications can modulate potency

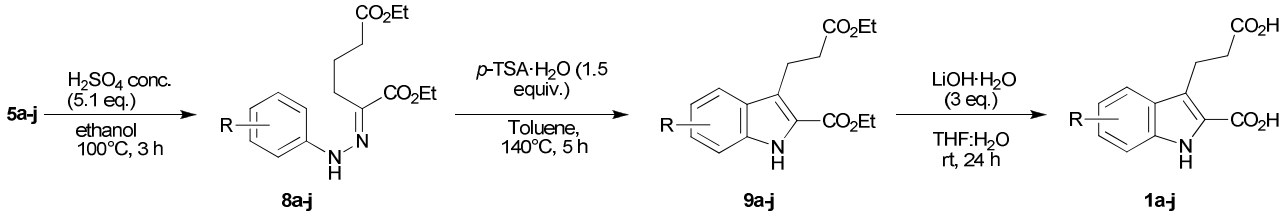
and even abolish their activity.



Scheme 1. General Japp–Klingemann/Fischer indole synthesis pathway for the preparation of indole derivatives **1**

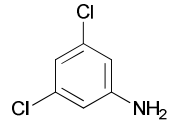
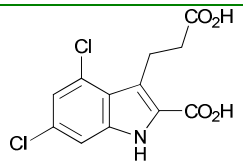
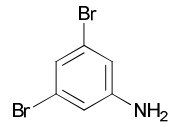
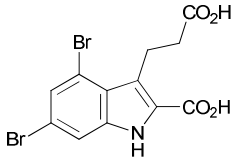
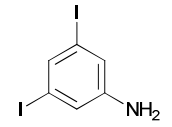
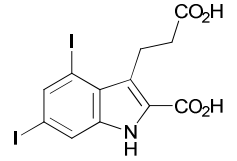
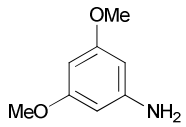
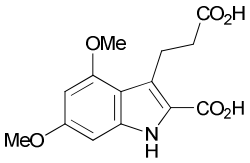
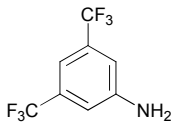
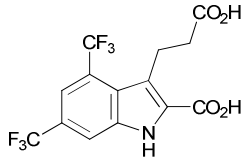
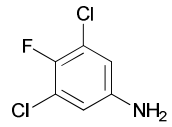
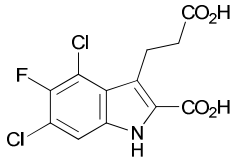
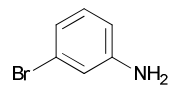
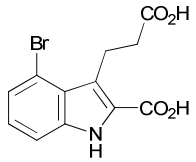
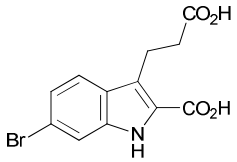
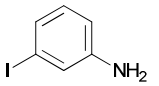
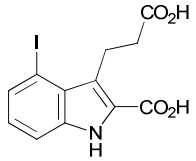
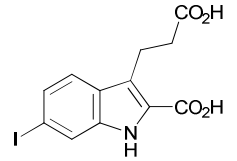


Scheme 2. Different cyclization methods of compound **5a**



Scheme 3. Improved procedure for the synthesis of 3-(2-carboxy-indol-3-yl)propionic acid derivatives

Table 1. Synthesized indole derivatives (**1a–j**), overall yields, melting points, and potency to stimulate GPR17

Product	Aniline Derivative	Product (1a-j)	m.p. (°C)	Yield (%)[a]	EC_{50} ± SEM (μM)[b]
1a			275–277	75	**0.331** ± 0.087
1b			259–260	63	**0.202** ± 0.063
1c			280–282	35	**4.79** ± 0.877
1d			239–241	36	>>100[c]
1e			249–251	45	**17.1** ± 4.12
1f			292–294	65	**52.0** ± 5.50
1g			235–237	23 (69% total)	**45.5** ± 8.66
1h			234–235	46 (69% total)	**2.24** ± 0.68
1i			246–248	30 (90% total)	**36.8** ± 1.81
1j			230–232	60 (90% total)	**0.715** ± 0.128

[a]Total isolated yield; [b]potency to induce calcium mobilization in 1321N1 astrocytoma cells transfected with the human GPR17; [c]also no antagonistic activity was observed.

Experimental Part

General: All materials were used without prior purification. Thin-layer chromatography was performed using TLC aluminum sheets silica gel 60 F_{254}. Synthesized compounds were visualized under UV light (254 nm). ^{1}H- and ^{13}C-NMR data were measured in DMSO-d_6 as a solvent. Chemical shifts are reported in parts per million (ppm) relative to the deuterated solvent (DMSO-d_6), δ ^{1}H: 2.49 ppm, ^{13}C: 39.7 ppm, coupling constants J are given in Hertz and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), sext (sextet), m (multiplet), br (broad). The purities of isolated products were determined by ESI–mass spectra obtained on an HPLC–MS instrument (LC–MS) using the same procedure as previously published.[10] The purity of the compounds was determined at 254 nm. For further proof of purity of the final compounds (**1a-j**), elemental analysis and ^{1}H-NMR were determined. Melting points were measured on a melting point apparatus and are uncorrected.

General procedures for the synthesis of the products 1a–j

General procedure A.

(i) Preparation of benzenediazonium salt derivatives (mixture I): To a well stirred suspension of aniline derivatives (10 mmol) in 16.6 ml aq. HCl (5 M) at 0–5 °C was dropwise added a solution of sodium nitrite (1.38 g, 20 mmol, 2 equiv.) in 8 ml water, previously cooled to 0–5 °C in an ice bath. The addition of sodium nitrite solution was slow, in order to keep the temperature of the mixture below 8 °C. The resulting orange-red mixture was stirred at 0–5 °C for additional 20 min in an ice bath.

(ii) Preparation of 2-(ethoxycarbonyl)cyclopentanone anion (mixture II): 2-(Ethoxycarbonyl)cyclopentanone (2.512 ml, 1.344 g, 15 mmol) was dissolved in ethanol (4.2 ml) and cooled to 0–5 °C. Then, a potassium hydroxide solution (5.040 g, 90 mmol, 6 equiv.) in water (5 ml) previously cooled to 0–5 °C was added dropwise within ca. 30 min in order to keep the temperature below 8 °C. The mixture turned to a white-milky appearance, and the final mixture was stirred at 0–5 °C for further 30 min.

(iii) Synthesis of compounds 5a–j: Ice (50 g) was added to mixture II with stirring at 0–5 °C in an ice bath, followed by the addition of mixture I, and stirring continued for 1 h at 40 °C. The combined mixtures were then let to cool to rt and the pH was subsequently adjusted to 4–5 by adding 1 M aq. HCl. The desired product was extracted with diethyl ether (3 × 50 ml). The combined organic layers were collected, dried over magnesium sulfate, filtered, and the filtrate was evaporated to dryness yielding gummy material (95–100%). This material was used without further purification for the next step.

General procedure B. Synthesis of compounds 8a–j: Compound **5a–j** (10 mmol), obtained from general procedure A, was dissolved in absolute ethanol (100 ml) followed by the addition of concentrated sulfuric acid (2.7 ml, 50.5 mmol, 5.1 equiv.). The mixture was then allowed to reflux for 1 h at 100 °C. Then the ethanol was evaporated and the residue was treated with 100 ml of ice-water. The aqueous solution was extracted with dichloromethane (3 × 50 ml); the organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography using 20% of ethyl acetate in cyclohexane yielding a white solid in 85–100% yield.

General procedure C. Synthesis of indole *di*–ethyl esters (9a–j): A mixture of *p*-toluenesulfonic acid (2.954 g, 15 mmol, 1.5 equiv.) and 100 ml of dry toluene was refluxed for 1 h at 140 °C; water was continuously removed by means of a Dean-Stark trap. Subsequently, 10 mmol of the starting material **8a–j** dissolved in a minimum amount of dry toluene (ca. 15 ml) was added and the mixture was refluxed for 5 h. Then it was allowed to cool down to rt, and toluene was removed under reduced pressure and the residue was dissolved in dichloromethane and washed with water. The organic layer was dried over magnesium sulfate, filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 20% of ethyl acetate/cyclohexane as eluent.

General procedure D. Saponification of indole diethyl esters 9a–j yielding the final products **1a-j:** Compound (**9a–j**, 10 mmol) was dissolved in 25 ml tetrahydrofurane (THF) with stirring at rt. Then a solution of 1.26 g of lithium hydroxide trihydrate (3 equiv.) in 25 ml water was added and the resulting mixture was left to stir at rt for 24 h. After completion of the reaction THF was removed under reduced pressure, the pH was adjusted to 4–5, and the product was extracted with diethyl ether (3 × 30 ml). The organic layers were dried over magnesium sulfate, filtered, and evaporated to dryness to yield the final products (**1a–j**) as solids in excellent isolated yield (95–100%).

Analytical data of the synthesized products 1a–j:

3-(2-Carboxyethyl)-4,6-dichloro-1*H*-indole-2-carboxylic acid (**1a**): ^{1}H-NMR (DMSO–d_6):) δ 2.47 (m, 2 H, 2′-H); 3.49 (m, 2 H, 1′-H); 7.14 (d, 1 H, ^{2}J = 1.8, 5-H); 7.38 (d, 1 H, ^{2}J = 1.8, 7-H); 11.95 (s, 1 H, NH); 12.70 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–*d6*) δ 20.21 (C-2′), 36.24 (C-1′), 111.38 (C5), 120.65 (C7), 121.06 (C4), 122.24 (C6), 126.55 (C3a), 127.35 (C7a) 128.75 (C2) 137.41 (C3), 162.68 (2′-CO_2H), 173.69 (2-CO_2H). LC-MS (*m/z*): 300 $[M]^-$. Purity (LC-MS): 98.5%.

3-(2-Carboxyethyl)-4,6-dibromo-1*H*-indole-2-carboxylic acid (**1b**): ^{1}H-NMR (MeOH–d_4): δ 2.67 (t, 2H, ^{3}J = 8.4 Hz, CH_2); 3,71 (t, 2H, ^{3}J = 8.4 Hz, CH_2); 7.42 (d, 1H, ^{4}J = 1.6 Hz, C7-H); 7,62 (d, 1H, ^{4}J = 1.6 Hz, C5-H). ^{13}C-NMR (MeOH–d_4): δ 21.4 (C-2′); 37.9 (C-1′); 115.9 (C-7); 117.0 (C-4); 118.8 (C-6); 123.9 (C-3); 125.5 (C-2); 127.9 (C-3a); 128.2 (C-5); 139.6 (C-7a); 164.6 (2′-CO_2H); 170.1 (2-CO_2H). LC-MS (*m/z*): 392.0 $[M]^+$; 390.0 $[M]^-$. Purity (LC-MS): 98.8 %.

3-(2-Carboxyethyl)-4,6-diiodo-1*H*-indole-2-carboxylic acid (**1c**): ^{1}H-NMR (DMSO–d_6) δ 2.48 (m, 2 H, 2′-H); 3.49 (m, 2 H, 1′-H); 7.78 (dd, 2 H, J = 1.7 Hz, 5-H, 7-H); 11.80 (s, 1 H, NH); 12.68 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 18.9 (C-2′); 36.4 (C-1′), 88.3 (C-4); 89.5 (C-6); 121.4 (C-7); 122.0 (C-3); 126.2 (C-2); 126.4 (C-3a); 137.8 (C-7a); 138.1 (C7); 162.7 (2′-

CO_2H); 173.58 (2-CO_2H). LC-MS (*m/z*): 503 $[M+NH_4^+]^+$, 486 $[M]^+$, 484 $[M]^-$. Purity (LC-MS): 97.9 %.

3-(2-Carboxyethyl)-4,6-dimethoxy-1*H*-indole-2-carboxylic acid (**1d**): ^{1}H-NMR (DMSO–d_6) δ 2.43 (t, 2 H, ^3J = 8.3 Hz, H-1'), 3.34 (t, 2 H, ^3J = 8.3 Hz, H-2'), 3.74, 3.81 (2 s, each 3 H, OCH_3), 6.11 (d, 1 H, ^4J = 2.0 Hz, H-5), 6.40 (d, 1 H, ^4J = 2.0 Hz, H-7), 11.15 (s, 1 H, NH), 12.24 (s, 2 H, CO_2H). ^{13}C-NMR (DMSO–d_6) δ 21.3 (C-1'), 36.0 (C-2'), 55.3, 55.4 (2 C, OCH_3), 86.7 (C-5), 92.1 (C-7), 112.3 (C-3a), 121.6 (C-3), 122.6 (C-2), 138.2 (C-7a), 155.9 (C-4), 159.2 (C-6), 163.1, 174.2 (2 C, CO_2H). Elemental analysis for $C_{14}H_{15}NO_6$: Calcd.: C 57.34; H 5.16; N 4.78; O 32.73; found: C 57.47; H 5.22; N 4.61. LC-MS (*m/z*): 294.30 $[M]^+$, 292.25 $[M]^-$. Purity (LC-MS): 98.9%.

3-(2-Carboxyethyl)-4,6-di(trifluoromethyl)-1*H*-indole-2-carboxylic acid (**1e**): ^{1}H-NMR (DMSO–d_6) δ 2.43 (m, 2 H, 2'-H); 3.33 (m, 2 H, 1'-H); 7.67 (s, 1 H, 1 H, 5-H); 8.07 (s, 1 H, 7-H); 12.67 (s, 1 H, NH); 12.92 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 20.0 (C-2'); 35.0 (C-1'); 114.6 (C-4); 115.2 (C-6); 119.5 (C-7); 120.9, 121.8, 122.1, 122.3 (CF_3-C-4); 122.7 (C-2); 123.1 (C-3); 123.3 (C-3a); 123.5, 123.8, 124.9, 125.2 (CF_3-C-6); 129.1 (C-5); 136.2 (C-7a); 160.7 (2'-CO_2H); 172.0 (2-CO_2H). Elemental analysis for $C_{14}H_9F_6NO_4$: Calcd.: C, 45.54; H, 2.46; N, 3.79, found: C, 45.94; H, 2.54; N, 3.82. LC-MS (*m/z*): 387 $[M+NH_4^+]^+$, 370 $[M]^+$, 368 $[M]^-$. Purity (LC-MS): 98.9%.

3-(2-Carboxyethyl)-4,6-dichloro-4-fluoro-1*H*-indole-2-carboxylic acid (**1f**): ^{1}H-NMR (DMSO–d_6) δ 2.48 (m, 2 H, 2′-H); 3.48 (m, 2 H, 1′-H); 7.49 (dd, 1 H, ^2J = 6 Hz, 7-H); 11.98 (s, 1 H, NH); 12.72 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 20.1 (C-2′); 36.1 (C-1′); 112.5 (C-7); 113.0, 113.2, 117.6, 117.7, 121.3, 121.4 (C-5); 122.3 (C-4); 127.7 (C-6); 132.5 (C-3); 147.2 (C-3a); 149.0 (C-2); 162.5 (2′-CO_2H), 173.6 (2-CO_2H). LC-MS (*m/z*): 337 $[M+NH_4^+]^+$, 320 $[M]^+$, 318 $[M]^-$. Purity (LC-MS): 98.6%.

3-(2-Carboxyethyl)-4-bromo-1*H*-indole-2-carboxylic acid (**1g**): ^{1}H-NMR (DMSO–d_6) δ 2.49 (m, 2 H, 2′-H); 3.54 (m, 2 H, 1′-H); 7.11 (dd, 1 H, *J* = 7.5 Hz, 6-H); 7.25 (dd, 1 H, *J* = 1.8 Hz, 5-H); 7.42 (dd, 1 H, *J* = 1.8 Hz, 7-H); 11.82 (s, 1 H, NH); 12.49 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 19.9 (C-2′), 36.4 (C-1′), 112.5 (C-7); 114.4 (C-4); 121.2 (C-3); 124.2 (C-2); 124.3 (C-5); 125.4 (C-6); 125.8 (C-3a); 137.6 (C-7a); 163.0 (2′-CO_2H); 173.8 (2-CO_2H). Elemental analysis for $C_{12}H_{10}BrNO_4$: Calcd.; C, 46.18; H, 3.23; N, 4.49, found C, 46.33; H, 3.59; N, 4.58. LC-MS (*m/z*): 329 $[M+NH_4^+]^+$, 312 $[M]^+$, 312 $[M]^-$. Purity (LC-MS): 99.1%.

3-(2-Carboxyethyl)-6-bromo-1*H*-indole-2-carboxylic acid (**1h**): ^{1}H- NMR (DMSO–d_6) δ 2.50 (m, 2 H, 2′-H); 3.23 (m, 2 H, 1′-H); 7.16 (dd, 1 H, *J* = 1.8 Hz, 5-H); 7.54 (dd, 1 H, *J* = 1.8 Hz, 7-H); 7.64 (dd, 1 H, *J* = 8.5 Hz, 4-H); 11.57 (s, 1 H, NH); 12.54 (b, 1 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 19.9 (C-2′); 35.2 (C-1′); 114.9 (C-7); 117.6 (C-4); 121.6 (C-3); 122.5 (C-6, C-5); 125.0 (C-2); 126.2 (C-3a); 136.8 (C-7a); 163.0 (2′-CO_2H); 174.0 (2-CO_2H). LC-MS (*m/z*): 329 $[M+NH_4^+]^+$, 312 $[M]^+$, 312 $[M]^-$. Purity (LC-MS): 98.9%.

3-(2-Carboxyethyl)-4-iodo-1*H*-indole-2-carboxylic acid (**1i**): ^{1}H- NMR (DMSO–d_6) δ 2.49 (m, 2 H, 2′-H); 3.35 (m, 2 H, 1′-H); 6.94 (t, 1 H, *J* = 8 Hz, 6-H); 7.45 (d, 1 H, *J* = 8.2 Hz, 5-H); 7.55 (d, 1 H, *J* = 8.2 Hz, 7-H); 11.72 (s, 1 H, NH); 12.60 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 19.00 (C-2′); 36.54 (C-1′); 86.3 (C-4); 113.1 (C-7); 121.7 (C-3); 125.8 (C-3a); 125.9 (C-6); 126.5 (C-2); 131.7 (C-5); 137.0 (C-7a); 163.0 (2′-CO_2H); 173.70 (2-CO_2H). Elemental analysis for $C_{12}H_{10}INO_4$: Calcd.: C, 40.14; H, 2.81; N, 3.90, found: C, 40.58; H, 3.04; N, 3.85. LC-MS (*m/z*): 377 $[M+NH_4^+]^+$, 360 $[M]^+$, 358 $[M]^-$. Purity (LC-MS): 98.8%.

3-(2-Carboxyethyl)-6-iodo-1*H*-indole-2-carboxylic acid (**1j**): ^{1}H- NMR (DMSO–d_6) δ 2.49 (m, 2 H, 2′-H); 3.22 (m, 2 H, 1′-H); 7.31 (dd, 1 H, *J* = 1.8 Hz, 5-H); 7.50 (d, 1 H, *J* = 8.5 Hz, 4-H); 7.74 (d, 1 H, *J* = 1.8 Hz, 7-H); 11.52 (s, 1 H, NH); 12.53 (b, 2 H, 2CO_2H). ^{13}C-NMR (DMSO–d_6) δ 19.9 (C2′); 35.2 (C1′); 89.6 (C-6); 121.0 (C-7); 121.6 (C-3); 122.7 (C-4); 124.6 (C-2); 126.5 (C-3a); 127.9 (C-5); 137.3 (C-7a); 163.0 (2′-CO_2H); 174.0 (2-CO_2H). LC-MS (*m/z*): 377 $[M+NH_4^+]^+$, 360 $[M]^+$, 358 $[M]^-$. Purity (LC-MS): 97.8%.

Biological evaluation

1321N1 astrocytoma cells stably transfected with the human GPR17 were used for fluorimetric measurement of intracellular calcium release induced by the test compounds in analogy to previously described procedures.[2,11]

Conclusions

In conclusion, we have developed a fast, efficient, and high-yielding procedure for the synthesis of 3-(2-carboxy-4,6-dichloro-indol-3-yl)propionic acid (**1a**, MDL29,951), which will allow the preparation of multi-gram amounts of this recently discovered potent GPR17 agonist. The developed method was used for the total synthesis of 10 indole derivatives, eight of which are new compounds (**1b**,**c**,**e**,**f-j**), while two derivatives (**1a**,**d**) had been previously reported.[9] Compound **1b** (3-(2-carboxy-4,6-dibromo-indol-3-yl)-propionic) showed the highest potency of the tested compound series at human GPR17 expressed in 1321N1 astrocytoma cells with an EC_{50} value of 202 nM. Steep SARs have been discovered for this class of compounds and further, more extensive exploration is warranted. New ligands for GPR17 are of great interest as pharmacological tools and as potential drug candidates.

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Notes and references

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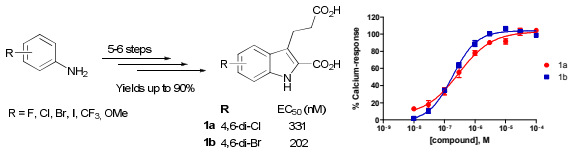
Graphical Abstract



Several *tri*– and *tetra*–substituted indole derivatives were synthesized and evaluated as human GPR17 agonists. Steep structure-activity relationships were observed.