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Thienopyrrole acetic acids as antagonists of the CRTH2 receptor

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ABSTRACT

The bioisosteric replacement of the indole core of CRTH2 antagonists using thienopyrroles was investigated, resulting in potent antagonists with good selectivity over DP1. Early ADME/PK assessment of this chemotype demonstrated bioavailability in mice.

Prostaglandin D2 (PGD_2) is the major prostanoid released by mast cells during allergic attacks.[1,2] In human patients, allergen challenge leads to a rapid increase in the production of PGD_2 in the airway of asthmatics,[3] in the nasal mucosa of allergic rhinitics[4] and in the skin of patients suffering from atopic dermatitis.[5] Two high affinity binding G-protein-coupled receptors, the DP1 receptor and the chemoattractant receptor-homologous expressed on Th2 cells (CRTH2 or DP2), have been shown to mediate the effects of PGD_2. The later receptor is expressed on cell types associated with allergic inflammation, such as Th2 cells, basophils and eosinophils and its activation by PGD_2 triggers the migration[6,7] and prevents the apoptosis of these cell types.[8] In vivo reports in rodents have highlighted the role of CRTH2 in promoting chronic allergic skin inflammation[9] and eosinophilic airway inflammation.[10] CRTH2 antagonists have shown efficacy in a murine model of allergic rhinitis.[11] More recently, an orally bioavailable CRTH2 antagonist (OC000459) successfully completed Phase IIa trials demonstrating efficacy in asthma and allergic rhinoconjunctivitis[12] thus unambiguously establishing CRTH2 as a central player of airway inflammation.

A number of medicinal chemistry programs started after the identification of non-steroidal anti-inflammatory drug indomethacin (**1**, Fig. 1) as an agonist of the CRTH2 receptor,[13,14] and that of thromboxane receptor antagonist ramatroban (**2**, Fig. 1) as an antagonist of the CRTH2 receptor.[15] As a result a large number of CRTH2 antagonists known to date are indole acetic acids[16,17] bearing the acetic acid moiety either at position 3, exemplified with compound **3** (Fig. 2)[18] or at position 1, exemplified with compound **4** (Fig. 2).[19]

A variety of fused 6–5-membered ring chemotypes have arisen such as 7-azaindole acetic acids,[20] benzimidazolyl acetic acids,[21] spiro-indolinone acetic acids,[22] and indolizine acetic acids,[23] however, to the best of our knowledge, fused 5,5-membered ring systems have not been reported.

As a template for fused 5,5-membered ring systems, we have investigated the bioisosteric replacement of the classical indole core of CRTH2 antagonists with two regioisomeric thienopyrroles.

The affinity of the thienopyrroles towards the CRTH2 receptor was evaluated in a radioligand binding assay using membranes prepared from HEK293.F cells transiently transfected with Hu-CRTH2 cDNA.[24]

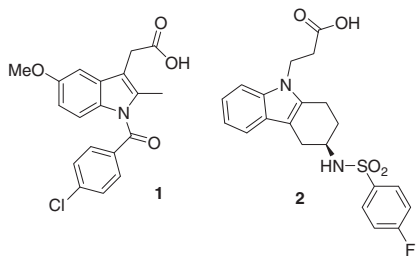

Figure 1. Structure of indomethacin (**1**) and ramatroban (**2**).

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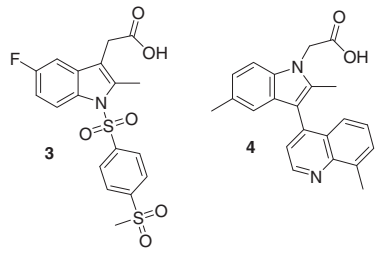


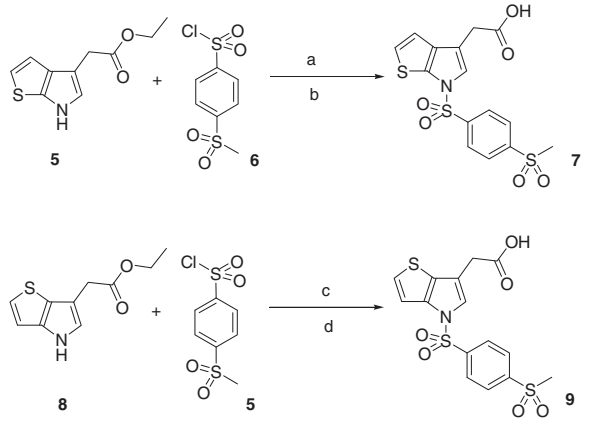
Figure 2. Representative examples of indole-1 and indole-3 acetic acids CRTH2 antagonists.

The unsubstituted methyl 2-(6*H*-thieno[2,3-*b*]pyrrol-4-yl)acetate (**5**) and methyl 2-(4*H*-thieno[3,2-*b*]pyrrol-6-yl)acetate (**8**) were synthesized as previously described,[25] starting from the Boc-protected iodothiophenes using a one-pot allylation with ethyl 4-bromocrotonate followed with an intra-molecular Heck cyclization. Subsequently **5** and **8** were reacted with the 4-(methylsulfonyl)benzene-1-sulfonyl chloride and saponified to give compound **7** and **9** (Scheme 1).

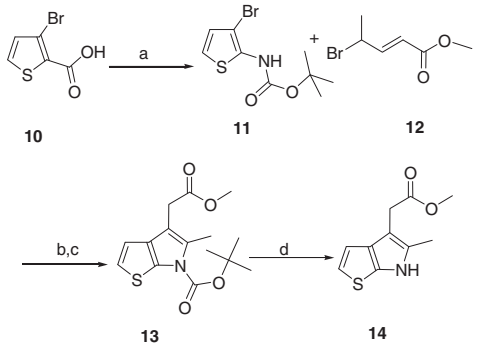
In the radioligand binding assay, thienopyroles **7** and **9** were found to be weak, equipotent binders with IC_{50}s of 4 μM and 5 μM, respectively, unambiguously showing the bioequivalency of both regioisomers. As a result the remainder of the study was conducted in a single series, the 6*H*-thieno[2,3-*b*]pyrroles.

With indole acetic acids, the methyl group α to the pyrrole nitrogen is an important structural feature since its removal resulted in a five-fold loss in potency as reported earlier.[19] We proceeded to introduce a comparable methyl group in the 6*H*-thieno[2,3-*b*]pyrrole core. The methyl 2-(5-methyl-6*H*-thieno[2,3-*b*]pyrrol-4-yl)acetate (**14**) was synthesized following the route highlighted above for the synthesis of **5**, using the (*E*)-methyl 4-bromopent-2-enoate (**12**)[26] in the allylation step (Scheme 2).

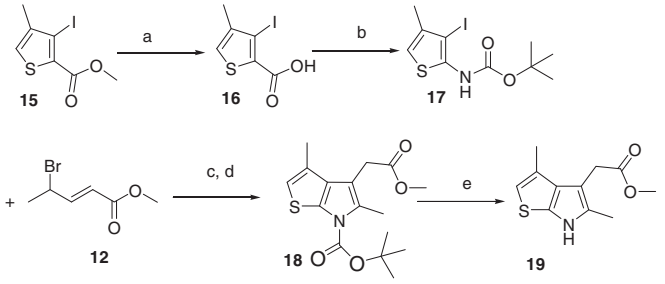
We also introduced a methyl group on the thiophene ring in order to mimic the substitution pattern of known potent 2-(2,5-dimethyl-1*H*-indol-1-yl)acetic acids antagonists such as **4**.[19] The methyl 2-(3,5-dimethyl-6*H*-thieno[2,3-*b*]pyrrol-4-yl)acetate (**19**) (Scheme 3) and the methyl 2-(2,5-dimethyl-6*H*-thieno[2,3-*b*]pyrrol-4-yl)acetate (**24**) (Scheme 4) cores were prepared using our standard route using the *tert*-butyl 3-iodo-4-methylthiophen-2-ylcarbamate (**17**) and *tert*-butyl 3-bromo-5-methylthiophen-2-ylcarbamate (**22**) as starting materials. The *tert*-butyl 3-iodo-4-methylthiophen-2-ylcarbamate (**17**) was obtained from the commercially available methyl 3-iodo-4-methylthiophene-2-carboxylate (**15**) through a saponification/Curtius rearrangement sequence while the *tert*-butyl 3-bromo-5-methylthiophen-2-ylcar-



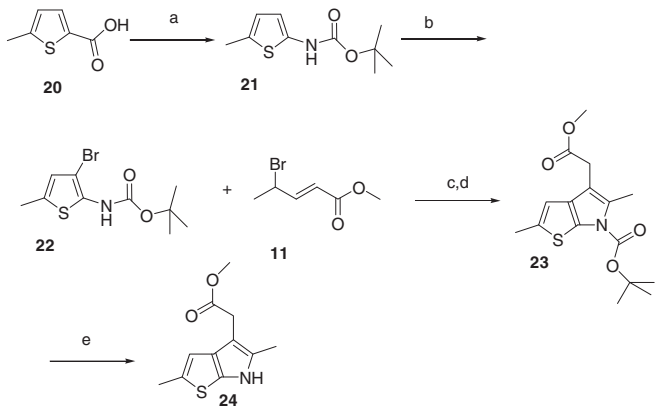
Scheme 1. Reagents and conditions: (a) *t*-BuOK, 18-crown-6, 0 °C–rt, 15%; (b) KOH, EtOH/THF: 1/1, rt, 64%; (c) NaH, THF, rt; (d) KOH, EtOH/H_2O, rt, 13%.



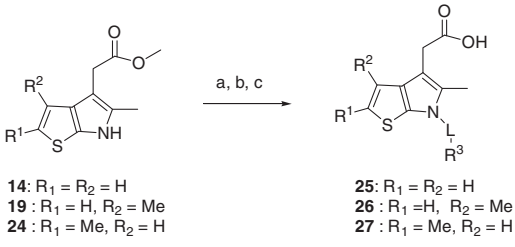
Scheme 2. Reagents and conditions: (a) diphenylphosphoryl azide, Et_3N, *t*-BuOH, 65 °C, 84%; (b) K_2CO_3, DMF, rt; (c) $Pd_2(OAc)_3$, PPh_3, 85 °C, 36%; (d) silica gel, 85 °C under vacuum, 46%.



Scheme 3. Reagents and conditions: (a) NaOH, THF/MeOH, rt, 100%; (b) diphenylphosphoryl azide, Et_3N, *t*-BuOH, 65 °C, 83%; (c) K_2CO_3, DMF, rt; (d) $Pd_2(OAc)_3$, PPh_3, 85 °C, 28%; (e) silica gel, 85 °C under vacuum, 67%.



Scheme 4. Reagents and conditions: (a) diphenylphosphoryl azide, Et_3N, *t*-BuOH, 65 °C, 75%; (b) Br_2, MeOH, 0 °C, 76%; (c) K_2CO_3, DMF, rt; (d) $Pd_2(OAc)_3$, PPh_3, 85 °C,25%; (e) silica gel, 85 °C under vacuum, 64%.



Scheme 5. Reagents and conditions: L = SO_2: (a) *t*-BuOK, THF, 0 °C; (b) sulfonyl chloride, rt; (c) KOH, MeOH/H_2O: 1/1, rt; L = CH_2: NaH, DMF, 0 °C, 1 h; (b) benzyl halide, NaI, rt; (c) NaOH, MeOH, rt.

bamate (**22**) was obtained via Curtius rearrangement of the commercially available 5-methylthiophene-2-carboxylic acid (**20**) followed by a bromination.

Final derivatization of thienopyrroles **14**, **19** and **24** with a variety of sulfonyl chlorides or benzyl halides, followed by saponification afforded the analogs **25**, **26** and **27** (Scheme 5).

The substitution pattern of the thienopyrrole core was found to be critical for activity. As expected, the introduction of the methyl group α to the pyrrole nitrogen of **7**, led to **25e** and a 46-fold improvement in affinity to the receptor with an IC_{50} of 0.09 μM (Table 1).

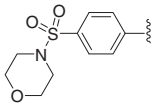
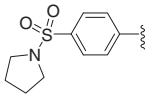
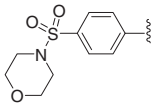
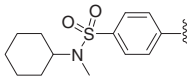
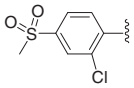
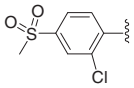
This methyl group most likely forces the carboxylic acid to adopt an optimal position to interact with the receptor.

Contrarily to the SAR reported in the indole series, an additional methyl on the core at position 2 (**27a**, **27b**) or at position 3 (**26a**, **26b**) of the thiophene ring was not tolerated and led to significant losses in activity compared to the desmethyl analogs (14-fold and 33-fold between **27a**/**25k** and **27b**/**25l**, respectively, and 15-fold and 30-fold between **26a**/**25e** and **26b**/**25k**, respectively).

The linker L could be changed from a sulfone to a methylene without affecting the affinity to the receptor, as shown with the 4-(methylsulfonyl)phenyl-analogs **25e** and **25k**, with IC_{50}s of 0.09 μM and 0.05 μM, respectively. The more sterically hindered 4-(morpholinosulfonyl)phenyl-analogs **25f** and **25i** were also found to be equipotent with IC_{50}s of 0.23 μM and 0.07 μM, respectively.

The substitution pattern of the phenyl ring (R3) was found to be important. The unsubstituted analog (**25a**) as well as the analogs substituted in the *para* position of with fluorine (**25b**), a trifluoromethyl-group (**25c**), or a cyano-group (**25d**) showed only weak activity. Compounds bearing a sulfone or sulfonamide at the *para* position of the phenyl group were the most active.

Table 1
Receptor assay data for thienopyroles

	L	R1	R2	R3	Hu-CRTH2 IC_{50}[a] (μM)
25a	SO_2	H	H	Ph	2.75
25b	SO_2	H	H	*p*-F–C_6H_4	1.63
25c	SO_2	H	H	*p*-CF_3–C_6H_4	1.13
25d	SO_2	H	H	*p*-CN–C_6H_4	3.00
25e	SO_2	H	H	*p*-SO_2Me–C_6H_4	0.09
25f	SO_2	H	H		0.23
25g	SO_2	H	H		0.39
25h	CH_2	H	H	*p*-SO_2NMe_2 –C_6H_4	0.19
25i	CH_2	H	H		0.07
25j	CH_2	H	H		0.02
25k	CH_2	H	H	*p*-SO_2Me–C_6H_4	0.05
25l	CH_2	H	H		0.01
26a	SO_2	H	Me	*p*-SO_2Me–C_6H_4	1.30
26b	CH_2	H	Me	*p*-SO_2Me–C_6H_4	1.50
27a	CH_2	Me	H	*p*-SO_2Me–C_6H_4	1.8
27b	CH_2	Me	H		0.33

[a] Values are means of $n = 2$–4 experiments.

Table 2
Selected in vitro data

	RLM/HLM[a] (%)	Dofetilide IC_{50} (μM)
25e	100/100	>100
25j	57/62	31
25k	90/91	31
25l	99/97	45

[a] % remaining after 30 min incubation.

The preferred substituents for that position were found to be the (*N*-cyclohexyl-*N*-methylsulfonyl)phenyl in **25j** and the 2-chloro-4-(methylsulfonyl)phenyl in **25l** with IC_{50}s of 0.02 μM and 0.01 μM, respectively.

Antagonistic activity of these thienopyrroles was verified for analog **25k** in a calcium flux assay using CHO/Ga16 cells stably transfected with Hu-CRTH2.[27] In this assay, **25k** displayed an IC_{50} of 0.13 μM.

Selectivity over the DP1 receptor[24] was evaluated for a sample of analogs (**25e**, **25j**, **25k** and **25l**) which were found to have no activity on this receptor (IC_{50} >50 μM).

An early in vitro ADME assessment showed good stability in rat and human liver microsomes as well as no significant activity in a dofetilide binding assay (Table 2).

Thienopyrrole **25e** was dosed orally, at 30 mpk as a solution in Tween 80 (0.2%)/HPMC (0.5%), in female balbc mice to provide an early assessment on bioavailability. Compound **25e** displayed an encouraging profile with a C_{max} of 7.8 μg/mL at 0.5 h and a significant systemic exposure of 16.4 μg.h/mL.

In conclusion, thienopyrrole acetic acids provide potent CRTH2 antagonists. They are selective over DP1, display good metabolic stability and do not inhibit dofetilide binding. This chemotype is also attractive as a result of its bioavailability which was demonstrated by dosing **25e** orally in mice.

Acknowledgments

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27. Calcium flux assays were performed in CHO cells expressing human CRTH2 and the Gα16 coupling protein using a FLIPR instrument (Molecular Devices). All compounds were dissolved in DMSO and assays run at a final DMSO concentration of 1% (v/v). PGD2 was purchased from Cayman Chemicals and used at a final assay concentration of 1 μM.