Bioorganic & Medicinal Chemistry Letters 21 (2011) 734–737

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The identification of substituted benzothiophene derivatives as PGE_2 subtype 4 receptor antagonists: From acid to non-acid ☆

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ARTICLE INFO

Article history:
Received 30 September 2010
Revised 23 November 2010
Accepted 29 November 2010
Available online 3 December 2010

Keywords:
PGE_2 subtype 4 receptor
EP_4
Antagonist

ABSTRACT

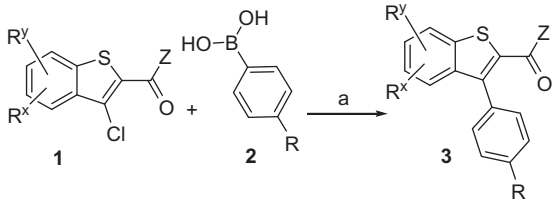
We disclose herein our preliminary SAR study on the identification of substituted benzothiophene derivatives as PGE_2 subtype 4 receptor antagonists. A potent EP_4 antagonist **6a** (K_i = 1.4 nM with 10% HSA) was identified. Furthermore, we found that an acidic group was not essential for the EP_4 antagonizing activity in the series and neutral replacements were identified. This opens a new direction for future EP_4 antagonist design.

Prostaglandins, a family of lipid mediators produced from arachidonic acid by the combined action of cyclooxygenases and distinct synthases, play key regulatory roles in the human body. NSAIDS mediated inhibition of their production, by blocking the action of cyclooxygenases, provides therapeutics benefits in managing inflammatory diseases[1] but causes side effects,[2–4] possibly due to the reduced production of beneficial lipid mediators that are not associated with the target disease. In order to take advantage of the associated therapeutic benefits while delineating the side effects, efforts to block the interaction of certain lipid mediators with their respective receptors become an alternative to current therapies and led to the search of PGE_2 subtype 4 receptor (EP_4) antagonists.[5] Potentially, EP_4 antagonists could exhibit therapeutic effect in various diseases such as inflammatory conditions[6,7] or cancers,[8–12] while displaying an improved side effect profile. Indeed, the anti inflammatory effects of EP_4 antagonist was demonstrated preclinically in various diseases models.[7,13,14] Previous publications[15–17] from this research center documented our commitment to the discovery of potent and selective EP_4 antagonists as novel therapeutic agents. In this manuscript we will disclose our efforts towards the identification of substituted benzothiophene derivatives as EP_4 antagonists and the interesting SAR that led to the discovery of non-acidic EP_4 antagonists.

The routes used to synthesize the EP_4 antagonists reported herein are outlined in Schemes 1–6. Compound **1** was prepared from the corresponding substituted 3-chloro-1-benzothiophene-2-carbonyl chloride by reacting with methanol and DMAP or with dimethylamine in THF. The Suzuki coupling reaction between compound **1** and a properly substituted boronic acid was catalyzed by a palladium complex formed in situ from palladium(II) acetate and DavePhos at room temperature in the presence of cesium fluoride. The presence of a small amount of water is necessary for obtaining the coupling product **3** in good yield. Thus, compounds **3a–l** were obtained as advanced intermediates for further chemical transformations (Scheme 1).

Treatment of **3a–d** with 5 equiv of tributyltin azide in reflux toluene, provided the corresponding tetrazoles **4a–d** (Scheme 1). Hydrolysis of **3e** with 5 equiv of sodium hydroxide (2 N) in a 2:1 solvent mixture of THF and methanol provided compound **4e** (Scheme 2).

The intermediate **3g** was transformed into other advanced intermediates **10a–c** by a two-step sequence. First, **3g** was hydrolyzed to the corresponding carboxylic acid under the aforementioned ester



Scheme 1. Reagents and conditions: (a) 4-R-Ar-$B(OH)_2$, $Pd(OAc)_2$ (0.06 equiv), DavePhos (0.09 equiv), CsF (3 equiv), 1% H_2O/1,4-dioxane, rt.

☆ In memory of legacy *Merck Frosst Centre for Therapeutic Research*.

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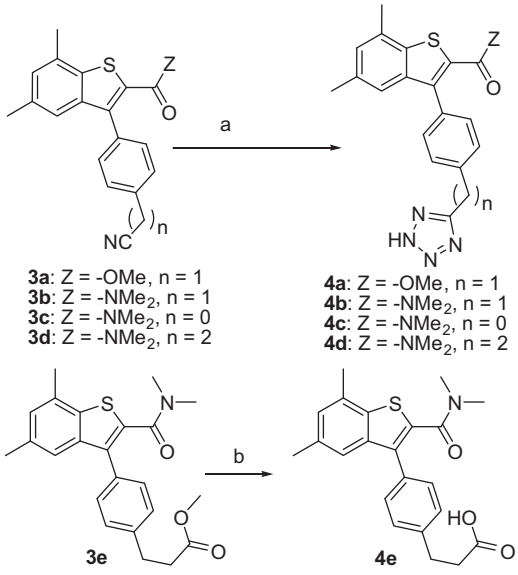
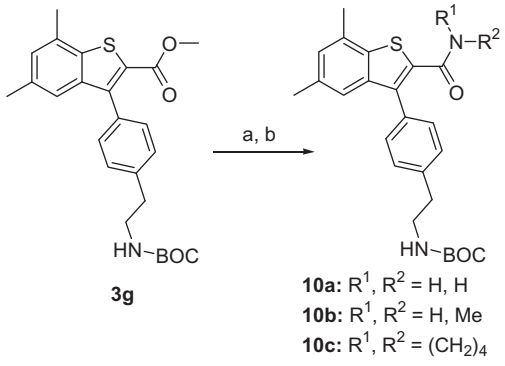
doi:10.1016/j.bmcl.2010.11.118



Scheme 2. Reagents and conditions: (a) Bu_3SnN_3, toluene, reflux; (b) NaOH, THF/MeOH.



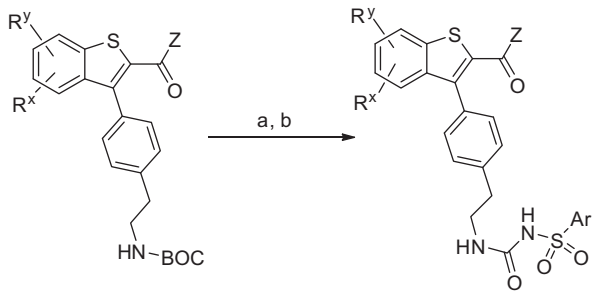
Scheme 3. Reagents and conditions: (a) NaOH, THF/MeOH; (b) EDCI, DMAP, amine, THF.

hydrolysis conditions for **3e**; then the obtained acid was converted to amide **10a–c** by EDCI and DMAP promoted coupling with proper amines (Scheme 3).

When the BOC-protected intermediates **3f–k** and **10a–c** were treated with a 50% TFA in dichloromethane at room temperature, the corresponding amines were obtained in quantitative yields. These amines were further elaborated into substituted benzenesulphonylureas **6a–c**, **9**, **11a–c** and **12a–d** by treatment with substituted benzenesulfonyl isocyanates.

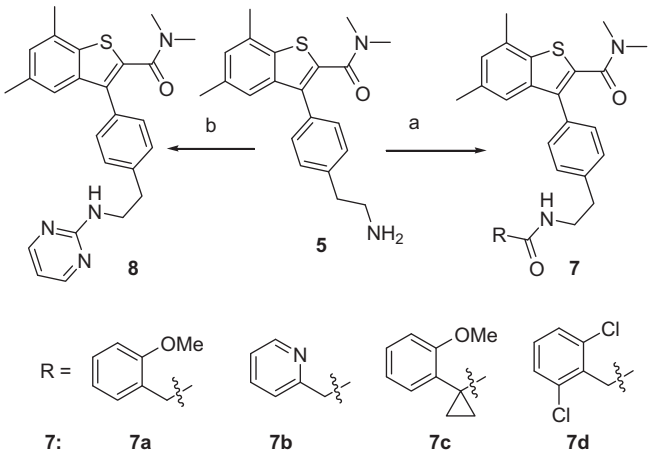
Furthermore, the intermediate **5** obtained from the TFA treatment of **3f** was transformed into amides **7a–d** by an EDCI/DMAP promoted coupling with proper carboxylic acids, or into compound **8** by heating with 10 equiv of 2-chloropyrimidine in the presence of Hünig's base (Scheme 5).

Finally, intermediate **3l** (Scheme 6) was treated with triphenylphosphine dibromide formed in situ to provide the corresponding benzyl bromide which was further reacted with reagent **13**[18] in DMF to afford sulfone **14**. Heating **14** with 2 equiv of sodium methoxide in reflux methanol yielded a sodium sulphinate intermediate, which was treated with NCS followed by ammonia gas in sequence to furnish compound **15**. Coupling of carboxylic acid **16a** or **16b** with sulfonamide **15** in the presence of EDCI and DMAP gave acylsulfonamide **17a and 17b** (Scheme 6).



-Z	R^x, R^y	Compound	-Ar
$-NMe_2$	5-Me, 7-Me	**3f** → **6a**	4-MeC_6H_4
$-NMe_2$	5-Me, 7-Me	**3f** → **6b**	4-ClC_6H_4
$-NMe_2$	5-Me, 7-Me	**3f** → **6c**	2-ClC_6H_4
-OMe	5-Me, 7-Me	**3g** → **9**	4-MeC_6H_4
$-NMe_2$	5-Cl, 7-Cl	**3h** → **12a**	4-MeC_6H_4
$-NMe_2$	5-F, 7-F	**3i** → **12b**	4-MeC_6H_4
$-NMe_2$	5-CF_3, 7-Cl	**3j** → **12c**	4-MeC_6H_4
$-NMe_2$	4-Me, 7-Me	**3k** → **12d**	4-MeC_6H_4
$-NH_2$	5-Me, 7-Me	**10a** → **11a**	4-MeC_6H_4
-NHMe	5-Me, 7-Me	**10b** → **11b**	4-MeC_6H_4
	5-Me, 7-Me	**10c** → **11c**	4-MeC_6H_4

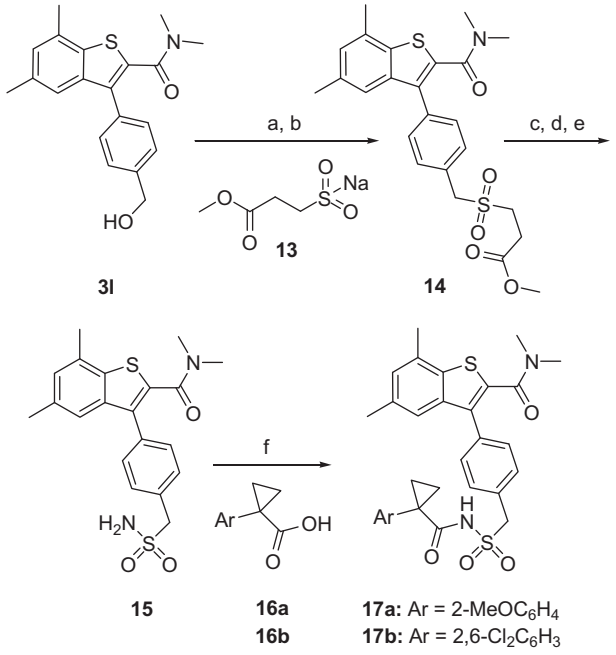
Scheme 4. Reagents and conditions: (a) TFA/DCM (1:1); (b) $ArSO_2NCO$, NEt_3, DCM.



Scheme 5. Reagents and conditions: (a) EDCI, DMAP, carboxylic acid or amine, THF; (b) 2-chloropyrimidine, DIPEA (10 equiv), reflux.

The synthesized compounds were evaluated in a PGE_2 subtype 4 receptor binding assay in the absence or presence of 10% HSA and the results are summarized in Table 1.[19] The purpose of using HSA in the binding assay is to evaluate the plasma protein shift potential of these compounds.

Compound **4a** was considered as the lead in the series. It has good intrinsic affinity (1.9 nM) for the EP_4 receptor but was found to be highly shifted (632-fold) in the presence of HSA. Interestingly, when the methoxycarbonyl moiety, at the 2-position of benzothiophene in **4a**, was replaced by a dimethylaminocarbonyl (**4b**),



Scheme 6. Reagents and conditions: (a) PPh_3, Br_2, NEt_3, DCM; (b) **13**, DMF, 85 °C; (c) MeONa, MeOH, reflux.; (d) NCS, DMF; (e) NH_3 (gas), THF; (f) EDCI, DMAP, **16a** and **16b**, THF.

Table 1
EP_4 receptor binding affinities[b]

Entry	Compound	EP_4 K_i[a] (nM)		
		0% HSA	10% HSA	Shift (folds)
1	**4a**	1.9	1200	632
2	**4b**	1.1	25	22
3	**4c**	160	2300	14
4	**4d**	25	543	22
5	**4e**	10	270	27
6	**9**	0.97	39	40
7	**6a**	0.54	1.4	2.6
8	**6b**	0.4	2.2	5.5
9	**6c**	0.42	2.7	6.4
10	**17a**	0.33	5.2	16
11	**17b**	0.44	5.5	13
12	**11a**	3.8	650	171
13	**11b**	0.5	134	268
14	**11c**	0.41	11	27
15	**12a**	0.59	17	29
16	**12b**	0.53	40	75
17	**12c**	0.55	8.7	16
18	**12d**	0.77	19	25
19	**3f**	68	1991	29
20	**7a**	0.2	3.4	17
21	**7b**	2.3	40	17
22	**7c**	3	17	5.7
23	**7d**	1.6	17	11
24	**8**	0.98	42	42

[a] Average of three or more assays.
[b] A table with structure available as Supplementary data.

the intrinsic affinity was slightly improved to 1.1 nM but the protein shift was reduced to 22-fold. Modification of the linker length between the tetrazole and the phenyl ring in compound **4b** led to compounds **4c** and **4d**, resulted in loss of potency while maintaining a similar shift to **4b**. Replacing the tetrazole group in **4d** with a carboxylic acid (**4e**) resulted in a slight improvement in potency but none in reducing protein shift. Compounds containing two other types of acidic surrogates, benzenesulfonylurea as in compounds **9** and **6a–c** and acylsulfonamides as in compounds **17a** and **17b**, were prepared. We found that they both were beneficial in maintaining potency on the EP_4 receptor and lowering protein shift. A dramatic improvement in plasma protein shift was observed with p-toluenesulfonylurea by comparing compounds **4a** with **9** (from 632-fold to 40-fold) and **4b** with **6a** (from 22-fold to 2.6-fold), respectively. The improvement in intrinsic potency brought by this acid surrogate is approximately a twofold. SAR modifications on the 2-position of the benzothiophene moiety of compound **6a** were also explored. Based on assay results obtained with **6a**, **11a–c**, we concluded that (1) a small alkyl substituent on the amide nitrogen was beneficial for intrinsic potency; (2) a tertiary amide is optimal for minimizing the protein shift; (3) the *N*,*N*-dimethylaminocarbonyl group in **6a** is the optimized substituent.

Table 2
The binding activities of **6a** against a full panel of prostanoid receptors in absence of HSA

Receptors	EP_4	EP_1	EP_2	EP_3	$CRTH_2$	DP	FP	IP	TP
K_i[a] (nM)	0.48	>9500	3330	530	>7230	73	>8700	>7700	61

[a] Average of two or more assays.

Our further SAR exploration was focused on the substituents on the phenyl ring of the benzothiophene pharmacophore. It was found that, in comparison to the 5-Me/7-Me in **6a**, other substituent combination such as of 5-Cl/7-Cl in **12a**, 5-F/7-F in **12b**, 5-CF3/7-Cl in compounds **12a–c** had little effect on intrinsic potency but all had detrimental effect on protein shift. The same phenomena was observed with the change of substituent pattern from 5,7-dimethyl in **6a** to 4,7-dimethyl in **12d**. One interesting discovery during our SAR study is that intermediate **3f** was active in binding assay which suggests that an acidic group is not a requirement for the EP_4 antagonizing activity in the series. Thus we devoted some limited efforts to further investigate this and identified that amides **7a–d** and compound **8** showed reasonable intrinsic affinities. The best amide analog was **7a**, which had a K_i of 0.2 and 3.4 nM in the absence and presence of HSA, respectively.

All the EP_4 ligands reported herein were determined to be full EP_4 antagonist when evaluated in human HEK-293 cell based functional assay. For example, compounds **6a** and **7a** has an IC_{50} = 1.2 and 2.4 nM, respectively, in the absence of HSA. They were also evaluated against other prostanoid receptors and showed similar selectivity profile. To illustrate this, the binding activities of **6a** against all prostanoid receptors are summarized in Table 2.[20] The selectivities of compound **6a** for EP_4 over the DP (152 folds) and TP (127 folds) receptors are moderate but acceptable, good over EP_3 (>1000 folds) receptor and excellent over the remaining prostanoid receptors (>6900 folds).

In summary, we have disclosed our preliminary SAR study on the identification of substituted benzothiophene derivatives as PGE_2 subtype 4 receptor antagonists. Highly potent EP_4 antagonist **6a** (K_i = 1.4 nM with 10% HSA) was identified. Furthermore, it was also found that an acidic group is not essential for the EP_4 binding activity in the series and non-acidic analogs were identified. This opens a new direction for future EP_4 antagonist design.

References

1. Rao, P. P. N.; Knaus, E. E. *J. Pharm. Pharm. Sci.* **2008**, *11*, 81s–110s.
2. Meek, I. L.; van de Laar, M. A. F. J.; Vonkeman, H. E. *Pharmaceuticals* **2010**, *3*, 2146–2162.
3. Allegaert, K. L.; de Hoon, J.; Debeer, A.; Gewillig, M. *Expert Opin. Drug Safety* **2009**, *8*, 669–681.
4. Abdel-Tawab, M.; Zettl, H.; Schubert-Zsilavecz, M. *Curr. Med. Chem.* **2009**, *16*, 2042–2063.
5. Jones, R. L.; Giembycz, M. A.; Woodward, D. F. *Br. J. Pharmacol.* **2009**, *158*, 104–145.
6. For a recent review, see: Sakata, D.; Yao, C.; Narumiya, S. *J. Pharm. Sci.* **2010**, *112*, 1–5.

7. Attur, M.; Al-Mussawir, H. E.; Patel, J.; Kitay, A.; Dave, M.; Palmer, G.; Pillinger, M. H.; Abramson, S. B. *J. Immunol.* **2008**, *181*, 5082–5088.
8. Terada, N.; Shimizu, Y.; Kamba, T.; Inoue, T.; Maeno, A.; Kobayashi, T.; Nakamura, E.; Kamoto, T.; Kanaji, T.; Maruyama, T.; Mikami, Y.; Toda, Y.; Matsuoka, T.; Okuno, Y.; Tsujimoto, G.; Narumiya, S.; Ogawa, O. *Cancer Res.* **2010**, *70*, 1606–1615.
9. Kundu, N.; Ma, X.; Holt, D.; Goloubeva, O.; Ostrand-Rosenberg, S.; Fulton, A. M. *Breast Cancer Res. Treat.* **2009**, *117*, 235–242.
10. Robertson, F. M.; Simeone, A.-M.; Mazumdar, A.; Shah, A. H.; McMurray, J. S.; Ghosh, S.; Cristofanilli, M. *J. Exp. Ther. Oncol.* **2008**, *7*, 299–312.
11. Oh, J.-M.; Kim, S.-H.; Lee, Y.-I.; Seo, M.; Kim, S.-Y.; Song, Y.-S.; Kim, W.-H.; Juhnn, Y.-S. *Carcinogenesis* **2009**, *30*, 141–149.
12. Robertson, F. M.; Simeone, A.-M.; Lucci, A.; McMurray, J. S.; Ghosh, S.; Cristofanilli, M. *Cancer* **2010**, *116*, 2806–2814.
13. Okumura, T.; Murata, Y.; Taniguchi, K.; Murase, A.; Nii, A. *J. Pharm. Pharmacol.* **2008**, *60*, 723–730.
14. Clark, P.; Rowland, S. E.; Denis, D.; Mathieu, M.-C.; Stocco, R.; Poirier, H.; Burch, J.; Han, Y.; Audoly, L.; Therien, A. G.; Xu, D. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 425–434.
15. Colucci, J.; Boyd, M.; Berthelette, C.; Chiasson, J.-F.; Wang, Z.; Ducharme, Y.; Friesen, R.; Wrona, M.; Levesque, J.-F.; Denis, D.; Mathieu, M.-C.; Stocco, R.; Therien, A. G.; Clarke, P.; Rowland, S.; Xu, D.; Han, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3760–3763.
16. Blouin, M.; Han, Y.; Burch, J.; Farand, J.; Mellon, C.; Gaudreault, M.; Wrona, M.; Levesque, J.-F.; Denis, D.; Mathieu, M.-C.; Stocco, R.; Vigneault, E.; Therien, A.; Clark, P.; Rowland, S.; Xu, D.; O'Neill, G.; Ducharme, Y.; Friesen, R. *J. Med. Chem.* **2010**, *53*, 2227–2238.
17. Burch, J. D.; Belley, M.; Fortin, R.; Deschênes, D.; Girard, M.; Colucci, J.; Farand, J.; Therien, A. G.; Mathieu, M.-C.; Denis, D.; Vigneault, E.; Lévesque, J.-F.; Gagné, S.; Wrona, M.; Xu, D.; Clark, P.; Rowland, S.; Han, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2048–2054.
18. Baskin, J. M.; Wang, Z. *Tetrahedron Lett.* **2002**, *43*, 8479–8483.
19. For assay details, please see previous publications (Refs. 15–17).
20. Unfortunately, the evaluation of **7a** against other receptors was done with $n = 1$. Although the results are not qualified to be reported, they do show that **7a** and **6a** have similar selectivity profiles.