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Evaluation of WO 2012/177618 A1 and US-2014/0179750 A1: Novel small molecule antagonists of PGE_2 receptor EP2

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Abstract

Recent studies underscore that prostaglandin-E_2 (PGE_2) exerts mostly proinflammatory effects in chronic CNS and peripheral disease models, mainly through a specific prostanoid receptor EP2. However, very few highly characterized EP2 receptor antagonists have been reported until recently, when Pfizer and Emory University published two distinct classes of EP2 antagonists with good potency, selectivity and pharmacokinetics. The purpose of this article is to evaluate recently published patents WO 2012177618 A1 and US-2014/0179750 A1 from Emory, which describe a number of cinnamic amide- and amide-derivatives as a potent antagonists of EP2 receptor, and their neuroprotective effects in *in vitro* and in an *in vivo* model. A selected compound from this patent(s) also attenuates prostate cancer cell growth and invasion *in vitro*, suggesting these compounds should be developed for therapeutic use.

Keywords

Neuroinflammation; status epilepticus; anti-cancer; neuroprotection; cinnamic amides; amides; competitive antagonism

1. Introduction

Cyclooxygenase-2 (COX-2) evolved as one of the major contributors to the inflammation following a brain or peripheral injury. Thus COX-2 inhibitors were developed and used in animal models and humans in the past[1, 2]. However, COX-2 catalyzes the synthesis of five prostanoids (PGD_2, PGE_2, PGF_2, PGI_2 and TXA_2), which activate a total of 11 prostanoid receptors (DP1 and DP2 activated by PGD_2; EP1, EP2 EP3 and EP4 by PGE_2; FPα and FPβ by PGF_2; IP by PGI_2; TPα and TP by TXA_2)[3–5]. Some of these receptors share common downstream signaling pathways. For example, EP2, EP4, DP1 and IP stimulate adenylate cyclase to elevate cAMP (*via* G_s coupled-protein), which promotes protein kinase A (PKA), and the exchange protein activated by cAMP (Epac) mediated signaling[6, 7]. Others such as DP2 and EP3 (*via* G_i coupled-protein) impede cAMP signaling; EP1, FP and TP receptors

competing interests disclosure

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promote (*via* G_q coupled-protein) calcium mediated signaling. Individually these receptors display 'Jekyll and Hyde nature', similar to COX-2, depending on the disease condition[3, 4]. Although, COX-2 inhibitors proved to be efficacious in ameliorating inflammation and pain in humans with osteoarthritis and rheumatoid arthritis[8, 9], they have not provided a clear benefit to the rodent models of inflammatory neurodegenerative disease epilepsy[10], and to humans with Alzheimer's diseases[11, 12] and ALS[13]. Instead, they resulted in adverse cardiovascular effects upon chronic use[14]. One of the important reasons for these adverse effects was due to inhibition of IP receptor[15, 16]. As a result, two COX-2 drugs rofecoxib (Vioxx) and valdecoxib (Bextra) were withdrawn from the USA market. Induction of COX-2 following a brain injury or excessive neuronal activity in the brain is often associated with induction of a membrane bound prostaglandin E synthase-1 (mPGES-1), which produces PGE_2 from COX-2 derived intermediate PGH_2. Thus, it appears a future anti-inflammatory therapy should be targeted through a specific prostanoid receptor or a prostanoid synthase enzyme downstream of COX-2, rather than generic block of entire COX-2 cascade[3, 4, 17–19].

PGE_2 is the major product of COX-2, but, it activates four receptors EP1-EP4. Studies show that each of these four receptors display ('yin-yang' nature) either protective or deleterious role depending on disease model[20]. EP2 receptor is widely distributed in the brain and periphery[21]. In the brain, EP2 is expressed on both neurons and microglia cells[19, 22]. It has been demonstrated that acute activation of EP2 was beneficial in stroke and glaucoma models[22, 23], whereas chronic activation was deleterious in models of Alzheimer's, Parkinson's and ALS diseases[18, 19, 24]. Furthermore, studies indicate that EP2 mediates tumorigenesis, and promotes tumor angiogenesis by attenuating apoptosis[25–27]. EP2 inhibition has been shown to impair several cell survival pathways and activates apoptotic pathways in a model of endometriosis[28] suggesting 'Jekyll and Hyde' nature either pro-apoptotic, or anti-apoptotic signaling leading to a beneficial outcome in two different disease conditions. However, a vast majority of the studies are conducted with EP2 gene knockout models and use of poorly selective or in vivo unstable EP2 agonists (e.g. PGE_2 and butaprost (Figure 1)). Pharmacological inhibition studies were limited until recently when a Emory University group published key results demonstrating proof of concept that a short term exposure of EP2 antagonist is anti-inflammatory in a pilocarpine induced acute brain injury model of status epilepticus[29], and, is anti-proliferative in vitro cultures[30], subsequent to the filing of the patents WO 2012/177618 A1 and US-2014/0179750 A1[31, 32], which are the subjects of current discussion below.

2 Chemistry

Initial hits **3** and **4** (Scheme 1) were identified though a high-throughput screening campaign by using a TR-FRET assay on human EP2 receptors expressed on C6-glioma cell line. These two compounds belong to a cinnamic amide chemical class, where one of amide ($CONH_2$) proton is substituted by a two carbon linker with an indole ring at the end. Medicinal chemistry on these hits generated number of compounds with modification on phenyl ring replacing one or two methoxyl groups of **3** and **4** with one or two fluorines, or a chlorine, bromine, or methyl group. Indole ring was also decorated with one fluorine atom, a methyl or trifluoromethyl group. Interestingly, these modifications retained the EP2 potency at nanomolar level (Schild $K_B \leq 50$ nM).

Cinnamic amides may act as Michael acceptors and pose a potential threat for drug development, thus investigators also developed several amide analogs as EP2 antagonists. About 150 analogs have been synthesized that fall in either one of the Markush structures **M1–M6** (Figure 2). A majority of the structural modifications are envisioned, for example substitution on the indole ring and replacements for indole ring (**M1** and **M4**), right side phenyl ring, and also at the linker region (**M4** and **M5**). Compound(s) belongs to each claim (**M1–M6**) are synthesized and characterized by *1H*-NMR, LCMS, HRMS, and elemental analysis.

The synthesis was straight forward and completed in 3 steps for **3**, **4** and many other derivatives. Briefly, 2-methyl indole was alkylated using sodium hydride as a base, and the resulting cyanide product was reduced with lithium aluminum hydride to synthesize amine precursor which was used to couple to either substituted cinnamic acids or benzoic acids, benzamidazole- and quinoline-carboxylic acids to furnish final cinnamic amides or amides (Scheme 1). Moreover, isomeric 3-indole derivatives and synthetically challenging benzofuran replacements (belongs to **M1–M3**) were also synthesized. Furthermore, derivatives with one methylene and three methylenes (linker) between indole and cinnamic amide were also synthesized analogously.

It is noteworthy to draw few comparisons between structures from the Emory patents[31, 32] to the structures previously published in patents from Bayer Schering Pharma[34]. As shown in Figure 3, Emory group also created several isomeric 3-indole derivatives as illustrated by **6** and **7** (along with novel 1–indoles **3** and **4** derivatives), which partially overlap with previously published dimainopyrimidines (e.g. **8**) with tryptamine moiety[34]. However, the cinnamic amide moiety makes **6**, **7** and their derivatives structurally novel. Likewise, the only amide compound **5** partially overlaps with previously published structure **9** in an abandoned patent[35] (personal communication from Emory University OTT). Nonetheless, structures such as **10** and **11**, which emerged from the **5** by the SAR study, are completely novel and may serve as the leads for further development.

3. Biology

Unlike several other previous patents, particularly on the EP2 antagonists[34–39], which reported relatively little or no biology, the Emory group[31, 32] presented a significant biological data to suggest EP2 antagonists are therapeutically useful. First, a cAMP-mediated TR-FRET assay was used to determine the potency. Schild K_B which was defined as 2-fold rightward shift of PGE_2 EC_{50} on human EP2 and EP4 receptors; or a rightward shift of BW25C (DP1 agonist) EC_{50} on DP1 receptors, expressed on C6-glioma cells was used to characterize the potency and selectivity of the compounds. The data indicate that several compounds including **3** are >10-fold selective to EP2 against DP1 receptor and >1000-fold selective against EP4 receptor. But recent reports from Emory laboratory suggest that the selectivity against DP1 receptor has been improved to 1000-fold[40] and a vast majority of the compounds display high selectivity against EP4 and IP receptors and insignificant cytotoxicity on parent C6-glioma cells[40, 41]. However, only the compound **3** and its trifluoromethylindole derivative **12** (Figure 4) were used to demonstrate a proof of concept either in vitro or in vivo models.

Status epilepticus (SE), a continuous seizure activity for more than 30 min, causes a significant inflammatory injury to the brain and is associated with significant delayed mortality (30 day mortality is 35%) in humans and laboratory rodent models (30–40%). Mouse and rat models of SE exhibit higher levels of COX-2 (and hence PGE_2) along with several inflammatory mediators, starting from 2h until 4 days. These agents play a deleterious role in over activation of brain resident macrophage microglia and astrocytes, subsequently leading to a robust neuronal death, memory, and behavioral deficits. Thus, SE model presents an opportunity to test whether EP2 receptor plays a proinflammatory role and whether an EP2 antagonist blunts EP2 mediated deleterious pathology after SE. The data presented in the patents[31, 32] suggest that a brief exposure of EP2 antagonist an hour after mice entered into SE by pilocarpine (a muscarinic receptor agonist), suppresses the delayed mortality by 30%, accelerates the recovery of mice by day 4 (determined by weight regain and nest building), suppresses the upregulation of several proinflammatory mediators including IL-1β, IL-6 and TNF-α, breakdown of blood brain barrier and neurodegeneration (by 50–66%) in hippocampus sub-regions (CA1, CA3 and hilus), in comparison to a vehicle treatment suggesting EP2 antagonist is useful to mitigate the inflammatory response after brain injury. The details of the study were published recently[29, 42].

A significant body of evidence suggests that upregulation of COX-2 correlates well with high levels of PGE_2 in tumor tissues[43–45], and administration of PGE_2 enhances the colon cancer in an azoxymethane-induced colon tumor model[46], suggesting antagonism of PGE_2 receptors could be a novel strategy for anti-cancer drug development. EP2 gene knockout models also established that EP2 receptor plays pivotal role in tumorigenesis and metastasis[25–27]. Thus, EP2 can be targeted by small molecule antagonist. While *in vivo* biological studies are still to be conducted, the early results presented in this patent and subsequently in a recent publication[30] demonstrate that an EP2 antagonist **3** attenuates EP2 agonist butaprost (Figure 1) mediated PC3 prostate cancer cell growth and invasion in vitro.

4. Expert opinion

The patents[31, 32] from the Emory laboratory underscore the importance of EP2 antagonists for therapeutic discovery, but they also raise a number of issues to be resolved before an EP2 antagonist is taken forward for human use. First, it should be determined whether a pharmacological inhibition of basal EP2 receptor activity in normal and healthy mice will have any detrimental effects, because EP2 global knockout mice displayed several adverse effects such as reduced fertility and pups with reduced litter size[47, 48]; systolic hypertension when fed high salt-diet[48]; cognitive, social memory deficits; impaired spatial learning[49, 50], suggesting EP2 plays a physiological role. Thus, it is important to demonstrate whether chronic, study state exposure of EP2 antagonist in rodents will have any adverse effects on all these fronts, particularly on maintenance of arterial blood pressure, ovulation, and fertility. However, studies from the Dingledine laboratory[51] established that EP2 inhibition recapitulates many features of COX-2 inhibition restricted to forebrain neurons in the SE model[51]; several other studies from the laboratories of Andreasson[18, 19, 52, 53] and Montine[54–56] demonstrated that EP2 exacerbates the inflammatory signaling and oxidative stress, drives brain micro environment for neurotoxicity and suppress the beneficial functions of microglia. Thus, EP2 antagonism should be explored as a novel therapeutic

strategy, not only for anti-inflammation and neuroprotection, but also for anti-cancer and endometriosis[3, 4].

Until now, very few selective EP2 antagonists have been discovered, including a brain permeable compound **12** with brain-to-plasma ratio (1.7) and plasma half-life 1.7 h. In the SE model study, three injections of **12** at 4, 21 and 30 hrs (5 mg/kg, ip) after SE seem beneficial, but it is difficult to ascertain whether a steady state inhibition of EP2 receptor was achieved by this regimen. It is unclear whether the efficacy can be repeated in this model if and when a novel compound with a prolonged plasma half-life and brain pharmacokinetics is used. Moreover, from the bioactivity, compound **12** is only 10-fold selective to EP2 over DP1, thus it is also possible that **12** may be working through DP1 receptors. Furthermore, the data is insufficient to ascertain the biological effect by **12** in the SE model is only through its interaction with EP2 receptor. A related compound **14**, which shares structural similarity with **12** (Figure 4) is also available for use in peripheral disease models. Compound **14** has a prolonged plasma half-life (2.7 h) and about 500-fold higher concentrations in plasma than its EP2 Schild K_B (8.8 nM) until 8 h after ip injection[57].

While Bayer Schering Pharma has not advanced any of their products, Pfizer seems to be a real competitor in the development of EP2 antagonists. Pfizer created a compound **13**, an azetidine carboxylic acid derivative with high selectivity to EP2 against all other prostanoid receptors and showed that it attenuates a butaprost induced cutaneous blood flow in rats[58]. However, its brain penetration properties were not reported to suggest whether it will be a useful compound for in vivo use in brain injury models and it is unknown whether Pfizer is exploring EP2 antagonists in any CNS models.

Therapeutic applications of a clinically useful EP2 antagonist would be enormous. Although the lead antagonist **13** may potentially result in idiosyncratic drug reactions due to the acrylamide and the trimethoxyphenyl functional groups[59, 60], the compounds **10** and **11** will not. Overall, as it was claimed in the patents[31, 32, 34], an optimized EP2 antagonist may be useful for treating a variety of inflammatory diseases such as arthritis and IBD; neurodegenerative diseases such as SE, epilepsy, AD, PD, and ALS; women's diseases such as menorrhagia, dysmenorrhea, and endometriosis; and other diseases such as cancer and COPD.

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Abbreviations

cAMP	cyclic adenosine monophosphate
ALS	amyotrophic lateral sclerosis
TR-FRET	time-resolved fluorescence resonance energy transfer

OTT	office of technology transfer
POC	proof of concept
SE	status epilepticus
AD	Alzheimer's disease
PD	Parkinson's disease
COPD	chronic obstructive pulmonary disease
IBD	inflammatory bowel disease

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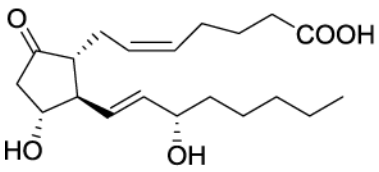
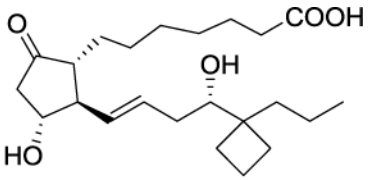


Prostaglandin-E_2 (PGE_2)

Receptor	*Ki* (nM)
EP1	9.1
EP2	4.9
EP3	0.33
EP4	0.79
DP	307
FP	119
IP	>100,000
TP	>29,000



Butaprost, Free Acid

Receptor	*Ki* (nM)
EP1	27,721
EP2	91
EP3	1,643
EP4	19,104
DP	12,097
FP	>100,000
IP	>54,836
TP	>19,667

Figure 1.

Structures and bioactivity of EP2 receptor agonist PGE_2 (endogenous) and synthetic derivative butaprost free acid. Values are obtained from[33].

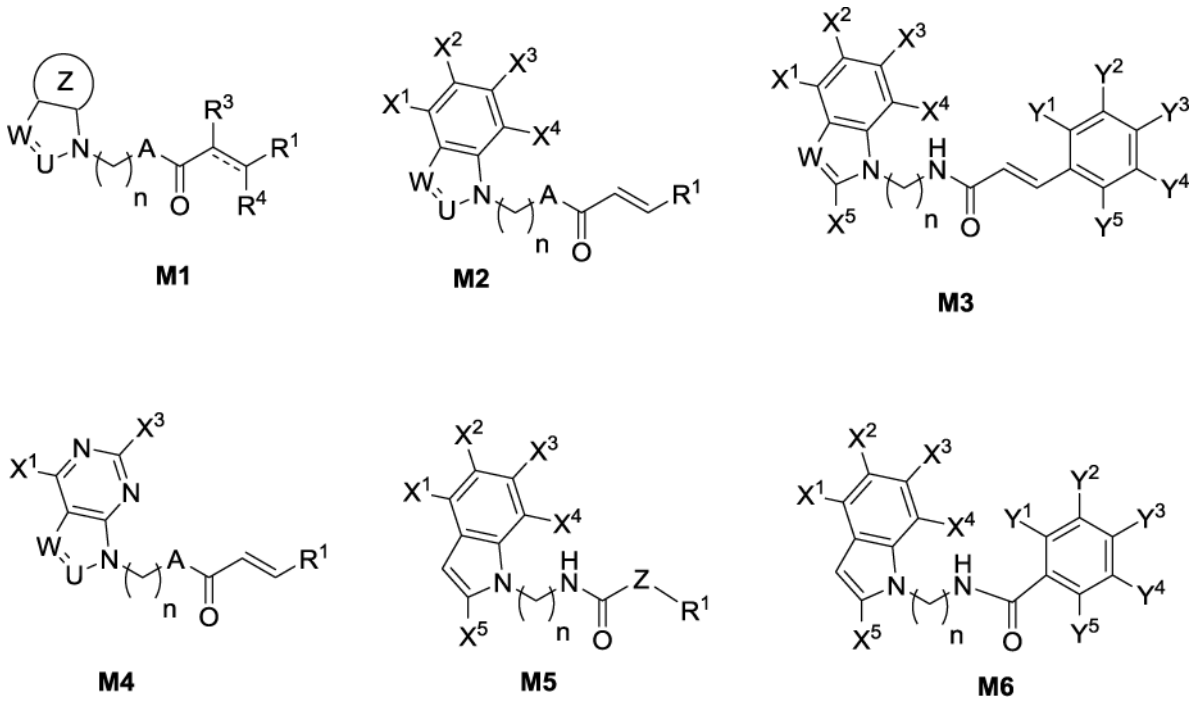


Figure 2.

Key Markush structures illustrated in the patent application[31, 32] to cover the structural modifications possible on this scaffold to protect the intellectual property

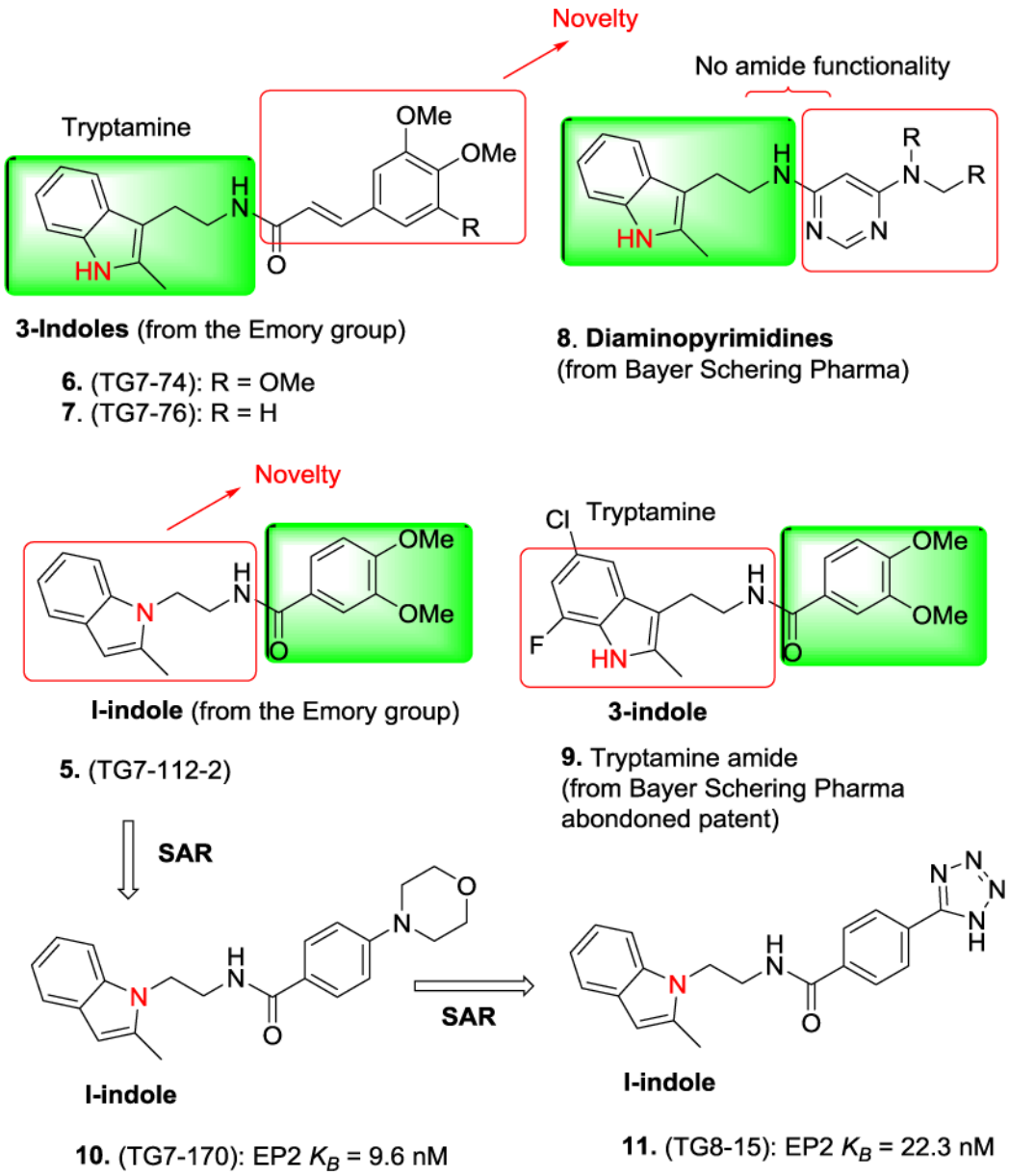
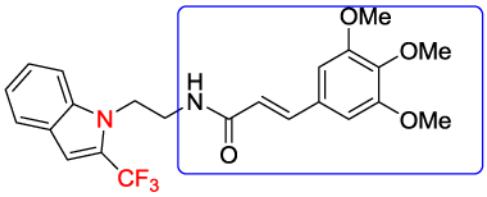
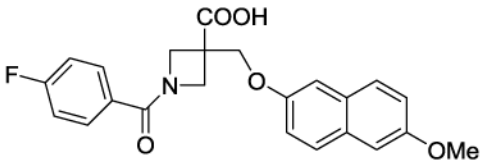


Figure 3.

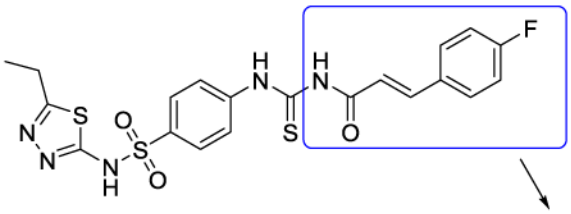
Structural comparison between compounds from the Emory patents[31, 32] to the one published in patents from Bayer Schering Pharma[34, 35]. SAR = Structure activity relationship study. Filled green boxes are used to indicate similarity, whereas the unfilled red boxes are used to indicate the differences.



12. (TG6-10-1); Emory lead EP2 antagonist.



13. PF-04418948; Pfizer lead EP2 antagonist.



14. (TG6-129); Selective EP2 antagonist from Emory group

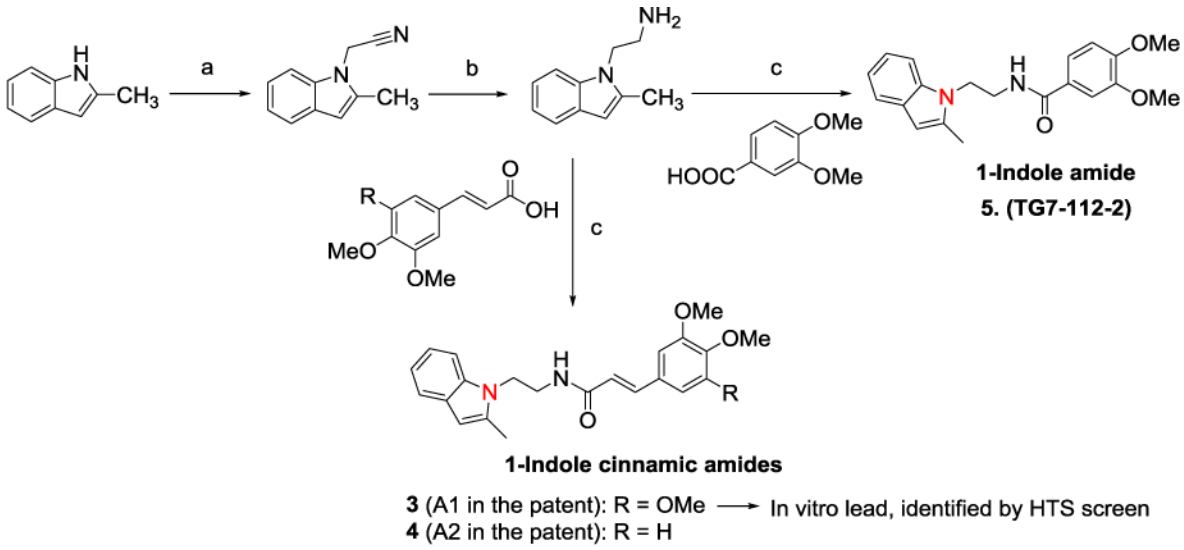
acrylamide moiety which overlaps with structure **12**

Compound	EP2 K_B	*Plasma* $T_{1/2}$	*B/P Ratio*
12	17.8 nM	1.7 h (ip & oral)	1.7
13	1.8 nM	8.8 h (oral)	NR
14	8.8 nM	2.7 h (ip & oral)	< 0.01

ip = intraperitoneal, NR = not reported

Figure 4.

Lead EP2 antagonists currently available for in vivo use. EP2 Schild K_B (derived from a cAMP-mediated-TR-FRET assay) and pharmacokinetics are shown in inset box. Blue boxes are used to indicate the similarity.



Scheme 1.

Synthesis of 1-indole cinnamic amide and amide EP2 antagonists. Reagents and conditions: a. NaH, bromoacetonitrile, DMF, 75% b. Lithium aluminum hydride (LAH), tetrahydrofuran (THF), c. a substituted cinnamic acid or a benzoic acid or a heterocyclic acid, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI), dimethylaminopyridine (DMAP), CH_2Cl_2, 70–80%.