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REVIEW

Prostanoid receptor antagonists: development strategies and therapeutic applications

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Identification of the primary products of cyclo-oxygenase (COX)/prostaglandin synthase(s), which occurred between 1958 and 1976, was followed by a classification system for prostanoid receptors (DP, EP_1, EP_2 . . .) based mainly on the pharmacological actions of natural and synthetic agonists and a few antagonists. The design of potent selective antagonists was rapid for certain prostanoid receptors (EP_1, TP), slow for others (FP, IP) and has yet to be achieved in certain cases (EP_2). While some antagonists are structurally related to the natural agonist, most recent compounds are 'non-prostanoid' (often acyl-sulphonamides) and have emerged from high-throughput screening of compound libraries, made possible by the development of (functional) assays involving single recombinant prostanoid receptors. Selective antagonists have been crucial to defining the roles of PGD_2 (acting on DP_1 and DP_2 receptors) and PGE_2 (on EP_1 and EP_4 receptors) in various inflammatory conditions; there are clear opportunities for therapeutic intervention. The vast endeavour on TP (thromboxane) antagonists is considered in relation to their limited pharmaceutical success in the cardiovascular area. Correspondingly, the clinical utility of IP (prostacyclin) antagonists is assessed in relation to the cloud hanging over the long-term safety of selective COX-2 inhibitors. Aspirin apart, COX inhibitors broadly suppress all prostanoid pathways, while high selectivity has been a major goal in receptor antagonist development; more targeted therapy may require an intermediate position with defined antagonist selectivity profiles. This review is intended to provide overviews of each antagonist class (including prostamide antagonists), covering major development strategies and current and potential clinical usage.
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Abbreviations: Ang II, angiotensin II; BMP, bone morphogenetic protein; BP, blood pressure; ClogP, predicted *n*-octanol/water partition coefficient; COX, cyclo-oxygenase; CPC, chronic persistent cough; PG, prostaglandin; $EP^{+/+}$/$EP^{-/-}$, system involving wild-type/homozygous gene-deleted EP receptor; FA, free acid; NSAID, non-steroidal anti-inflammatory agent; PAF, platelet-activating factor; PGIS, prostacyclin synthase; PLC, phospholipase C; PRP, platelet-rich plasma; rc, recombinant; SAR, structure–activity relationships; TM, transmembrane; TXA_2, thromboxane A_2; TXS, thromboxane synthase

Introduction

The pharmacological classification for prostanoid receptors was developed on the basis that each receptor preferentially recognizes one of the major natural prostaglandins (PGs). Thus, PGD_2 preferentially activates the DP receptor, PGE_2 preferentially activates the EP receptor, with the same applying to $PGF_{2\alpha}$/FP receptor and PGI_2 (prostacyclin)/IP receptor (Coleman *et al.*, 1994b). In the case of the TP receptor, both thromboxane A_2 (TXA_2) and its precursor PGH_2 are potent agonists. There are two distinct subtypes of DP receptor, DP_1 and DP_2; the latter has also been called CRTh2 (chemoattractant receptor-homologous molecule expressed on T helper 2 cells). Of the four EP receptor subtypes, EP_1 and EP_3 generally elicit excitatory actions, while EP_2 and EP_4 elicit inhibitory actions on cell function. All prostanoid receptors belong to the G protein-coupled receptor superfamily of cell-surface

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receptors. Each has seven transmembrane (TM)-spanning segments and may couple to one or more signal-transduction processes. In addition, mRNA splicing variants have been discovered for DP_1, EP_1, EP_3, EP_4, FP and TP receptors (see Pierce and Regan, 1998).

The repertoire of bioactive oxygenated lipids and signal transduction mechanisms is expanded by homodimerization and heterodimerization between different prostanoid receptors. For example, an isoprostane binding site is created by IP/TPα heterodimerization (Wilson *et al.*, 2004). Moreover, the prostamide (prostaglandin-ethanolamide) recognition site appears to result from heterodimerization of wild type and alternatively spliced FP receptor variants (Liang *et al.*, 2008). Prostanoid receptors may also complex with non-prostanoid receptors, for example, EP_1 receptor with β_2-adrenoceptor, but discussion of these interactions is outside the scope of this review. The receptor/second messenger nomenclature used in the review conforms to this journal's Guide to Receptors and Channels (Alexander *et al.*, 2008).

Assay systems and antagonist development

The initial pharmacological differentiation of prostanoid receptors relied heavily on isolated tissue studies, with ileum, trachea and vas deferens of the guinea pig being especially important (Jones *et al.*, 1982; Coleman *et al.*, 1984; 1987; 1994a; Dong *et al.*, 1986; see Chen *et al.*, 2001 for experimental details). Isolated tissue preparations are still used today as they often reflect phenomena that occur in the integrated, living mammal. In addition, they still provide evidence for new receptor entities, as shown by the critical role of the cat iris preparation in the elucidation of prostamide pharmacology (Matias *et al.*, 2004; Woodward *et al.*, 2007; 2008). Studies on isolated and cultured cells have also been important in building the pharmacological classification of prostanoid receptors (Coleman *et al.*, 1984; Eglen and Whiting, 1988; Woodward *et al.*, 1995a,b).

Although of low throughput, isolated tissue studies have been critical to the discovery of prostanoid antagonists, particularly for DP_1, EP_1 and TP receptors. However, it is remarkable that antagonists for other receptors (EP_2, EP_3, FP and IP) have been slow to emerge following discovery of the natural ligand/receptor; several reasons may account for this situation. Partial agonism is found in closely related analogues of the natural ligands for DP_1 and TP receptors (Table 1), thereby providing inroads to pure antagonists. However, partial agonism may not always be readily recognized. For example, taprostene, an early analogue of PGI_2 (Müller *et al.*, 1983), was only shown to be an IP partial agonist in 2004 (Chan and Jones, 2004). Furthermore, partial agonism may not always translate into pure antagonism. Thus, some of the many 'non-prostanoid prostacyclin mimetics' synthesized (see later) show IP partial agonism (Merritt *et al.*, 1991a; Jones *et al.*, 1997; Seiler *et al.*, 1997; Kam *et al.*, 2001), but IP antagonists have apparently not emerged from this grouping. Moreover, a

Table 1 Prostanoid receptor agonists relevant to defining antagonist profiles

Prostanoid receptor	*Full agonist*		*Partial agonist*
	High selectivity	*Moderate selectivity*	
DP_1	BW-245C		BW-192C86[a]
DP_2	15(*R*) PGD_2, 15(*R*)-15-methyl PGD_2 13,14-dihydro-15-oxo PGD_2		
EP_1	ONO-DI-004	17-Phenyl PGE_2	Iloprost[b,c]
EP_2	ONO-AE1-259, CAY-10399[d]	Butaprost-FA, CP-533536[e] 19(*R*)-hydroxy PGE_2[f]	
EP_3	ONO-AE-248, SC-46275	Sulprostone, MB-28767	ONO-AP-324[g]
EP_4	ONO-AE1-329, tetrazolo PGE_1[h]	(PGE_2)[i]	
FP	Fluprostenol, latanoprost-FA	Cloprostenol	AL-8810[j,k,l]
IP	Cicaprost	AFP-07, iloprost	Octimibate[m], taprostene[n]
TP	STA_2, U-46619		CTA_2[o], PTA_2[o,p], U-44069[p]

Information on the non-referenced agonists may be obtained from Jones (2004) and this journal's Guide to Receptors and Channels edited by Alexander *et al.* (2008). In older publications, fluprostenol = ICI-81008, cloprostenol = ICI-80996, cicaprost = ZK-96480 and iloprost = ZK-36374.
FA, free acid.
[a]Series of bicyclic-hydantoin prostanoids (Leff and Giles, 1992).
[b]Dong *et al.* (1986).
[c]Boie *et al.* (1997).
[d]Tani *et al.* (2002).
[e]Paralkar *et al.* (2003).
[f]Woodward *et al.* (1993a).
[g]Jones *et al.* (1998).
[h]Analogue 19a in Billot *et al.* (2003).
[i]Utility on high-sensitivity EP_4 systems.
[j]Griffin *et al.* (1999).
[k]Woodward *et al.* (2007).
[l]Sharif *et al.* (2008).
[m]Merritt *et al.* (1991a,b).
[n]Chan and Jones (2004).
[o]Armstrong *et al.* (1985).
[p]Jones *et al.* (1982).

large range of compounds block TP receptors (see later); this expansiveness may simply not apply to other prostanoid receptors. Finally, there has not been widespread commercial drive to develop EP_2 antagonists, for example, owing to a perceived lack of therapeutic utility (see later).

Dramatic progress in antagonist development was seen following cloning of the various prostanoid receptors in the early 1990s: DP_1 (Boie *et al.*, 1995), DP_2 (Hirai *et al.*, 2001), EP_1 (Funk *et al.*, 1993a), EP_2 (Regan *et al.*, 1994), EP_3 (Yang *et al.*, 1994), EP_4 (Bastien *et al.*, 1994), FP (Abramovitz *et al.*, 1994), IP (Namba *et al.*, 1994), TP (Hirata *et al.*, 1991). Stable overexpression of each prostanoid receptor in carrier cell lines allowed high-throughput radioligand binding and functional studies using 96- or even 384-well plate format. Thus, chemical library screening resulted in the discovery of new non-prostanoid scaffolds as leads, from which potent and selective agonists and antagonists were designed. Inspection of Figures 1–8 will reveal the prevalence of aryl-sulphonamido linkages in the various antagonist classes. Non-prostanoid structures provide a practical approach to obtaining new prostanoid-based therapies, as the issues of bioavailability, metabolic stability and cost of synthesis that surround PG analogues are avoided.

Selective agonists for prostanoid receptors

Selective agonists are the pharmacological counterparts of antagonists and, as such, fulfil an important role in receptor characterization. In terms of prostanoid receptors, three of the natural ligands, PGH_2/TXA_2 and PGI_2, are unstable under physiological conditions and are usually replaced by U-46619 and cicaprost respectively (Table 1). In addition, there are potential problems with certain EP agonists synthesized as C1 methyl esters, for example, butaprost (EP_2) and SC-46275 (EP_3); full agonist potency is only realized after (enzymatic) hydrolysis of the ester within the tissue. Ono Pharmaceuticals have synthesized selective agonists for EP_1 (ONO-DI-004), EP_2 (ONO-AE1-259), EP_3 (ONO-AE-248) and EP_4 (ONO-AE1-329) receptors (Suzawa *et al.*, 2000). However, the rather modest potencies of ONO-DI-004 and ONO-AE-248 may restrict their utility in full Schild antagonism protocols (R.L. Jones *et al.*, 2008, submitted). Partial agonists for DP_1, EP_1, EP_3, FP, IP and TP receptors are known (Table 1); their use in antagonist protocols may present difficulties of interpretation.

As drugs, notably for systemic administration, selective prostanoid mimetics are a high-risk proposition because of the myriad of unwanted side effects that may occur. As such, the future of prostanoid-based therapies appears to reside in the main in selective PG synthase inhibitors and prostanoid receptor antagonists.

Antagonist protocols

The Schild protocol for inferring the nature of competition and determining the affinity constant of an antagonist remains the gold standard (see Colquhoun, 2007). It is applicable to both isolated tissue and the ever-increasing number of recombinant (rc) receptor/cell-based assay methods usually involving Ca^{2+} mobilization or cAMP generation. While the latter methods are given to high throughput and precision, care must be taken with high-affinity antagonists in the Ca^{2+} assays as the agonist response is usually measured as the peak of the transient Ca^{2+} signal, which may occur before re-equilibration of antagonist occupancy is complete. The majority of the data given in Table 2 relate to pA_2 values derived using the Schild protocol, with emphasis on human, guinea pig and rat isolated preparations. Binding data (preferably pK_i) are given where functional information is not available. There are considerable binding data on mouse prostanoid receptors, but little affinity data in functional systems.

Inhibition-curve (or Cheng-Prusoff) protocols have been used infrequently in prostanoid receptor studies. Bley *et al.* (2006) estimated the pA_2 of the IP antagonists RO-1138452 and RO-3244794 using carbacyclin as the fixed-concentration agonist in a human rc-IP receptor – cAMP assay, but failed to use the modified form of the Cheng-Prusoff equation (Craig, 1993; Lazareno and Birdsall, 1993; Leff and Dougall, 1993). These protocols have the advantages of operating over a lower agonist concentration range than Schild protocols and providing direct observation of the rate of onset of antagonism. The latter is important in recognizing the slow approach to steady state that occurs with high-affinity antagonists at low concentration, and also with highly lipophilic antagonists (Jones *et al.*, 2008) that regularly emerge from combinatorial chemistry – high-throughput screening. Ultimately, it is important and even preferable to define the pharmacology in the human target tissue; human rc-receptor assays are a useful accompaniment. Successful drugs require the correct pharmacological attributes, but physical chemical properties are also important.

DP_1 receptor antagonists

Development

Antagonists for, what we now know to be, the DP_1 receptor subtype, were first described in the 1970s and early 1980s. Examples include N-0164 (MacIntyre and Gordon, 1977), diphloretin phosphate (Westwick and Webb, 1978) and desacetyl-1-nantradol (Horne, 1984), but none of these compounds exhibit the potency and selectivity essential for unambiguous receptor classification. The simple xanthone-carboxylic acid AH-6809 (Keery and Lumley, 1988) has sufficient DP_1 affinity (pA_2 = 5.9–6.6, Table 2), but it has been mainly employed as an EP_1 antagonist (see later). Indeed, the hydantoin derivative, BW-A868C (Figure 1) has been the only selective, surmountable and competitive DP_1 antagonist (pA_2 > 9 for the human subtype) readily available to pharmacologists (Giles *et al.*, 1989; Lydford *et al.*, 1996c). The N-benzyl substituent is crucial for antagonist activity; in a related series of bicyclic-hydantoin analogues a progression from full agonism to virtually pure antagonism is seen with hydrogen, methyl, ethyl and *n*-propyl substituents on N^{10} (Giles and Leff, 1992). BW-A868C also has low affinity (pA_2 = 5.1) for the EP_4 subtype (Lydford *et al.*, 1996c). ZK-138357 (Schering AG) is a moderate-affinity DP_1 antagonist (Table 2) with some structural similarity to BW-A868C.

Recently, the potential pathological role of PGD_2, especially in allergic disorders, has been revived resulting in the discovery and evaluation of highly selective DP_1 antagonists of

Table 2 Affinities of prostanoid receptor antagonists in functional isolated preparations

Antagonist	*Species*	*Tissue system*	*Agonist*	*pA₂*	*Reference*
DP_1 receptor					
AH-6809	Human	Neutrophil/superoxide release	BW-245C	6.55	Wheeldon and Vardey (1993)
			BW-245C	6.59	Lydford *et al.* (1996a)
		Platelet/aggregation	PGD_2	6.27[a]	Keery and Lumley (1988)
	Cow	Embryonic tracheal fibroblast/cAMP	PGD_2	6.36	Ito *et al.* (1990)
	Rabbit	Saphenous vein	BW-245C	5.93	Lydford *et al.* (1996c)
BW-A868C	Human	Neutrophil/superoxide release	BW-245C	9.46	Lydford *et al.* (1996a)
		Platelet/aggregation	BW-245C	9.26	Giles *et al.* (1989)
		Pulmonary vein	PGD_2	7.84	Walch *et al.* (1999)
		Uterus (non-pregnant)	BW-245C	8.3	Senior *et al.* (1992)
		Uterus (pregnant)	BW-245C	8.6	Senior *et al.* (1993)
	Cow	Embryonic tracheal fibroblast/cAMP	BW-245C	8.0	Crider *et al.* (1999)
	Dog	Nasal vein	BW-245C	7.3	Liu *et al.* (1996a)
		Tracheal epithelium/Cl^- secretion	BW-245C	8.16	Liu *et al.* (1996b)
	Rabbit	Jugular vein	BW-245C	8.73	Giles *et al.* (1989)
		Saphenous vein	BW-245C	8.50	Lydford *et al.* (1996c)
MK-0524 (Laropiprant)	Human	rc-DP_1/HEK-293E/binding	[^{3}H]-PGD_2	*10.5*[b]	Sturino *et al.* (2007)
		Platelet/cAMP	PGD_2	10.05[c]	Sturino *et al.* (2007)
ONO-AE3-237	Human	rc-DP_1/CHO/binding	[^{3}H]-PGD_2	*7.74*	Torisu *et al.* (2004c)
S-5751	Human	Platelet/cAMP	PGD_2	9.02[b,c]	Arimura *et al.* (2001)
	Guinea pig	Platelet/cAMP	PGD_2	7.50[b,c]	Arimura *et al.* (2001)
ZK-138357	Human	Neutrophil/superoxide release	BW-245C	7.25	Lydford *et al.* (1996a)
	Rabbit	Saphenous vein	BW-245C	5.05	Lydford *et al.* (1996a)
	Rat	Peritoneal mast cell	BW-245C	~6.0	Chan *et al.* (2000)
Compound **1**	Human	Platelet/binding	[^{3}H]-PGD_2	*6.22*	Mitsumori *et al.* (2003a)
Compound **2**	Human	Platelet/binding	[^{3}H]-PGD_2	*7.62*	Mitsumori *et al.* (2003a)
Compound **3**	Human	rc-DP_1/HEK-293-Gα15/Ca^{2+}	BW-245C	[~7.4]	Krauss *et al.* (2005)
Compound **4**	Human	rc-DP_1/CHO/binding	[^{3}H]-PGD_2	*8.27*	Torisu *et al.* (2004c)
Compound **5**	Human	rc-DP_1/HEK-293/binding	[^{3}H]-PGD_2	*9.0*	Beaulieu *et al.* (2008)
DP_2 receptor					
BAY-u3405 (Ramatroban)	Human	rc-DP_2/CHO/GTPγS binding	PGD_2	7.44	Mathiesen *et al.* (2006)
		Eosinophil/shape change	PGD_2	~8.0[a]	Mathiesen *et al.* (2006)
K-117	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	*8.26*	Mimura *et al.* (2005)
K-604	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	*7.96*	Mimura *et al.* (2005)
TM-30089[d] (CAY-10471)	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	*8.74*	Mathiesen *et al.* (2006)
				9.22	Ulven and Kostenis (2005)
Compound **6**	Human	rc-DP_2/pre-B L1.2/Ca^{2+}	PGD_2	[6.8]	Bauer *et al.* (2002)
Compound **7**	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	*8.64*[c]	Birkinshaw *et al.* (2006)
Compound **8**	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	[*9.40*][c]	Bonnert and Rasul (2004)
Compound **9**	Human	rc-DP_2/CHO/binding	[^{3}H]-PGD_2	*7.17*	Armer *et al.* (2005)
		Eosinophil/shape change	PGD_2	7.13[c]	Armer *et al.* (2005)
		Th2-lymphocyte/chemotaxis	PGD_2	7.17[c]	Armer *et al.* (2005)
Compound **10**	Human	rc-DP_2/CHO/Ca^{2+}	PGD_2	[8.53]	Fretz *et al.* (2005)
Compound **11**	Human	rc-DP_2/HEK-293/binding	[^{3}H]-PGD_2	[*9.0*]	Bonnert *et al.* (2005c)
EP_1 receptor					
AH-6809	Human	rc-EP_1/HEK-293E/reporter gene	Iloprost	~6.4	Durocher *et al.* (2000)
		Pulmonary vein	Sulprostone	5.52	Walch *et al.* (2001)
	Guinea pig	Ileum	PGE_2	6.8	Coleman *et al.* (1987)
		Ileum	PGE_2	7.39	Eglen and Whiting (1988)
		Ileum	PGE_1	7.42	Eglen and Whiting (1988)
		Ileum	16,16-DM PGE_2	7.59	Eglen and Whiting (1988)
		Trachea	16,16-DM PGE_2	7.48	Eglen and Whiting (1988)
		Trachea	17-Phenyl PGE_2	7.35	Lawrence *et al.* (1992)
GW-848687	Human	rc-EP_1/not given/reporter gene	PGE_2	9.1	Giblin *et al.* (2007)
MF-266-1	Human	rc-EP_1/HEK-293/Ca^{2+}	PGE_2	7.8	Clark *et al.* (2008)
ONO-8711	Mouse	rc-EP_1/CHO/binding	[^{3}H]-PGE_2	*8.77*	Watanabe *et al.* (1999)
	Human	rc-EP_1/CHO/binding	[^{3}H]-PGE_2	*9.22*	Watanabe *et al.* (1999)
ONO-8713	Mouse	rc-EP_1/not given/binding	[^{3}H]-PGE_2	9.5	Narumiya and Fitzgerald (2001)
SC-19220	Guinea pig	Ileum	PGE_2	5.5	Sanner (1969)
		Ileum	PGE_2	5.6	Bennett and Posner (1971)
		Trachea	$PGF_{2\alpha}$	6.6	Farmer *et al.* (1974)
SC-51089	Human	rc-EP_1/HEK-293E/reporter gene	Iloprost	6.94	Durocher *et al.* (2000)
	Guinea pig	Ileum	PGE_2	6.5	Hallinan *et al.* (1993)
		Ileum	PGE_2	6.7	Sametz *et al.* (2000)
SC-51322	Human	rc-EP_1/HEK-293E/reporter gene	Iloprost	8.80	Durocher *et al.* (2000)
	Guinea pig	Ileum	PGE_2	8.1	Hallinan *et al.* (1994)
		Trachea	17-Phenyl PGE_2	8.45	Hung *et al.* (2006)
Compound **13**	Mouse	rc-EP_1/CHO/Ca^{2+}	PGE_2	8.25[c]	Naganawa *et al.* (2006)
Compound **14**	Human	rc-EP_1/HEK-293E/binding	[^{3}H]-PGE_2	*8.0*	Ruel *et al.* (1999)
Compound **15**	Human	rc- EP_1/CHO/Ca^{2+}	PGE_2	8.2	Hall *et al.* (2007b)
EP_2 receptor					
AH-6809	Human	rc-EP_2/COS-7/cAMP	PGE_2	~6.5	Woodward *et al.* (1995)
		Bronchus	PGE_2	5.78	Norel *et al.* (1999)
	Guinea pig	Trachea	PGE_2	5.7[e]	KJ Ong and RL Jones (unpublished)

Table 2 *Continued*

Antagonist	*Species*	*Tissue system*	*Agonist*	*pA₂*	*Reference*
EP_3 receptor					
DG-041	Human	rc-EP_3/Chem-1/Ca^{2+}	PGE_2	8.09[c]	Singh *et al.* (2009)
L-798106	Guinea pig	Aorta	17-Phenyl PGE_2[f]	7.96	Jones *et al.* (2008)
		Trachea	Sulprostone	7.82	Clarke *et al.* (2004)
		Vas deferens	Sulprostone	7.48	Clarke *et al.* (2004)
L-826266	Human	rc-EP_3/HEK-293E/Ca^{2+}	PGE_2	7.97	R.L. Jones *et al.* (2008, submitted)
		Erythroleukaemia cell/cAMP	Sulprostone	8.35	Clark *et al.* (2008)
	Guinea pig	Aorta	17-Phenyl PGE_2	7.58	R.L. Jones *et al.* (2008, submitted)
ONO-AE3-240	Mouse	rc-EP_3/not given/Ca^{2+}	PGE_2	8.8	Amano *et al.* (2003)
Compound **17**	Human	Erythroleukaemia cell/cAMP	Sulprostone	6.89	Gallant *et al.* (2002)
Compound **18**	Human	rc-EP_3/HEK-293E/binding	[^{3}H]-PGE_2	*7.7*	Juteau *et al.* (2001)
Compound **19**	Human	rc-EP_3/HEK-293E/cAMP	PGE_2	8.22	Belley *et al.* (2005)
EP_4 receptor					
AH-23848	Sheep	Ductus arteriosus	PGE_2	~5.2[a]	Bouayad *et al.* (2001)
	Human	Middle cerebral artery	PGE_2	5.7	Davis *et al.* (2004)
	Pig	Saphenous vein	PGE_2	5.0	Coleman *et al.* (1994a)
	Rabbit	Saphenous vein	PGE_2	4.96	Lydford *et al.* (1996b)
	Mouse	rc-EP_4/CHO/cAMP	PGE_2	5.3	Nishigaki *et al.* (1995)
BGC-20-1531	Human	rc-EP_4/HEK-293E/cAMP	PGE_2	7.6	Maubach *et al.* (2009)
		Middle cerebral artery	PGE_2	7.8	Maubach *et al.* (2009)
	Dog	Middle meningeal artery	PGE_2	7.7	Maubach *et al.* (2009)
CJ-023423	Human	rc-EP_4/HEK-293/cAMP	PGE_2	8.3	Nakao *et al.* (2007)
	Rat	rc-EP_4/HEK-293/cAMP	PGE_2	8.2	Nakao *et al.* (2007)
CJ-042794	Human	rc-EP_4/HEK-293/cAMP	PGE_2	8.6	Murase *et al.* (2008b)
	Rat	rc-EP_4/HEK-293/cAMP	PGE_2	8.7	Murase *et al.* (2008a)
GW-627368	Human	rc-EP_4/HEK-293/cAMP	PGE_2[b]	7.9	Wilson *et al.* (2006)
		Pulmonary vein	ONO-AE1-329	7.06	Foudi *et al.* (2008)
	Pig	Saphenous vein	PGE_2	9.2	Wilson *et al.* (2006)
	Rabbit	Saphenous vein	PGE_2[g]	≥8.5	Jones and Chan. (2005)
L-161982	Human	rc-EP_4/HEK-293/cAMP	PGE_2	~8.5	Machwate *et al.* (2001)
		BEAS-2B cell/CRE reporter	ONO-AE1-329	9.14	L.M. Ayer and M.A. Giembycz (unpublished)
		Middle cerebral artery	PGE_2	8.4	Davis *et al.* (2004)
	Rat	rc-EP_4/HEK-293/binding	[^{3}H]-PGE_2	*7.50*	Machwate *et al.* (2001)
		Periosteal cell/cAMP	PGE_2	7.0[c]	Machwate *et al.* (2001)
MF-498	Human	rc-EP_4/HEK-293/cAMP	PGE_2	8.77[c]	Clark *et al.* (2008)
ONO-AE2-227	Mouse	rc-EP_4/CHO/cAMP	PGE_2	8.0[c]	Mutoh *et al.* (2002)
ONO-AE3-208	Mouse	rc-EP_4/not given/binding	[^{3}H]-PGE_2	*8.89*	Kabashima *et al.* (2002)
Compound **20**	Human	rc-EP_4/HEK-293E/cAMP	PGE_2	8.49[c]	Burch *et al.* (2008)
FP receptor					
AS-604872	Human	rc-FP/HEK-293E/PI	$PGF_{2\alpha}$	7.33[c]	Cirillo *et al.* (2007)
THG-113	Pig	Retinal blood vessel	$PGF_{2\alpha}$	[6.34][c]	Peri *et al.* (2006)
THG-113.31	Pig	Retinal blood vessel	$PGF_{2\alpha}$	[8.00][c]	Peri *et al.* (2006)
THG-113.824	Pig	Retinal blood vessel	$PGF_{2\alpha}$	[8.96][c]	Peri *et al.* (2006)
THG-113.825	Pig	Retinal blood vessel	$PGF_{2\alpha}$	7.21[c]	Peri *et al.* (2006)
Prostamide F receptor					
AGN-204396	Cat	Iris sphincter	Prostamide $F_{2\alpha}$	5.64	Woodward *et al.* (2007)
IP receptor					
RO-1138452	Human	rc-IP/CHO/cAMP	Carbacyclin	9.0	Bley *et al.* (2006)
		Pulmonary artery	Cicaprost	8.20	Jones *et al.* (2006)
	Guinea pig	Aorta	Cicaprost	8.39	Jones *et al.* (2006)
	Rabbit	Mesenteric artery	Cicaprost	8.12	Jones *et al.* (2006)
RO-3244794	Human	rc-IP/CHO/cAMP	Carbacyclin	8.5	Bley *et al.* (2006)
		BEAS-2B cell/CRE reporter	Taprostene	9.24	L.A. Ayer and M.A. Giembycz (unpublished)
Compound **21**	Rat	rc-IP/not given/cAMP	(Not given)	[8.12][h]	Keitz *et al.* (2004)
Compound **22**	Rat	UMR-106 osteosarcoma cell/cAMP	Iloprost	6.41	Nakae *et al.* (2005)
Compound **23**	Rat	UMR-106 osteosarcoma cell/cAMP	Iloprost	6.32	Nakae *et al.* (2005)
Compound **24**	Human	Platelet membrane/cAMP	Iloprost	7.8[c]	Brescia *et al.* (2007)
TP receptor					
AA-2414	Human	Bronchus	U-46619	7.7	Itoh *et al.* (1993)
(Seratrodast)	Pig	Coronary artery	U-44069	9.0	Imura *et al.* (1990)
	Guinea pig	Aorta	U-46619	8.5	Zhang *et al.* (1996)
		Trachea	U-46619	7.69	Ashida *et al.* (1989)
	Rat	Aorta	U-46619	7.8	Zhang *et al.* (1996)
AH-23848	Human	Bronchus	U-46619	8.5	Coleman and Sheldrick (1989)
		Bronchus	U-46619	6.9	McKenniff *et al.* (1988)
		Hand vein	U-46619	8.4	Arner *et al.* (1991)
		Lung parenchyma	U-46619	8.7	McKenniff *et al.* (1988)
		Platelet/aggregation	U-46619	8.05	Tymkewycz *et al.* (1991)
	Guinea pig	Lung parenchyma	U-46619	8.7	McKenniff *et al.* (1988)
		Trachea	U-46619	8.7	McKenniff *et al.* (1988)
		Trachea	U-46619	9.76	Tymkewycz *et al.* (1991)

Table 2 *Continued*

Antagonist	*Species*	*Tissue system*	*Agonist*	*pA_2*	*Reference*
	Rat	Aorta	U-46619	8.47	Tymkewycz *et al.* (1991)
		Lung parenchyma	U-46619	6.9	Norman *et al.* (1992)
		Platelet/aggregation	U-46619	8.19	Tymkewycz *et al.* (1991)
BAY-u3405	Human	Bronchus	U-46619	8.8	McKenniff *et al.* (1991)
(Ramatroban)		Pulmonary vein	U-46619	8.94	Walch *et al.* (2001)
	Guinea pig	Lung parenchyma	U-46619	7.7	Norman *et al.* (1992)
		Trachea	U-46619	8.7	McKenniff *et al.* (1991)
	Rat	Lung parenchyma	U-46619	8.6	McKenniff *et al.* (1991)
BM-13177	Human	Platelet/aggregation	U-46619	6.31	Karasawa *et al.* (1991a)
(Sulotroban)	Guinea pig	Aorta	U-46619	5.58	Karasawa *et al.* (1991b)
	Rabbit	Jugular vein	U-46619	6.01	Giles *et al.* (1989)
BM-13505	Human	Hand vein	U-46619	7.9	Arner *et al.* (1991)
		Platelet/aggregation	U-46619	7.75	Karasawa *et al.* (1991a)
		Uterus (non-pregnant)	U-46619	7.4	Senchyna and Crankshaw (1996)
	Guinea pig	Aorta	U-46619	6.89	Dubé *et al.* (1992)
		Aorta	U-46619	7.22	Karasawa *et al.* (1991b)
		Aorta	U-46619	7.3	Zhang *et al.* (1996)
		Lung parenchyma	U-46619	7.0	Norman *et al.* (1992)
		Trachea	U-46619	7.73	Dubé *et al.* (1992)
		Trachea	U-46619	7.5	Ogletree and Allen (1992)
	Rat	Aorta	U-46619	8.2	Zhang *et al.* (1996)
		Aorta	U-46619	8.6	Ogletree and Allen (1992)
		Lung parenchyma	U-46619	7.5	Norman *et al.* (1992)
BMS-180291	Human	Platelet membrane/binding	[^{3}H]-SQ-29548	*8.4*	Ogletree *et al.* (1993)
(Ifetroban)	Guinea pig	Aorta	U-46619	9.8	Zhang *et al.* (1996)
	Rat	Aorta	U-46619	9.5	Zhang *et al.* (1996)
CV-4151	Human	Platelet/aggregation	U-46619	5.2	Watts *et al.* (1991)
	Rabbit	Aorta	U-44069	5.9	Imura *et al.* (1988)
EP-092	Human	Bronchus	U-46619	7.37	Featherstone *et al.* (1990)
		Bronchus	U-46619	6.8	McKenniff *et al.* (1988)
		Lung parenchyma	U-46619	8.9	McKenniff *et al.* (1988)
		Platelet /aggregation	U-46619	7.73	Tymkewycz *et al.* (1991)
		Uterine artery	U-46619	8.5	Baxter *et al.* (1995)
	Guinea pig	Lung parenchyma	U-46619	8.7	McKenniff *et al.* (1988)
		Trachea	U-46619	7.29	Featherstone *et al.* (1990)
		Trachea	U-46619	8.7	McKenniff *et al.* (1988)
		Trachea	U-46619	8.02	Tymkewycz *et al.* (1991)
	Rat	Aorta	U-46619	8.55	Tymkewycz *et al.* (1991)
		Lung parenchyma	U-46619	7.1	Norman *et al.* (1992)
		Platelet aggregation	U-46619	7.80	Tymkewycz *et al.* (1991)
EP-169	Human	Platelet/aggregation	U-46619	8.30	Tymkewycz *et al.* (1991)
		Pulmonary artery	U-46619	~8.2	Qian *et al.* (1994)
	Guinea pig	Trachea	U-46619	8.77	Tymkewycz *et al.* (1991)
	Rat	Aorta	U-46619	8.73	Tymkewycz *et al.* (1991)
		Platelet/aggregation	U-46619	8.48	Tymkewycz *et al.* (1991)
Glibenclamide	Human	Internal mammary artery	U-46619	6.3[i]	Stanke *et al.* (1998)
		Saphenous vein		6.7[i]	Stanke *et al.* (1998)
	Dog	Coronary artery	U-46619	6.2	Cocks *et al.* (1990)
	Rabbit	Aorta	U-46619	6.08[a]	Pfister *et al.* (2004)
	Guinea pig	Aorta	U-46619	<5.0	Kemp and McPherson (1998)
	Rat	Aorta	U-46619	6.13	Kemp and McPherson (1998)
GR-32191	Human	Bladder (detrusor)	U-46619	8.27	Palea *et al.* (1998)
(Vapiprost)		Bronchus	U-46619	8.77	Featherstone *et al.* (1990)
		Bronchus	U-46619	8.40	Armour *et al.* (1989)
		Platelet/binding	[^{3}H]-GR-32191	*8.66*	Armstrong *et al.* (1993)
		Pulmonary artery	U-46619	8.18	Lumley *et al.* (1989)
		Saphenous vein	U-46619	8.93	Furci *et al.* (1991)
		Umbilical artery	U-46619	8.0	Boersma *et al.* (1999)
		Uterine artery	U-46619	8.5	Baxter *et al.* (1995)
		Uterus (non-pregnant)	U-46619	8.6	Senchyna and Crankshaw (1996)
		Uterus (pregnant)	U-46619	8.5[a]	Senior *et al.* (1993)
	Guinea pig	Aorta	U-46619	8.77	Lumley *et al.* (1989)
		Aorta	U-46619	9.4	Ogletree and Allen (1992)
		Trachea	U-46619	8.26	Featherstone *et al.* (1990)
		Trachea	U-46619	9.43	Tymkewycz *et al.* (1991)
		Trachea	U-46619	10.0	Ogletree and Allen (1992)
	Rat	Aorta	U-46619	7.87	Lumley *et al.* (1989)
		Aorta	U-46619	7.49	Furci *et al.* (1991)
		Aorta	U-46619	8.41	Tymkewycz *et al.* (1991)
		Aorta	U-46619	8.3	Ogletree and Allen (1992)
		Trachea	U-46619	8.31	Lydford and McKechnie (1994)
GR-83783[j]	Rat	Aorta	U-46619	7.5	Campbell *et al.* (1991a)
GR-108774[j]	Rat	Aorta	U-46619	9.2	Campbell *et al.* (1991b)
ICI-192605	Human	Platelet/aggregation	U-46619	8.16	Brewster *et al.* (1988)
		Umbilical artery	U-46619	8.1	Boersma *et al.* (1999)
		Umbilical vein	U-46619	9.07	Daray *et al.* (2003)
		Uterus (non-pregnant)	U-46619	9.2	Senchyna and Crankshaw (1996)
	Rat	Aorta	U-46619	8.4	Brewster *et al.* (1988)

Table 2 *Continued*

Antagonist	*Species*	*Tissue system*	*Agonist*	*pA₂*	*Reference*
I-PTA-OH	Guinea pig	Lung parenchyma	U-46619	5.6	Norman *et al.* (1992)
	Rat	Lung parenchyma	U-46619	5.8	Norman *et al.* (1992)
I-SAP	Human	Platelet/aggregation	U-46619	8.01[k]	Naka *et al.* (1992)
KW-3635	Human	Platelet/aggregation	U-46619	8.88	Karasawa *et al.* (1991a)
	Guinea pig	Aorta	U-46619	*7.74*	Karasawa *et al.* (1991b)
L-655240	Guinea pig	Aorta	U-44069	8.0	Hall *et al.* (1987)
		Aorta	U-44069	8.0	Hall *et al.* (1987)
ONO-11120	Human	Platelet/binding	[^{125}I]-PTA-0H	*7.71*[c]	Narumiya *et al.* (1986)
		Platelet/aggregation	U-46619	*7.49*	Tymkewycz *et al.* (1991)
	Guinea pig	Trachea	U-46619	8.07	Tymkewycz *et al.* (1991)
	Rat	Aorta	U-46619	*7.14*	Tymkewycz *et al.* (1991)
		Platelet/aggregation	U-46619	*7.38*	Tymkewycz *et al.* (1991)
ONO-NT-126	Human	Astrocytoma cell/PI	STA_2	10.0	Nakahata *et al.* (1990)
Ridogrel[j]	Human	Platelet/aggregation	U-46619	5.7	Watts *et al.* (1991)
	Rat	Tail artery	U-46619	5.5	Janssens *et al.* (1990)
(±)-S-145	Human	Astrocytoma cell/PI	STA_2	8.48	Nakahata *et al.* (1990)
(Domitroban)[l]		Platelet membrane/binding	[^{3}H]-(+)-*S*-145	*9.35*	Kishino *et al.* (1991)
	Rat	Aorta smooth muscle cell/binding	[^{3}H]-SQ-29548	*9.5*[a]	Hanasaki *et al.* (1988)
S-18886	Rabbit	Saphenous vein	U-46619	8.9	Cimetière *et al.* (1998)
(Terutroban)					
SQ-29548	Human	Astrocytoma cell/PI	STA_2	8.08	Nakahata *et al.* (1990)
		Immortalized ciliary epithelial cell/PI	U-46619	*7.7*[m]	Sharif *et al.* (2002)
		Corpus cavernosum	U-46619	9.0	Angulo *et al.* (2002)
		Umbilical artery	U-46619	*7.6*	Boersma *et al.* (1999)
		Umbilical vein	U-46619	*7.96*	Daray *et al.* (2003)
		Uterus (non-pregnant)	U-46619	8.2	Senchyna and Crankshaw (1996)
	Pig	Coronary artery	U-46619	8.8[a]	Kromer and Tippins (1996)
	Rabbit	Aorta	U-46619	*7.95*	Yoshida *et al.* (2007)
	Guinea pig	Aorta	U-46619	*7.96*	Dubé *et al.* (1992)
		Aorta	U-46619	8.9	Ogletree and Allen (1992)
		Aorta	U-46619	8.5	Zhang *et al.* (1996)
		Lung parenchyma	U-46619	7.7	Norman *et al.* (1992)
		Trachea	U-46619	8.70	Dubé *et al.* (1992)
		Trachea	U-46619	8.9	Ogletree and Allen (1992)
	Rat	Aorta	U-46619	9.2	Zhang *et al.* (1996)
		Lung parenchyma	U-46619	*7.2*	Norman *et al.* (1992)
SQ-30741	Human	Coronary artery	U-46619	7.54	Maassen VanDenBrink *et al.*, 1996)
	Human	Umbilical artery	U-46619	7.0	Boersma *et al.* (1999)
	Guinea pig	Aorta	U-46619	8.1	Ogletree and Allen (1992)
		Trachea	U-46619	8.6	Ogletree and Allen (1992)
	Rat	Aorta	U-46619	7.9	Ogletree and Allen (1992)
YM-158	Guinea pig	Trachea	U-46619	8.81[n]	Arakida *et al.* (1998)
Z-335	Human	Platelet membrane/binding	[^{3}H]-SQ-29548	*7.52*	Tanaka *et al.* (1998)
		Platelet/shape change[o]	U-46619	8.02	Yoshida *et al.* (2007)
	Rabbit	Aorta	U-46619	8.64	Yoshida *et al.* (2007)
ZD-1542[j]	Guinea pig	Lung parenchyma	U-46619	8.5	Brownlie *et al.* (1993)
		Trachea	U-46619	8.3	Brownlie *et al.* (1993)
	Rat	Aorta	U-46619	8.51	Brownlie *et al.* (1993)

pA_2 values relate to functional assays. Recombinant (rc-) systems: prostanoid receptor followed by the carrier cell line and second messenger measurement. Smooth muscle preparations: contraction or relaxation of induced tone. Platelets: all data from plasma-free platelet suspensions. Where pA_2 values are not available, pK_i/pK_D values derived from radioligand binding are given (italics). Affinity values in square brackets derive from the patent literature.
Structures of compounds: 1–5, Figure 1; 6–12, Figure 2; 13–15, Figure 3; 16–19, Figure 4; 20, Figure 5; 21–24, Figure 7.
BEAS, human bronchial epithelium; CHO, Chinese hamster ovary; COS-7, African green monkey kidney; HEK, human embryonic kidney; CRE, cAMP response element; 16,16-DM PGE_2, 16,16-dimethyl PGE_2; PI, phosphoinositide.
[a]Our calculation.
[b]Appreciable affinity for corresponding TP receptor.
[c]pIC_{50}.
[d]Insurmountable antagonism in functional DP_2 systems.
[e]1 μM SC-51322 present.
[f]Replacement for sulprostone, which had slow onset and offset.
[g]Possible interference by EP_2 system.
[h]pKi.
[i]pK_b for non-competitive antagonism.
[l]Combined TP antagonist/TXS inhibitor.
[k]Shape change seen.
[l]Data for (+)- and (–)-enantiomers in Kishino *et al.* (1991).
[m]Non-competitive antagonism.
[n]pA_2 = 8.87 for LTD_4 antagonism.
[o]Suppression of maximum aggregation response.

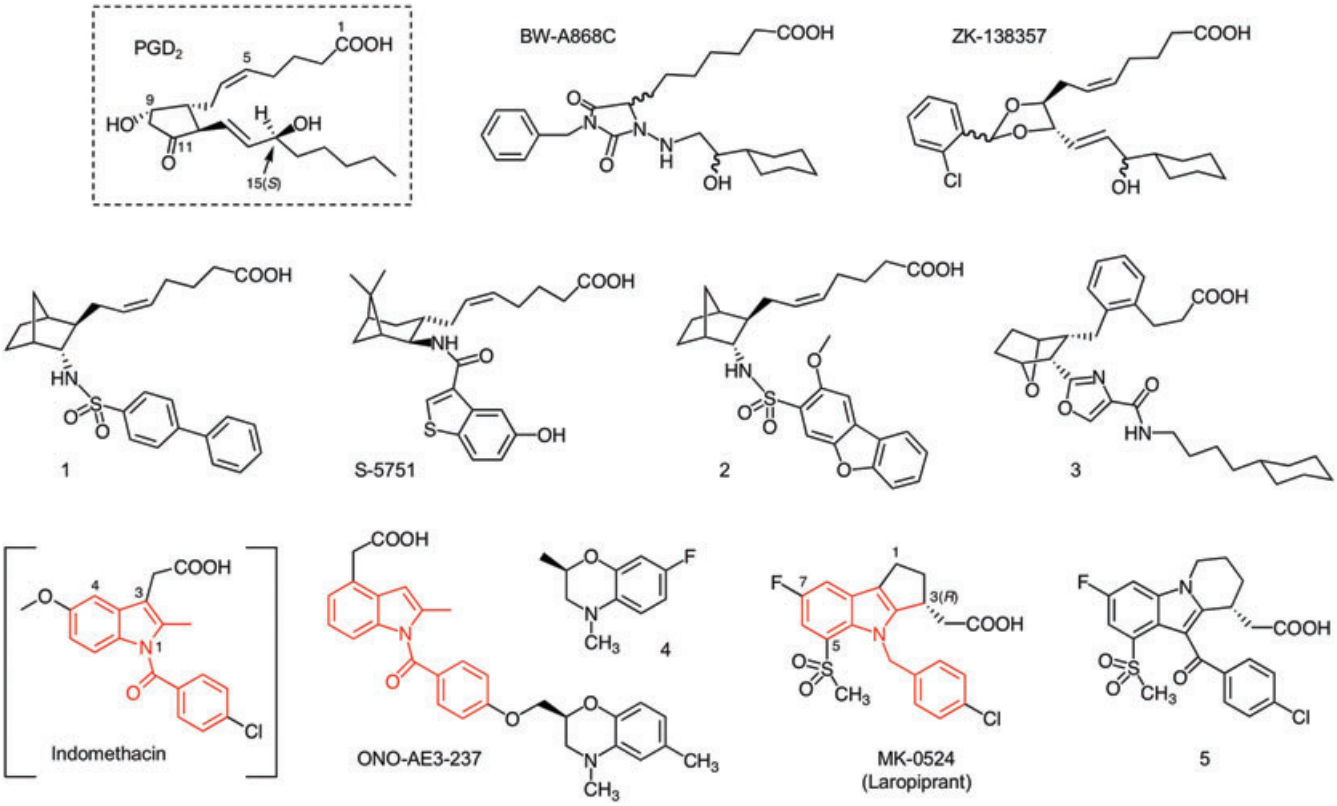

Figure 1 DP_1 receptor antagonists. The natural ligand PGD_2 is shown in the box; the *trans*-orientation of the α (upper) and ω (lower) side-chains and (*S*) configuration at C15 are found in primary products of all COX/synthase systems. BW-A868C and ZK-138357 are each composed of four diastereoisomers (chiral centres at C8/C15 and C10/C15 respectively); compound **1** is racemic. Indomethacin is a lead compound for the non-prostanoid antagonists shown in the lower row; *N*-benzoyl-2-methyl-indol-3-yl-acetic acid templates are shown in red. The 3(*S*)-enantiomer of MK-0524 has 320-fold lower affinity for the human DP_1 receptor (Sturino *et al.*, 2007).

several structural classes. The most important of these (Figure 1) and their therapeutic applications are described below.

Bicycloheptanes. Chemists in Shionogi have synthesized selective DP_1 antagonists containing a bicyclo[2.2.1]heptane ring system akin to that present in PGH_2 (see inset in Figure 8) (Tsuri *et al.*, 1997; Honma *et al.*, 1998; Mitsumori *et al.*, 2003a). An initial lead was the racemic compound **1** previously shown to be a TP antagonist (Narisada *et al.*, 1988). Subsequent structure–activity relationship (SAR) studies revealed that a 6,6-dimethylbicyclo[3.1.1]heptane (pinane) system could substitute for the bicycloheptane ring (Tsuri *et al.*, 1997; Mitsumori *et al.*, 2003b; Yoshikawa *et al.*, 2005) and carbonylamino or sulphonylamino linkages to the ω-aryl moiety were required for potent DP_1 antagonism (Tsuri *et al.*, 1997; Honma *et al.*, 1998; Mitsumori *et al.*, 2003b). Accordingly, S-5751 has high affinity for the DP_1 receptor ($pK_i = 8.8$) and is orally active in models of allergy and inflammation in the guinea pig (Tsuri *et al.*, 1997; Arimura *et al.*, 2001; Mitsumori *et al.*, 2003b; Yasui *et al.*, 2008). In addition, lead optimization of the (+)-isomer of compound **1**, which has much higher DP_1 selectivity than its mirror-image, led to the 1-methoxy-dibenzo[*b*,*d*]furan **2**. This antagonist is potent, orally bioavailable and efficacious in guinea pig models of conjunctivitis and allergen-induced bronchoconstriction (Mitsumori *et al.*, 2003a).

Allergan have also filed patents claiming DP_1 receptor antagonism based on a 1(*S*),4(*S*)-7-oxabicyclo[2.2.1]heptane scaffold (Krauss *et al.*, 2005). One of these, compound **3**, has a pA_2 of ~7.4 for the human rc-DP_1 receptor and represents a logical structure for lead optimization.

Indole acetic acids. A non-prostanoid exploited for DP_1 antagonism at Ono Pharmaceuticals is the cyclo-oxygenase (COX) inhibitor/non-steroidal anti-inflammatory drug (NSAID), indomethacin (Figure 1). Initial studies showed that the acetic acid moiety could be switched to position 4 on the indole ring (Torisu *et al.*, 2004a). Optimization led to the discovery of two benzoxazines, ONO-AE3-237 and compound **4**, with high DP_1 antagonist selectivity and (sub)nanomolar affinity (Torisu *et al.*, 2004b,c,d; Torisu *et al.*, 2005). Administered orally, both compounds effectively suppressed PGD_2- and allergen-induced vascular permeability in the guinea pig conjunctiva (Torisu *et al.*, 2004c). Pharmacokinetic studies on ONO-AE3-237 given by the oral (10 $mg \cdot kg^{-1}$) and intravenous (1 $mg \cdot kg^{-1}$) routes to fasted rats afforded plasma half-lives of 7.8 and 9.2 h respectively. The compound has a high volume of distribution indicating good tissue penetration and is 48% bioavailable at a dose of 10 $mg \cdot kg^{-1}$ p.o. (Torisu *et al.*, 2004c).

Merck Frosst (Wang *et al.*, 2002; Berthelette *et al.*, 2003) and Sanofi-Aventis (Yang *et al.*, 2008) also filed patents claiming DP_1 antagonists with further variation of the acetic acid position on the indole template. Screening of the Merck

compound collection identified a difluoro-indole that had low-nanomolar affinity for DP_1 and TP receptors (Sturino *et al.*, 2006) and this, and other analogues, were optimized resulting in two 7-methylsulphone derivatives with high DP_1 affinity (K_i ~2 nM) and at least 100-fold selectivity over other prostanoid receptors. However, both compounds displayed poor pharmacokinetics in the rat, in particular extensive biliary excretion (Sturino *et al.*, 2007). This liability was overcome when the 5-substituent and the 7-methylsulphone on the indole ring were replaced by methylsulphone and fluorine, respectively, to give MK-0542 (laropiprant; Figure 1). Laropiprant has very high affinity for the human rc-DP_1 receptor (pK_i = 10.5), with ~300-fold lower affinity for the corresponding TP receptor (Sturino *et al.*, 2007). Excellent pharmacokinetic profiles have been found in the rat, dog, monkey and man (Chang *et al.*, 2007; Karanam *et al.*, 2007; Sturino *et al.*, 2007; Lai *et al.*, 2008b). Given orally to healthy male volunteers, laropiprant at single doses up to 900 mg and multiple doses up to 450 mg is rapidly absorbed (T_{max} = 0.8–2 h), demonstrates dose-proportional systemic exposure, has a half-life of 12–18 h and is generally well tolerated; this pharmacokinetic profile is unaffected by food (Karanam *et al.*, 2007; Lai *et al.*, 2008b). At a dose of 6 mg, laropiprant was effective in antagonizing PGD_2-induced cAMP accumulation in human platelets *ex vivo*, indicating an interaction with the desired molecular target. Evidence for TP receptor blockade was also detected, but this effect was deemed not to be clinically relevant (Lai *et al.*, 2008b).

Merck Frosst has also disclosed 'backup' DP_1 antagonists, in which the indole template present in laropiprant is inverted. The tetrahydropyridoindole **5** (Figure 1) exhibited the best profile (pK_i = 9.0 and 6.8 at DP_1 and TP receptors respectively) and is considered a suitable candidate for development (Beaulieu *et al.*, 2008).

Other structural classes. Certain aminopyrimidines have also been claimed in the patent literature to be DP_1 antagonists but neither *in vitro* nor *in vivo* pharmacological data are yet available (Langevin *et al.*, 2007; Stefany *et al.*, 2007).

Therapeutic applications

PGD_2 is an established mediator of allergic disease. It is the major prostanoid released from mast cells (Lewis *et al.*, 1982; Peters *et al.*, 1982) and is also secreted, albeit in lower amounts, by T-lymphocytes of the Th2 subset (Tanaka *et al.*, 2000). In asthma, dermatitis and rhinitis, allergen challenge leads to the rapid production of PGD_2 (Naclerio *et al.*, 1983; Murray *et al.*, 1986; Charlesworth *et al.*, 1991) and PGD_2, itself, can reproduce many symptoms associated with allergic phenomena (see Pettipher, 2008). However, therapeutic/commercial success has not yet been attained with DP_1 antagonists probably because functional and subsequently molecular evidence emerged for a second subtype of PGD_2-sensitive receptor that is strongly implicated in several manifestations of allergic disease including eosinophil infiltration, mucus hyper-secretion and plasma extravasation (see next section).

In terms of allergic inflammation, activation of DP_1 receptors is known to mediate pathological changes in blood flow. In allergic rhinitis, vessels within the nasal mucosa become engorged leading to congestion and the release of plasma proteins, which contribute to enhanced nasal secretions. These effects are mimicked by PGD_2, which explains the limited efficacy of histamine H_1 antagonists in allergic rhinitis (see Pettipher, 2008). Sturino *et al.* (2007) have shown that laropiprant abolishes the marked increase in nasal airway resistance induced by intranasal instillation of PGD_2 in conscious sheep. Significantly, comparable data have also been obtained in 15 healthy, non-smoking male volunteers in whom laropiprant (25 mg or 100 mg q.d. for 3 days) significantly suppressed PGD_2-induced nasal congestion (Van Hecken *et al.*, 2007). Interestingly, PGD_2 fails to lower diastolic blood pressure (BP) in human volunteers (Heavey *et al.*, 1984) although (BW)-192C86, a DP_1 partial agonist (Gray *et al.*, 1992), is an effective depressor and BW-245C evokes adverse cardiovascular effects consistent with vasodilatation (Al Sinawi *et al.*, 1985). The reason for this discrepancy is unknown, but given that PGD_2 is a potent vasoconstrictor in several species (Jones, 1976; 1978) and the DP_2 receptor is expressed in the aorta (Nagata and Hirai, 2003) and potentially other blood vessels, its activation may oppose the BP-lowering activity of PGD_2 acting via the DP_1 receptor.

The ability of PGD_2 to promote adverse vasodilatation is also associated with the therapeutic use of niacin (vitamin B3), which, in high doses, is used clinically to lower plasma cholesterol. Niacin may also have anti-oxidant and anti-inflammatory activity and is used, in conjunction with statins, to treat dyslipidaemia (see Kamanna *et al.*, 2008). The adverse effects of niacin, particularly flushing, are due to receptor (GPR109A)-mediated release from Langherhans' cells of PGD_2 and PGE_2, which promote vasodilatation of dermal and cerebrovascular capillaries though activation of DP_1, EP_2 and EP_4 receptors. Merck (Paolini *et al.*, 2008) and Sanofi-Aventis (Harris, 2008) have claimed that a DP_1 antagonist could limit the cardiovascular liability of niacin if given as a combination therapy, thereby providing a better-tolerated drug. Indeed, laropiprant significantly reduced niacin-induced flushing in normal and dyslipidaemic subjects when compared with niacin alone (Cheng *et al.*, 2006; Lai *et al.*, 2007; Paolini *et al.*, 2008). Accordingly, these and other findings led Merck to develop the investigational combination product *Cordaptive*, which significantly reduced the vascular side effects of niacin in patients with primary hypercholesterolaemia and mixed dyslipidaemia (see Kamanna *et al.*, 2008). However, in April 2008 the US Food and Drug administration did not approve Merck's application to market *Cordaptive* (see http://www.merck.com/newsroom/press_releases/research_and_development/2008_0428.html), and at the same time, rejected the name of the combination therapy. The US Food and Drug administration's decision not to approve *Cordaptive*, now renamed *Tredaptive*, is unclear given that the European Medicines Agency has approved this new extended-release combination therapy (see http://www.emea.europa.eu/humandocs/Humans/EPAR/tredaptive/tredaptive.htm). One possibility is that the beneficial effects of laropiprant are not superior to aspirin (Kamanna *et al.*, 2008) and the risk/benefit ratio is a primary consideration when the long-term effects of a new drug class are unknown.

DP_1 antagonists may also have utility in the treatment of allergic asthma, although, currently, this is controversial (see

Pettipher, 2008). Persuasive evidence is available that activated mast cells, through their ability to generate PGD_2, promote T-lymphocyte polarization towards a Th2 phenotype (Faith *et al.*, 2005; Hammad and Lambrecht, 2008). Mechanistically, it is believed that mast cell-derived PGD_2 activates DP_1 receptors on dendritic cells within the respiratory mucosa. This interaction then suppresses the generation of interleukin (IL)-12 (which normally favours a Th1 cell bias) leading to Th2 dominance (Kitawaki *et al.*, 2006; Theiner *et al.*, 2006). A cycle of chronic immunological activation then ensues through further production of IgE and mast cell activation. Indeed, these data are consistent with allergic airway responses being less intense in DP_1 receptor-deficient mice when compared with wild-type animals (Matsuoka *et al.*, 2000). However, the role of DP_1 receptors in regulating allergic reactions is complex. For example, the administration to wild-type mice of the DP_1 agonist, BW-245C, paradoxically *reduced* pulmonary allergic responses whereas DP_1 receptor null mice were unaffected (Hammad *et al.*, 2007). To explain this apparently contradictory result it has been proposed that DP_1 receptor-mediated inhibition of IL-12 release from dendritic cells during host sensitization promotes Th2 polarization (i.e. is pro-inflammatory). In contrast, during maintained airway inflammation, PGD_2 by suppressing dendritic cell function reduces Th2 cell function (i.e. is anti-inflammatory). Indeed, the DP_1 antagonist, S-5751, *attenuated* rather than exacerbated allergen-induced inflammation in sensitized guinea pigs (Arimura *et al.*, 2001). Thus, the harmful/protective actions of PGD_2 may depend on when and where it is produced (Pettipher, 2008).

DP_2 receptor antagonists

Development

Studies conducted in the 1970s showed that PGD_2 elicited peripheral vasoconstrictor responses that were unlikely to be due to activation of, what we now understand to be, DP_1, FP or TP receptors (Jones, 1976; 1978). In particular, 15-oxo PGD analogues (Jones and Wilson, 1978) had unexpectedly high agonist potency. Moreover, evidence for DP receptor heterogeneity was provided in 1985 by the finding that PGD_2 and six related analogues evoked functional responses across a variety of PGD_2-sensitive systems that were not mimicked by the selective DP agonist, BW-245C (Narumiya and Toda, 1985). The results of several other studies also indicated the existence of multiple DP receptors (Woodward *et al.*, 1990a; 1993b; Rangachari and Betti, 1993; Fernandes and Crankshaw, 1995; Rangachari *et al.*, 1995). However, it was not until 1999 that the idea of multiple DP-receptors really began to gain general acceptance. Nagata *et al.* (1999b) identified a novel molecule expressed on the surface of minor populations

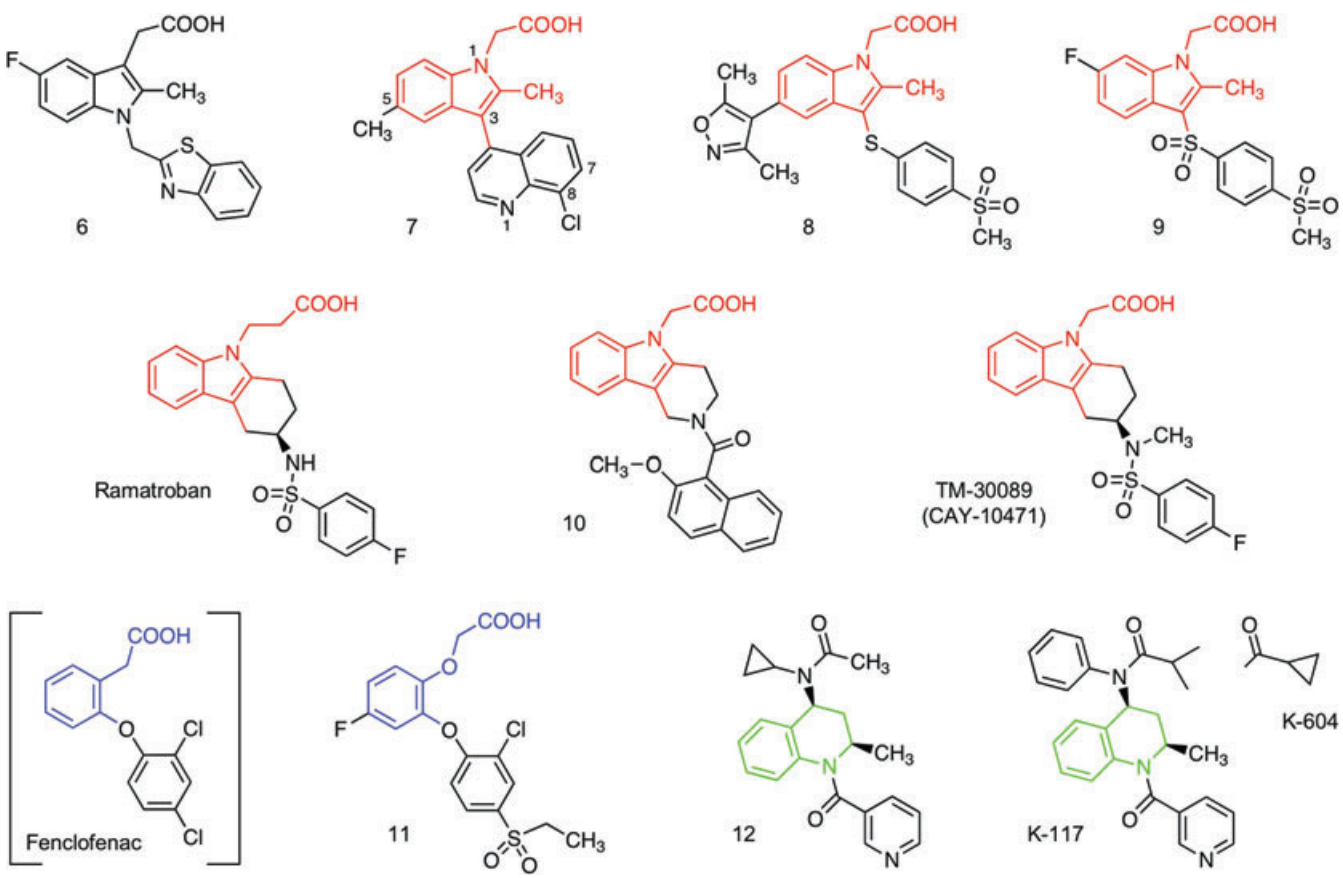

Figure 2 DP_2 receptor antagonists. *Inverted* 2-methyl-indole-acetic acid residues (compare with **6**) are highlighted in red; ramatroban has an extra methylene (C2a). The phenylacetic acid moiety is shown in blue in fenclofenac, a lead molecule for compound **11**. Compound **12**, K-117 and K-604 contain a tetrahydroquinoline residue (green).

of CD4+ T-lymphocytes, which resembled activated Th2 cells in that they released IL-4, IL-5 and/or IL-13 but not interferon-γ on stimulation. This orphan site was named 'chemoattractant receptor-homologous molecule expressed on Th2 cells', or CRTh2, as primary sequence analysis showed significant amino acid homology to receptors that mediate chemoattraction (Hirai *et al.*, 2001). Indeed, despite the ability of PGD_2 to interact with the DP_1 subtype and CRTh2 with comparably high affinity (K_i = 45 and 61 nM respectively), the two cognate receptors are quite dissimilar at the amino acid level (Hirai *et al.*, 2001). A follow-up study by the same investigators discovered a mast cell-derived factor that could increase the cytosolic free Ca^{2+} concentration in CRTh2-expressing cells (Nagata *et al.*, 1999a), which was identified as PGD_2 (Hirai *et al.*, 2001). This latter finding coincided with the publication of a pharmacological study in which Monneret *et al.* (2001) found that PGD_2 was chemotactic for eosinophils and also up-regulated the expression of CD11b and L-selectin by a mechanism that was neither mimicked by BW-245C nor blocked by the DP_1 antagonist, BW-A868C. Thus, a second DP receptor subtype (DP_2, *aka* CD294) was identified on Th2 cells and eosinophils that mediated responses diametrically opposite to the inhibitory effects classically associated with DP_1 agonism. Given that CRTh2 is now known to be ubiquitously expressed within (Nagata *et al.*, 1999a) and outwith (Hirai *et al.*, 2001; Nagata and Hirai, 2003; Kostenis and Ulven, 2006; Kim and Luster, 2007) the immune system (Sawyer *et al.*, 2002; Nagata and Hirai, 2003), the term 'DP_2' is a more appropriate designation and is used throughout this review.

The ability of PGD_2 to act as a chemoattractant for pro-inflammatory cells and to release Th2-like cytokines has resulted in a concerted effort by the pharmaceutical industry to synthesize selective DP_2 antagonists. Indeed, such compounds may be useful in suppressing a myriad of Th2-driven inflammatory pathologies including asthma, otitis, contact dermatitis and rhinitis. At the time of writing, in excess of 90 patents had been filed claiming selective DP_2 antagonists. In the sections below the main structural classes are described and affinity estimates of lead antagonists (Figure 2) are given in Table 2.

Indole acetic acids. In addition to providing a scaffold for the development of DP_1 antagonists, indomethacin (Figure 1) is also a selective, albeit weak, DP_2 agonist (Hirai *et al.*, 2002; Stubbs *et al.*, 2002). Exploiting this property, Pfizer first reported a benzothiazole derivative **6** that had a pA_2 of 6.8 and was ~40-fold selective for the DP_2 receptor (Bauer *et al.*, 2002). Subsequently, several patents describing highly potent and selective DP_2 antagonists were filed by AstraZeneca (Baxter *et al.*, 2003a,b; Birkinshaw *et al.*, 2003; Bonnert *et al.*, 2003; 2004; 2005a,b,c; Bonnert and Rasul, 2004). An initial hit was a 7-chloroquinoline derivative of indomethacin (pA_2 ~7 for human rc-DP_2 receptor), which also inhibited COX-1 with high potency (Birkinshaw *et al.*, 2006). Inversion of the indole template (Figure 2) and substitution of the 5-methoxy moiety by methyl increased antagonist potency by 23-fold and reduced COX-1 inhibition by a factor of 10 (Birkinshaw *et al.*, 2006). Addition of chlorine at position 8 of the quinoline to give **7** also increased DP_2 affinity by 13-fold (IC_{50} for inhibition of [^{3}H]-PGD_2 binding = 2.3 nM). This derivative has a preferred biological profile with relatively weak binding to plasma proteins and good bioavailability in rats (76%) and dogs (100%), with half-lives of 1.7 and 5.3 h respectively (Birkinshaw *et al.*, 2006). Compounds having an arylthio substituent on position 3 of the indole nucleus also display very potent antagonism at the DP_2 receptor; compound **8** has a binding IC_{50} of 0.4 nM (Bonnert and Rasul, 2004).

Related 1-acetic acid derivatives from Oxagen containing methylene or sulphonyl spacers between the aromatic moieties have been reported as potent DP_2 antagonists (Middlemiss *et al.*, 2005a,b,c,d; Armer *et al.*, 2006; Lovell, 2007), including compound **9**, which has a K_i of 68 nM and a DP_2/DP_1-selectivity ratio of approximately 150. Functionally, **9** potently inhibits DP_2 receptor-mediated human eosinophil shape change and Th2 cell chemotaxis with IC_{50} values of 74 and 67 nM respectively (Armer *et al.*, 2005). Moreover, this compound is metabolically stable, has no inhibitory effect on five of the major cytochrome P450 enzymes (1A2, 2C19, 2C9, 2D6, 3A4) and fails to induce CYP3A4, CYP1A and CYP2C9. In rats, **9** is 56% bioavailable and has a half-life of 5.5 h following oral administration. Oxagen has reported the development of a lead compound, ODC9101 (*aka* OC459), which is in Phase IIa clinical trials for asthma. According to the company's website, ODC9101 has completed safety evaluations, is orally active and suitable for once-a-day dosing. Oxagen has also reported the development of a pre-clinical back-up molecule, OC499, and DP_2 antagonists for non-oral delivery (OC1768) and topical administration (OC2125, OC2184; see http://www.oxagen.co.uk/pdfs/CRTH2summary.pdf). The structures of these compounds have not been disclosed.

Athersys has also described a series of indole acetic acid derivatives with potent DP_2 antagonist activity (Bennani *et al.*, 2006) including substituted 3-benzylphthalazin-1(2*H*)-ones which have radioligand binding IC_{50} values in the low nanomolar range; their development status is unknown.

Ramatroban and analogues. Ramatroban (Bay u3405) was originally described as a TP antagonist with a pA_2 of ~8.8 on human tissues (McKenniff *et al.*, 1991). Later studies revealed DP_2 antagonism (Sugimoto *et al.*, 2003), albeit of lower affinity (pA_2 = 7.44; Mathiesen *et al.*, 2006). The structural similarity of ramatroban to compounds **7–9** is clear. These observations were the impetus for the synthesis of compounds with increased selectivity for the DP_2 receptor (Arimura *et al.*, 2003). An example is **10**, in which the amide nitrogen atom is included in the tricyclic system. This compound has high affinity for the DP_2 receptor (pA_2 = 8.53) with significantly reduced TP receptor-blocking activity (Fretz *et al.*, 2005).

Other minor changes to ramatroban also resulted in compounds with a high degree of DP_2 selectivity. 7TM Pharma reported that *N*-methylating the sulphonamide or truncating the propionate moiety to acetate produces very selective (>1000-fold over DP_1 and TP), high-affinity DP_2 antagonists with K_i values of 1.9 (TM-30642) and 0.51 nM (TM-30643) respectively (Ulven and Kostenis, 2005). Furthermore, making both modifications to produce TM-30089 (*aka* CAY-10471)

preserved the DP_2 affinity of TM-30643 and further increased the DP_2/TP-selectivity ratio to >10 000 (Ulven and Kostenis, 2005). Interestingly, in functional studies (e.g. [^{35}S]-GTPγS binding/inositol phosphate accumulation; PGD_2-induced eosinophil shape change), ramatroban and TM-30642 are surmountable competitive antagonists whereas TM 30643 and TM 30089 suppress the maximal response in a concentration-dependent manner (Mathiesen *et al.*, 2006). The insurmountable behaviour of TM-30089 and TM-30643 may be due to its slow dissociation from the DP_2 receptor, which also results in long-lasting antagonism (Mathiesen *et al.*, 2006). Whether such pharmacological behaviour occurs *in vivo* and would be therapeutically advantageous is currently unclear. However, this could be a desirable property as slowly dissociating drugs should act much longer than would be predicted from their plasma half-lives (Mathiesen *et al.*, 2006). 7TM Pharma, in partnership with Ortho-McNeil-Janssen Pharmaceuticals, has a compound in late-stage lead optimization although neither the structure nor the profile of the antagonist has been disclosed (see http://www.7tm.com/News.aspx?M=News&PID=42&NewsID=39).

Phenyl acetic acids. Another NSAID, fenclofenac (Figure 2), provided the starting point for the synthesis of DP_2 antagonists based on a phenylacetic acid template. An initial hit claimed in the original patent filed by Pfizer (Bauer *et al.*, 2002) had a 4-chlorophenylthio substituent resulting in a functional DP_2/DP_1-selectivity ratio of 40. Several other companies, including AstraZeneca, have since filed patents for *bis*-ether derivatives such as **11**, which has a binding pIC_{50} of 9.0 (Bonnert *et al.*, 2005c).

Tetrahydroquinolines. Millennium and Warner-Lambert (now Pfizer) were the first to disclose DP_2 antagonists within the tetrahydroquinoline class (Awad *et al.*, 2004; Ghosh *et al.*, 2004; 2005; Kuhn *et al.*, 2004). These compounds are unique in that they are non-acidic indicating that a carboxylic acid moiety is not essential for DP_2 antagonism, as previously assumed (see Pettipher *et al.*, 2007). The 4-amino-tetrahydroquinoline **12** (Figure 2) is reported to gain access to the cerebrospinal fluid after oral dosing and also is efficacious in animal models of inflammation at an oral dose of 25 $mg \cdot kg^{-1}$ (Corradini *et al.*, 2005). However, at the time of writing detailed pharmacological data on non-acidic DP_2 antagonists is sparse. Researchers at Kyowa Hakko Kogyo have reported K_i values of 5.5 and 11 nM for K-117 and K-604 respectively, with minimal interaction with TP or DP_1 receptors at concentrations up to 1 μM (Mimura *et al.*, 2005).

Therapeutic applications

Arguably, allergic inflammation is the primary indication for antagonists that selectively block the DP_2 receptor. Indeed, the gene encoding this receptor shows a particularly strong association with asthma in Chinese and African-American populations (Huang *et al.*, 2004). Moreover, there is good evidence from *in vitro* and *in vivo* studies in laboratory animals that PGD_2, acting via the DP_2 receptor, can mediate many of the cardinal features of allergic airways inflammation (see Ulven and Kostenis, 2006; Pettipher, 2008 for detailed reviews). The most important observations that have led to this view can be summarized as follows:

1. PGD_2 and selective DP_2 agonists promote chemotaxis of eosinophils, basophils and CD4+ T-lymphocytes of the Th2 subset and this effect is abolished by a neutralizing anti-DP_2 receptor antibody (Hirai *et al.*, 2001; Monneret *et al.*, 2001).
2. PGD_2 promotes pulmonary eosinophilia in rats; this effect is mimicked by selective DP_2, but not DP_1, agonists and is abolished by ramatroban (Almishri *et al.*, 2005; Shiraishi *et al.*, 2005).
3. In guinea pigs, the DP_2 agonist, Δ^{12}-PGJ_2, mobilizes eosinophils from the bone marrow (Heinemann *et al.*, 2003).
4. The DP_2 agonist, 13,14-dihydro-15-oxo PGD_2, promotes pulmonary eosinophilia and exacerbates histopathology in a murine model of allergic asthma (Spik *et al.*, 2005).
5. Ramatroban and DP_2 antagonists *devoid* of TP receptor-blocking activity reduce pulmonary eosinophilia in several animal species in response to allergen challenge (Nagai *et al.*, 1995; Uller *et al.*, 2007; Pettipher, 2008).
6. PGD_2 promotes the production of Th2 cytokines *in vivo* including IL-4, IL-5 and IL-13 (Fujitani *et al.*, 2002) and *in vitro*, this can occur in the absence of allergen or co-stimulatory molecules (Xue *et al.*, 2005).
7. The expression of the DP_2 receptor on eosinophils is up-regulated in atopic individuals (see Kostenis and Ulven, 2006).
8. High concentrations of PGD_2 are present in the airways of asthmatic subjects after antigen challenge (Murray *et al.*, 1986).
9. The expression of the DP_2 receptor on Th2 T-lymphocytes is up-regulated in individuals sensitized to pollen or house dust mite or who have atopic dermatitis (Iwasaki *et al.*, 2002).

The proof of this line of argument is the marketing of ramatroban in Japan under the trade name *Baynas* for the treatment of perennial allergic rhinitis; its clinical efficacy (e.g. reduction of symptoms and of chronic nasal swelling) has been attributed to DP_2 receptor blockade (Terada *et al.*, 1998).

In addition to anti-allergic indications, DP_2 antagonists may have utility in combating neuropathic pain (Corradini *et al.*, 2005) where the up-regulation of COX-2 and the subsequent formation of PGs are central to disease pathophysiology (Camu *et al.*, 2003).

Hybrid DP_1/DP_2 receptor antagonists

In considering the SAR data described in the preceding sections, indole acetic acids may be a fruitful starting point for antagonists that block both DP_1 and DP_2 receptors. Indeed, in the context of allergic diseases, a hybrid antagonist may exert clinically relevant, beneficial effects that are not achieved when just one DP receptor is targeted. Thus, blockade of the DP_1 subtype would prevent PGD_2 from inhibiting the generation of IL-12 from dendritic cells, thereby inhibiting the polarization of T-lymphocytes to a Th2 phenotype that occurs during host sensitization. Antagonism of the DP_2 receptor

would, at the same time, suppress the chemotactic activity of PGD_2 towards eosinophils, basophils and T-lymphocytes and so reduce pulmonary leukocyte burden and inflammatory status. Theoretically, this latter action would overcome any DP_1 receptor-mediated anti-inflammatory effect of PGD_2 on dendritic cells during maintained inflammation (Pettipher, 2008). It is also tempting to speculate that a non-selective DP antagonist that also has TP receptor-blocking activity could be efficacious in allergic asthma. In this scenario, TP receptor blockade would reduce the ability of PGD_2 to induce bronchoconstriction, which in humans is mediated through the TP receptor (Beasley *et al.*, 1989). Again, indole acetic acids and ramatroban analogues may provide good templates for optimization.

EP_1 receptor antagonists

Development

Figure 3 shows the commonly used EP_1 antagonists; while some may be considered as prostanoids, none is structurally close to PGE_2. The first EP_1 antagonist was SC-19220, a dibenzoxazepine hydrazide (Sanner, 1969). Although of low affinity ($pA_2 = 5.5$), SC-19220 proved useful in the early characterization and elucidation of the roles of EP receptors. For example, on guinea pig trachea, 3–50 μM SC-19220 (and indomethacin) suppressed inherent tone, indicating that PGE_2 acting via EP_1 receptors is the likely mediator (Farmer *et al.*, 1974). Also, SC-19220 at 10 μM equally inhibited matching contractions of guinea pig trachea induced by 16,16-dimethyl PGE_2, iloprost and isocarbacyclin, but had no effect on contractions induced by U-46619 or histamine; these results demonstrated that certain PGI_2 analogues could potently activate EP_1 receptors (Dong *et al.*, 1986).

SC-19220 (7.5–30 $mg \cdot kg^{-1}$ i.p.) inhibited carrageenan-induced inflammation in the rat without suppressing PGE_2 levels at the injury site (Barbieri *et al.*, 1977). These and other observations were the stimulus for the synthesis of higher-affinity EP_1 antagonists with potential as anti-inflammatory/analgesic drugs in man; alteration of the acetyl group was the most rewarding strategy. SC-51089 was unusual in the series in releasing hydrazine and was dropped from development (Hallinan *et al.*, 1993). The thioether SC-51322, which is much more potent than the corresponding sulphone (Hallinan *et al.*,

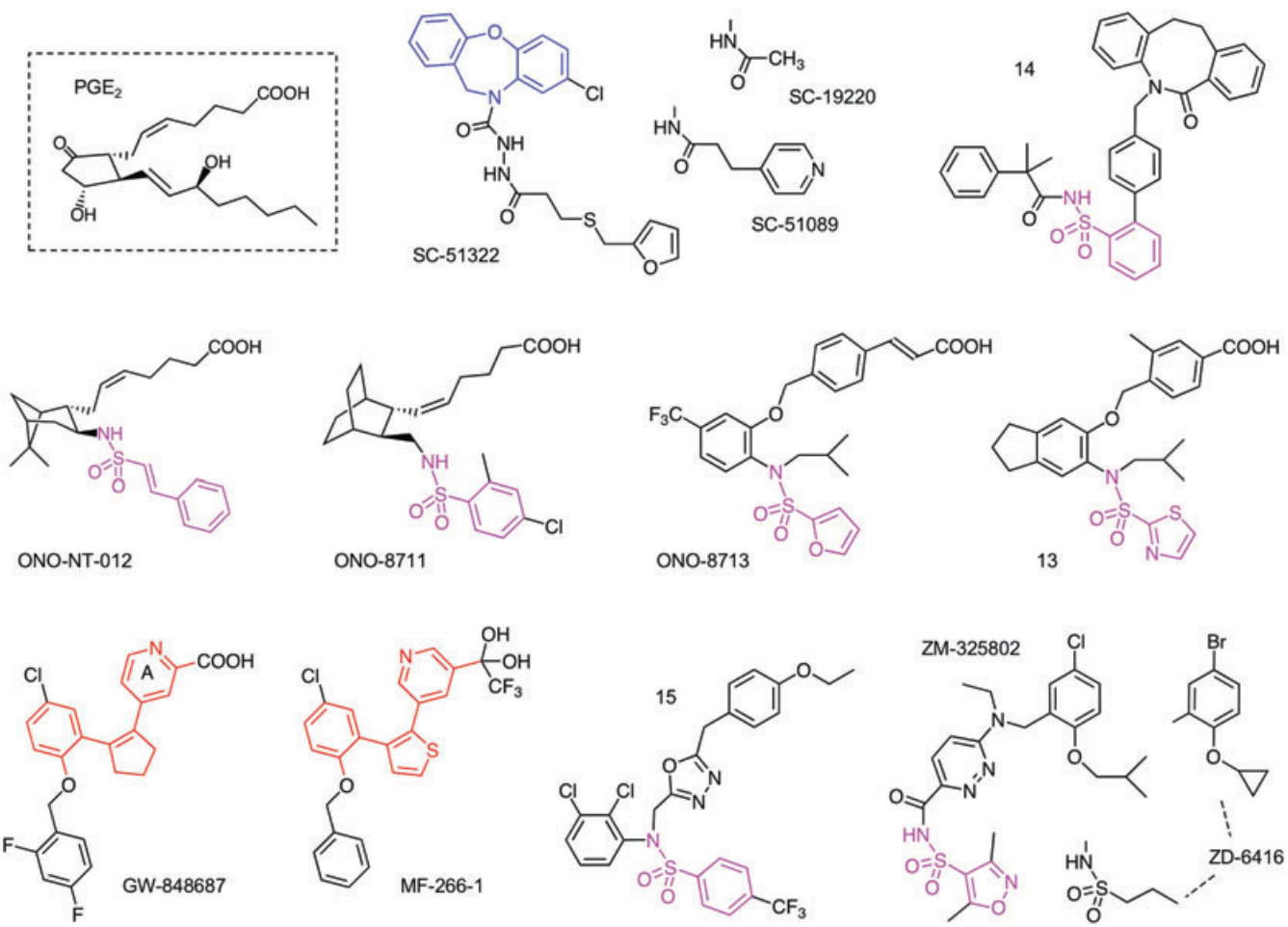

Figure 3 EP_1 receptor antagonists. The natural ligand PGE_2 is shown in the box. The dibenzoxazepine residue in SC-51322 is shown in blue. Aryl-sulphonamido residues in antagonists with prostanoid and non-prostanoid structures are shown in cerise; ONO-NT-012 contains a styryl-sulphonamido moiety. The 1,2-biaryl-cyclopentene pharmacophores in GW-848687 and MF-266-1 are shown in red. Ring A in GW-848687 is part of a picolinic acid (pyridine-2-carboxylic acid) residue.

1994), has become the agent of choice for receptor characterization. It behaved competitively over the range 25–625 nM in a human rc-EP_1 receptor – reporter gene assay (pA_2 = 8.8; Schild plot slope = 0.91; Durocher *et al.* (2000). In rat hepatocytes, SC-51322 at 100 nM abolished DNA synthesis induced by PGE_2 or 17-phenyl PGE_2 (Table 1), while 1 μM SC-51322 did not affect responses to either $PGF_{2\alpha}$ or PGI_2; the selective involvement of EP_1 receptors in the PGE response is clear (Kimura *et al.*, 2000; 2001). However, not all inferences about EP_1 receptor involvement are as secure. In a study on rat progenitor Leydig cells, 3 and 30 μM SC-51322 suppressed IL-1β expression induced by 10 μM 17-phenyl PGE_2 by 28% and 59% respectively (Walch *et al.*, 2003). Given that this system responded well to 100 nM cloprostenol, a potent FP agonist (Table 1), the action of 17-phenyl PGE_2 and its inhibition by SC-51322 may also have involved FP receptors. SC-51322 at 10 μM did not inhibit PGE_2-mediated inhibition of superoxide generation in human blood neutrophils (EP_2 system) (Kanamori *et al.*, 1997) or inhibit sulprostone-induced contraction of rat femoral artery (EP_3 system) at 1 μM (Hung *et al.*, 2006).

Another widely used EP_1 antagonist is AH-6809. At 0.1–10 μM, it blocked EP_1-mediated actions of PGE_2, 16,16-dimethyl PGE_2 or 17-phenyl PGE_2 in an apparently competitive manner (pA_2 = 7.4) (Coleman *et al.*, 1987; Eglen and Whiting, 1988; Lawrence *et al.*, 1992). It did not block EP_3 receptor-mediated contraction of guinea pig ileum (Lawrence *et al.*, 1992) and human pulmonary artery (Qian *et al.*, 1994) at 2 and 5 μM respectively and had no effect on the presynaptic EP_3 action of sulprostone on rat trachea at 3 μM (Racké *et al.*, 1992). However, AH-6809 blocks EP_2 receptors (see later) as well as DP_1 and TP receptors in human-washed platelets (Keery and Lumley, 1988) with pA_2 values of ~6.3 and ~5.9 respectively (our calculation). Of further concern is the inhibitory effect of AH-6809 (3–10 μM) on platelet-activating factor (PAF)- and ADP-induced aggregation, which was attributed to inhibition of phosphodiesterase(s) (Keery and Lumley, 1988). In our hands, AH-6809 at 3–10 μM caused similar partial block of the contractile actions of phenylephrine (α_1), histamine (H_1), U-46619 (TP) and ONO-AE-248 (EP_3) on guinea pig aorta (R.L. Jones *et al.*, 2009, submitted). AH-6809 has often been used at even higher concentrations, possibly because of its high water solubility. For example, 30–300 μM AH-6809 inhibited the contractile action of PGE_2 in pig large cerebral artery; EP_1 receptor involvement was inferred (Jadhav *et al.*, 2004). However, the pA_2 corresponding to 30 μM AH-6809 is only 5.2 (our calculation) and the block was insurmountable at the higher concentrations. With more potent and selective EP_1 antagonists now available, it is time to relegate AH-6809 to its place in the historical development of prostanoid antagonists.

The EP_1 antagonists developed by Ono Pharmaceuticals (Figure 3, middle row) demonstrate an interesting progression from the TP antagonist ONO-11120 (see Figure 8; Katsura *et al.*, 1983) to a related pinane analogue (ONO-NT-012) showing EP_1, FP and TP antagonism (and EP_3 agonism), to a bicyclo[2.2.2]octane analogue (ONO-8711) showing EP_1/EP_3 antagonism, and, finally, to the non-prostanoids ONO-8713 and compound **13** with high selectivity for the EP_1 receptor. ONO-8711, ONO-8713 and **13** have K_D values for mouse rc-EP_1 receptors of 1.7, 0.3 and 0.14 nM respectively (Watanabe *et al.*, 1999; 2000; Naganawa *et al.*, 2006). Small modifications to **13** can restore EP_3 antagonist affinity. In rat-cultured mesangial cells, ONO-8713 at 1 μM abolished induction of the transforming growth factor-β-fibronectin cascade elicited by PGE_2 under high-glucose conditions; a COX-2-PGE_2-EP_1 receptor drive was postulated to contribute to deleterious changes in diabetes (Makino *et al.*, 2002). Ohnishi *et al.* (2001) showed that 10 μM ONO-8713 partially inhibited PGE_2-induced exocytosis in mucous cells from guinea pig antrum (IC_{50} ~1 μM). However, 17-phenyl PGE_2 was a very weak agonist. The authors postulated that EP_1 and EP_4 receptors co-operate to sustain the high exocytotic response to PGE_2. Norel *et al.* (2004) also showed that 10 μM ONO-8713 partially suppressed the contractile action of sulprostone on human pulmonary vein; functional EP_1 receptors were postulated, even though the selective EP_1 agonist ONO-DI-004 was a very weak agonist. Prostanoid receptor binding data alone do not guarantee functional selectivity at these high antagonist concentrations; inclusion of control agonists (both prostanoid and non-prostanoid) in the system under test is essential.

Other pharmacophores for potent EP_1 antagonism, with inactivity against cytochrome P450 enzymes and good penetration into the central nervous system being secondary goals (see later), have emerged within the last 10 years. The series reported by Merck (Ruel *et al.*, 1999) contains a tricyclic system akin to that in the Searle series. Again EP_1/EP_3 selectivity can be readily modulated, as shown by replacement of the terminal phenyl group in compound **14** (K_i values for human EP_1 and EP_3 receptors = 10 and 4000 nM) by methyl (770 and 1000 nM). EP_1 antagonists with aryl groups attached to adjacent carbons of a 5-membered ring (cyclopentene, thiophene, pyrrole) have been reported by research groups at GlaxoSmithKline and Merck Frosst. GW-848687 has nanomolar affinity for the human EP_1 receptor, 30-fold lower affinity for the human TP receptor and >400 times lower affinity for other prostanoid receptors (Giblin *et al.*, 2007). The picolinic acid residue in GW-848687 (ring A) is highly acidic (p*K*a ~1.0) and there has been considerable SAR work by both research groups on modulating the acidity of this region. The Merck antagonist MF-266-1, with a *m*-$C(CF_3)$-$(OH)_2$ substituent (Figure 3), is a weak acid (p*K*a = 7.5 for first ionization) that retains high EP_1 affinity (Ducharme *et al.*, 2005; Clark *et al.*, 2008). A *m*-$C(CF_3)_2$-OH substituent in the Merck series and a *p*-$C(CF_3)_2$-OH substituent in the GlaxoSmithKline series resulted in lower EP_1 affinity (Ducharme *et al.*, 2005; Hall *et al.*, 2007a). Further modifications to ring A (e.g. *m*-NH(C=O)CH_2Ph/*p*-(C=O)NHCH(CH_3)Ph substituents) resulted in non-acidic relatives with high EP_1 potency. Compound **15** is also a non-acidic EP_1 antagonist (Hall *et al.*, 2007b).

Therapeutic applications

The upper portion of Table 3 shows that parenteral administration of EP_1 antagonists of different chemical classes suppressed the allodynic/hyperalgesic signs of inflammation in the rat and mouse. Intraplantar injection of these antagonists also opposed pain-producing stimuli, although at the mouse EP_1 receptor AH-6809 had minimal affinity, which suggests an off-target effect. That said, Khasar *et al.* (1993) showed that PGE_2 and SC-19220 are only mutually antagonistic when they

Table 3 Anti-nociceptive and anti-inflammatory activities of EP receptor antagonists

Route/species	*Experimental model*	*Noxious stimulus/site*	*Antagonist (nominal receptor)*	*Antagonist dosing*	*Efficacy*	*Reference*
Oral						
Guinea pig	Joint pain (mono-osteoarthritis)	Iodoacetate/shoulder joint	MF-498 (EP_4)	30 mg·kg^{-1}	+++	Clark *et al.* (2008)
	Thermal hyperalgesia	L-902688[a]/paw	MF-498 (EP_4)	0.1–30 mg·kg^{-1}	+++	Clark *et al.* (2008)
Rat	Writhing response	Acetic acid/peritoneal cavity	SC-19220 (EP_1)	50–300 mg·kg^{-1}	++	Drower *et al.* (1987)
	Behavioural response	Formalin/paw	SC-19220 (EP_1)	50–150 mg·kg^{-1}	++	Drower *et al.* (1987)
	Chronic mechanical/thermal hyperalgesia/allodynia	Nerve constriction/sciatic	ONO-8711 (EP_1)	10–100 mg·kg^{-1} per day	+++	Kawahara *et al.* (2001)
	Chronic hyperalgesia (weight-bearing)	Freund's adjuvant/knee joint	GW-848687 (EP_1)	30 mg·kg^{-1} per day	+++	Giblin *et al.* (2007)
	Chronic arthritis (oedema/radiology)	Freund's adjuvant/paw	Compound **20** (EP_4)	0.005 mg·kg^{-1} per day	+++	Burch *et al.* (2008)
	Local oedema	Freund's adjuvant/paw	MF-266-1 (EP_1) MF-266-3 (EP_3) MF-498 (EP_4)	Each 0.008–20 mg·kg^{-1} daily for 10 days	0 0 +++	Clark *et al.* (2008)
	Mechanical hyperalgesia	Carrageenan/paw	CJ-042794 (EP_4)	1–30 mg·kg^{-1}	+++	Murase *et al.* (2008a)
	Local oedema	Freund's adjuvant/paw	CJ-042794 (EP_4)	1–30 mg·kg^{-1}	+++	Murase *et al.* (2008a)
	Mechanical hyperalgesia	Carrageenan/paw	CJ-023423 (EP_4)	3–100 mg·kg^{-1}	+++	Nakao *et al.* (2007)
	Thermal hyperalgesia	PGE_2/paw	CJ-023423 (EP_4)	1–29 mg·kg^{-1}	+++	Nakao *et al.* (2007)
	Chronic inflammation (weight-bearing)	Freund's adjuvant/paw	CJ-023423 (EP_4)	10–57 mg·kg^{-1}	+++	Nakao *et al.* (2007)
	Chronic inflammation (oedema, histology)	Freund's adjuvant/paw	CJ-023423 (EP_4)	29–96 mg·kg^{-1}	+++	Okumura *et al.* (2008)
Mouse	Writhing response	Phenylbenzylquinone/ peritoneal cavity	SC-51089 (EP_1)	1–30 mg·kg^{-1}	+++	Hallinan *et al.* (1993)
	Writhing response	Phenylbenzylquinone/ peritoneal cavity	SC-51322 (EP_1)	1–30 mg·kg^{-1}	+++	Hallinan *et al.* (1994)
	Migration of Langerhans cells into lymph node	Fluorescein isothiocyanate/ skin	ONO-AE3-208 (EP_4)	10 mg·kg^{-1} twice daily	++	Kabashima *et al.* (2003)
	Ear swelling/histology	UVB/skin of ear	ONO-AE3-208 (EP_4)	10 mg·kg^{-1}	++	Kabashima *et al.* (2007)
Intravenous						
Rat	Sensory discharge	Acetic acid/bladder lumen	ONO-8711 (EP_1)	1–3 mg·kg^{-1}	++	Ikeda *et al.* (2006)
	Visceromotor reflex	Distension/bladder	DG-041 (EP_3)	10 mg·kg^{-1}	+++	Su *et al.* (2008a)
Intraperitoneal						
Rat	Mechanical hyperalgesia	Freund's adjuvant/paw	AH-23848 (EP_4)	0.1–10 mg·kg^{-1}	+++	Lin *et al.* (2006)
	Thermal hyperalgesia	Freund's adjuvant/paw	AH-23848 (EP_4)	0.1–10 mg·kg^{-1}	+++	Lin *et al.* (2006)
Mouse	Mechanical allodynia	Sulprostone/intrathecal	ZM-325802 (EP_1)	0.03 μg·kg^{-1}	+++	Gil *et al.* (2008)
Topical to skin						
Mouse	Oedema	Chronic UVB	ONO-8713 (EP_1)	96 nmol	++	Tober *et al.* (2006)
Intraplantar						

Rat	Paw-withdrawal	PGE_2/paw[b]	SC-19220 (EP_1)	2.3 nmol	++	Khasar *et al.* (1993)
	Mechanical hyperalgesia	Incision wound/paw	ONO-8711 (EP_1)	4.5–114 nmol	+++	Omote *et al.* (2001)
	Chronic mechanical hyperalgesia	Partial nerve transection/ sciatic	SC-19220 (EP_1)	2.3 nmol	+++	Syriatowicz *et al.* (1999)
	Chronic thermal hyperalgesia	Partial nerve transection/ sciatic	SC-19220 (EP_1)	2.3 nmol	++	Syriatowicz *et al.* (1999)
Mouse	Mechanical allodynia	PGE_2/paw	AH-6809 (EP_1)	100 nmol	0	Kassuya *et al.* (2007)
			L-826266 (EP_3)	0.1–10 nmol	+++	
			L-161982 (EP_4)	10 nmol	0	
		Carrageenan/paw	L-826266 (EP_3)	10 nmol	++	Kassuya *et al.* (2007)
			L-161982 (EP_4)	10 nmol	++	
	Paw-licking	PGE_2/paw	AH-6809 (EP_1)	10–100 nmol	0	Kassuya *et al.* (2007)
			L-826266 (EP_3)	3–30 nmol	++	
			L-161982 (EP_4)	1–30 nmol	+++	
Intrathecal						
Rat	Mechanical allodynia	Nerve ligation/L5 spinal root	SC-51322 (EP_1)	22–220 nmol	++	Hefferan *et al.* (2003); O'Reilly and Loomis (2007)
	Mechanical hyperalgesia	Incision wound/foot	ONO-8711 (EP_1)	114–228 nmol	++	Omote *et al.* (2002)
	Thermal hyperalgesia	Incision wound/paw	ONO-8711 (EP_1)	114–128 nmol	0	Ikeda *et al.* (2006)
	Flinching (late phase)	Formalin/paw	SC-58109 (EP_1)	65–650 nmol	++	Malmberg *et al.* (1994)
	Mechanical hyperalgesia (late phase)	Carrageenan/paw	ONO-8711 (EP_1)	2.28–228 nmol	+++	Nakayama *et al.* (2002)
	Mechanical hyperalgesia (late phase)	PGE_2/intrathecal	ONO-8711 (EP_1)	0.11–0.45 nmol	+++	Nakayama *et al.* (2004)
	Visceromotor reflex	Distension/bladder	L-798106 (EP_3)	10–300 nmol	+++	Su *et al.* (2008b)
			DG-041 (EP_3)	10–100 nmol	+++	Su *et al.* (2008b)
Mouse	Thermal hyperalgesia	PGE_2/intrathecal	AH-6809 (EP_1)	0.29–2.9 nmol	+++	Uda *et al.* (1990)
	Writhing response	PGE_2/intrathecal	AH-6809 (EP_1)	0.29–2.9 nmol	+++	Uda *et al.* (1990)
	Mechanical allodynia	PGE_2/intrathecal	AH-6809 (EP_1)	4.2 nmol	0	Minami *et al.* (1995)
	Thermal hyperalgesia	PGE_2/intrathecal[c]	AH-6809 (EP_1)	4.2 nmol	+++	Minami *et al.* (1995)
	Mechanical allodynia	PGE_2/intrathecal	ONO-NT-012 (?)[d]	3×10^{-5}–0.03 nmol	+++	Minami *et al.* (1995)
	Thermal hyperalgesia	PGE_2/intrathecal	ONO-NT-012 (?)	3×10^{-5}–0.03 nmol	0	Minami *et al.* (1995)
Topical to spinal tissue						
Rat	Mechanical allodynia	Bicuculline/L5–L6 spine	SC-51322 (EP_1)	0.22–17.5 nmol	+++	Zhang *et al.* (2001)

Upper and lower panels relate to parenteral and topical administration of antagonist.
[a]Selective EP_4 agonist.
[b]Intradermal.
[c]About 30-fold higher dose of PGE_2 required compared with mechanical allodynia.
[d]See EP_1 antagonist section for specificity; reported pA_2 of 9.96 in allodynia model is unlikely to be valid because antagonist concentrations in biophase are unknown.

are both injected intradermally and not subcutaneously. More recently, attention has focused on the role of EP receptors in central nociception (see Svensson and Yaksh, 2002; Hefferan *et al.*, 2003; Mebane *et al.*, 2003). Minami *et al.* (2001) showed that PGE_2 could induce hyperalgesia in the mouse when injected intrathecally over the dose-range 0.00035–350 pmol. Deletion of the EP_3 receptor gene removed the more sensitive component of the hyperalgesia. In addition, the selective EP_3 agonist ONO-AE-248 induced hyperalgesia at relatively low doses. Surprisingly, the EP_1 knock-out mouse showed a hyperalgesic response in the hot-plate test, thereby confounding the role of EP_1 receptors in mediating hyperalgesia (Minami *et al.*, 2001). EP_1 antagonists given intrathecally suppress flinching, and mechanical allodynia and hyperalgesia (Table 3), although in certain cases the doses required for these effects are high. For example, in the studies by Omote *et al.* (2002), 100 μg ONO-8711 was injected intrathecally in a volume of 10 μl, representing an injectate concentration of 23 mM! Even after dilution in the cerebrospinal fluid, EP_3 receptors are likely to be blocked as well.

Intravenous ONO-8711 also suppressed afferent nerve discharge to distension of the rat bladder sensitized with acetic acid (Ikeda *et al.*, 2006). The inference from these studies was that $PG(E_2)$ generated locally activates EP_1 receptors on peripheral sensory neurones. Again EP_1/EP_3 selectivity is critical, given that EP_3 agonists augment bradykinin-induced sensory nerve discharge (Kumazawa *et al.*, 1996). Topical ONO-8713 (like celecoxib) inhibited UV-B-induced skin inflammation and tumour development in the mouse (Tober *et al.*, 2006); the higher selectivity of ONO-8713 supports EP_1 receptor involvement. PGE_2 production, but not COX-1/COX-2 expression, was suppressed by ONO-8713; the mechanism is not clear.

Despite continuing synthesis of potent EP_1 antagonists with testing in analgesic/anti-inflammatory models, there has been little solid evidence of clinical efficacy. Sarkar *et al.* (2003) reported that ZD-6416, which is related to ZM-325802 (Figure 3; Shaw *et al.*, 1999; Jenkins *et al.*, 2001), inhibited upper oesophageal pain threshold to electrical stimulation in human volunteers. However, ZD-6416 does not appear to be particularly useful in the clinical setting (development profile obtained from Pharmaprojects; http://www.pharmaprojects.com).

While the preferred indication for EP_1 antagonists has been for pain, additional therapeutic uses in cancer, osteoporosis, arthritis, and neurodegenerative and renal disorders have been suggested. COX-2 inhibitors are of potential value in reducing colorectal adenomas which, in turn, has created interest in using prostanoid antagonists as an alternative. The cardiovascular risk associated with celecoxib in clinical trials involving colorectal adenoma prevention (Solomon *et al.*, 2005) would intensify interest in using prostanoid antagonists for this indication. ONO-8711 inhibited formation of colonic crypts (Kawamori *et al.*, 2001) and reduced the frequency of polyp formation in APC1309 mice (Watanabe *et al.*, 1999; Kitamura *et al.*, 2003b), and aberrant crypt foci in oxymetazaline-treated mice (Watanabe *et al.*, 1999). Correspondingly, aberrant crypt foci were reduced by 60% in $EP_1^{-/-}$ receptor mice (Watanabe *et al.*, 1999). COX-2 up-regulation has also been considered a target for drug treatment of pathologies involving neurological injury and neurodegeneration. The downstream effects of COX-2 neurotoxicity have been reported to be EP_1 receptor-mediated (Kawano *et al.*, 2006).

Blood pressure in the spontaneously hypertensive rat was reduced by SC-51322 ($10\ mg^{-1}{\cdot}kg^{-1}{\cdot}day^{-1}$, gavage) (Guan *et al.*, 2007). EP_1 receptors appear to regulate BP in the male but not the female mouse (Audoly *et al.*, 1999; Stock *et al.*, 2001). In a more detailed study, Guan *et al.* (2007) showed that SC-51322 and EP_1-receptor gene deletion blunted pressor responses to 17-phenyl PGE_2 and sulprostone, whereas the pressor response to the PGE_1 analogue MB-28767 (claimed to be 'a pure EP_3 agonist') was the same in $EP_1^{+/+}$ and $EP_1^{-/-}$ mice. While we agree with the authors' contention that both EP_1 and EP_3 receptors contribute to the pressor effects, the utility of MB-28767 may be compromised by its moderate TP agonism (Lawrence and Jones, 1992) EP_1-receptor gene deletion also reduced the elevated BP and cardiac hypertrophy following a 4-week infusion of angiotensin (Ang) II in the mouse, and 1 μM SC-51322 markedly attenuated the contractile action of Ang II on the isolated pre-glomerular arteriole (Guan *et al.*, 2007). How these findings for EP_1 receptors integrate with the modest hypertension and reduction of antihypertensive efficacy associated with NSAIDs and COX-2 inhibitors in the human setting (Johnson *et al.*, 1994; Ishiguro *et al.*, 2008) is not clear. Species and gender appear to be highly influential factors.

EP_2 receptor antagonists

Selective EP_2 receptor antagonists are essentially unavailable. This may be partly due to a reluctance to inhibit the potential anti-inflammatory actions of endogenous PGE_2 (see Teixeira *et al.*, 1997) mediated via EP_2 receptors (Noguchi *et al.*, 1999; Nataraj *et al.*, 2001).

Woodward *et al.* (1995b) showed that AH-6809 has modest affinity for human rc-EP_2 receptors and antagonized PGE_2-induced activation of adenylyl cyclase with a pA_2 of about 6.5. Lower affinities were found for inhibition of PGE_2-induced relaxation of human bronchus ($pA_2 = 5.78$; Norel *et al.*, 1999) and guinea pig trachea ($pA_2 = 5.6$, KJ Ong and RL Jones, unpubl. obs.) (Table 2). This is consistent with its utility for distinguishing EP_1 receptors from other EP subtypes in non-primate pharmacological preparations (Coleman *et al.*, 1987; Eglen and Whiting, 1988; Lawrence *et al.* 1992). However, given the poor selectivity of AH-6809 in the low micromolar range, findings arising from its use in high concentration as an EP_2 antagonist (e.g. 100 μM in Aronoff *et al.*, 2004) should be approached with caution. K_i values for rc-EP_2 receptors of about 1 μM have been found for some compounds in combinatorial studies (see Murase *et al.*, 2008b); it should be possible to build on these observations.

EP_3 receptor antagonists

Development

EP_3 antagonism in a series of biaryl-acylsulphonamides was reported in 2002 by Merck (Gallant *et al.*, 2002). The lead

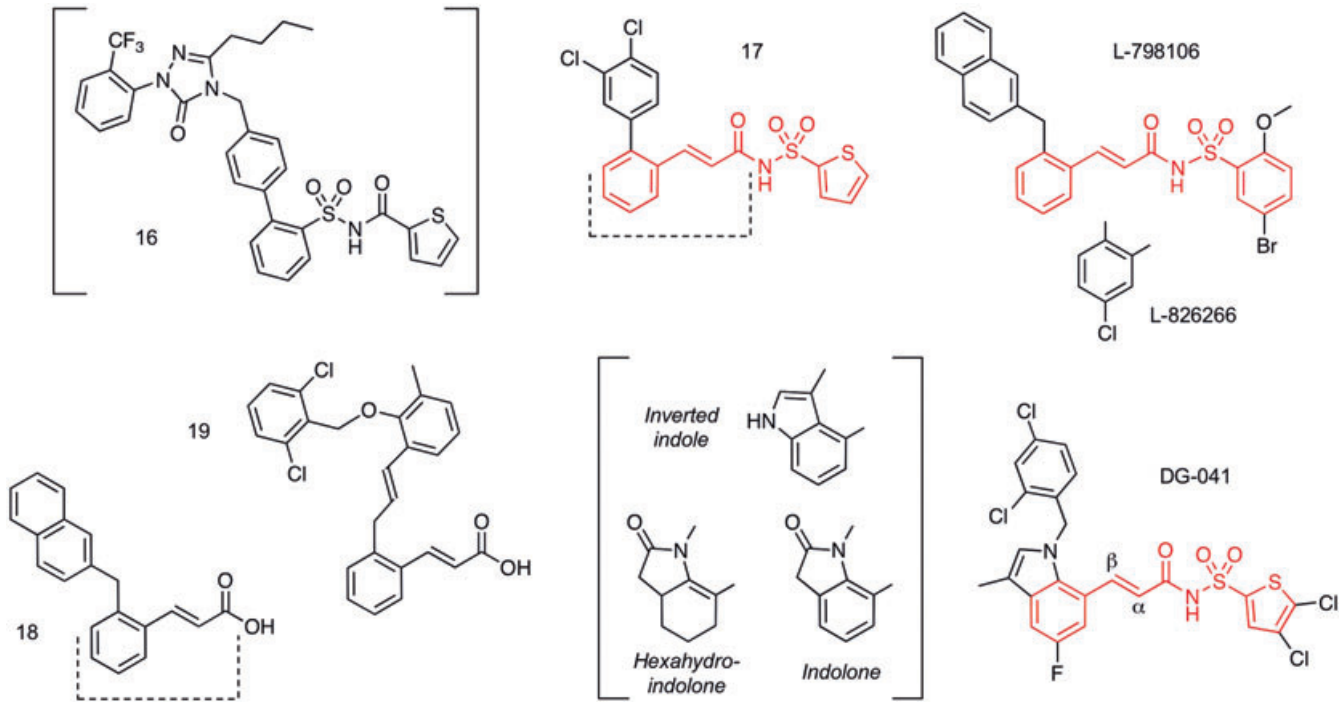

Figure 4 EP_3 receptor antagonists. The AT_1 receptor antagonist, compound **16**, is a lead molecule for the biaryl-ene-acyl-sulphonamide antagonists (pharmacophore in red). The left-hand portion of this pharmacophore corresponds to the cinnamic acid moiety in compounds **18** and **19** (see broken brackets). L-826266 is a chloro analogue of L-798106. The lower-middle brackets show modifications to the indole nucleus in the EP_3 antagonist series of DeCode Genetics.

compound was an AT_1 receptor antagonist **16** (Figure 4) with a binding K_D of 7 μM for the human EP_3 receptor. In a combinatorial approach, reversal of the acylsulphonamide and insertion of an ethylene spacer yielded **17** with an EP_3 K_D of 25 nM and minimal binding to other prostanoid receptors. A related analogue, L-798106, at 0.2 μM blocked the presynaptic (EP_3) inhibitory actions of sulprostone on guinea pig vas deferens and trachea with pA_2 values of 7.5 and 7.8 respectively (Clarke *et al.*, 2004). L-798106 (0.2–1 μM) also caused parallel displacement of log concentration–response curves for contraction of rat femoral artery by sulprostone (in synergy with phenylephrine) (Hung *et al.*, 2006). L-826266, a chloro analogue of L-798106, has been used in other studies despite the lack of detailed information on its basic pharmacology. Schlemper *et al.* (2005) showed that L-826266 at 30 μM inhibited both PGE_2- and bradykinin-induced relaxation of guinea pig trachea, and proposed that bradykinin induces *de novo* synthesis of PGE_2, which then activates EP_3 receptors. However, the specificity of L-826266 at this high concentration is unknown. An alternative explanation is that L-826266 modestly blocks EP_2 receptors thereby allowing the action of PGE_2 on the contractile EP_1 system in the trachea to dominate. Oliva *et al.* (2006) reported that L-826266 injected (as 0.1 μL of 1.25–5 mM solutions in 20% DMSO/PSS) into the periaqueductal grey matter of the mouse brain suppressed the late hyperalgesic response to intradermal formalin. However, the concentration of L-826266 at the site(s) of action is indeterminate and interpretation of the finding is difficult because similar high 'doses' of EP_1 and EP_4 antagonists and AH-6809 also suppressed the formalin response.

DeCode Genetics has described a series of related molecules containing an indole nucleus, from which DG-041 (Figure 4) was selected for clinical investigation (Singh *et al.*, 2009). DG-041 had a IC_{50} of 8.1 nM in a EP_3/Ca^{2+} flux FLIPR assay; corresponding values in DP_1 and DP_2 assays were 131 and >10 000 nM respectively (Singh *et al.*, 2009). SAR studies involved modifications to the terminal aryl moieties, together with the indole unit. Compounds with an inverted indole nucleus retain high EP_3 affinity (Zhou *et al.*, 2009a,b), as do indolones and hexahydro-indolones (O'Connell *et al.*, 2009). Saturation of the α.β-double bond also produced highly potent EP_3 antagonists, while further saturation of the remaining double bond in the hexahydro-indolone residue resulted in marked reduction in affinity. Much of DeCode's work was directed towards improving water solubility: predicted *n*-octanol/water partition coefficients (ClogP) for DG-041, L-798106 and L-826266 are 6.6, 6.9 and 7.4 respectively (ChemAxon freeware). In this context, our recent studies on L-798106 and L-826266 have shown a slowly developing block of EP_3 agonist-induced contraction on guinea pig aorta, affording pA_2 values of 7.96 and 7.58 respectively after 3-h contact (Jones *et al.*, 2008). As expected, highly potent antagonists such as BMY-180291 (TP, pA_2 = 9.8) and doxepin (histamine H_1, pA_2 = 9.6) also had slow onsets at low-nanomolar concentrations. However, the slow onsets of L-798106 and L-826266 may be related to their high lipophilicity rather than their (moderate) receptor affinity.

Merck-Frosst researchers have also identified EP_3 antagonism in *ortho*-substituted cinnamic acid derivatives, which correspond to the left-hand portions of the ene-acyl-

sulphonamide antagonists shown in Figure 4. Compound **18** has a binding K_D for the human EP_3 receptor of 20 nM (Juteau *et al.*, 2001), while compound **19** has the highest binding affinity (3 nM) and behaved as a pure antagonist in a human EP_3/adenylyl cyclase assay (pA_2 = 8.22; Belley *et al.*, 2005). Compound **19** is also highly lipophilic (ClogP = 8.29).

ONO-AE3-240 is reported to be a highly selective EP_3 antagonist (mouse EP_3/EP_1 selectivity ratio = 2500; Amano *et al.*, 2003), but its structure has not been disclosed.

Therapeutic applications

Like most prostanoid receptors, the EP_3 receptor has been implicated in pain of various aetiologies. These include allodynia produced by HIV-1 glycoprotein gp 120 (Minami *et al.*, 2003) and PGE_2 (Kassuya *et al.*, 2007), acute herpetic pain (Takasaki *et al.*, 2005), thermal hyperalgesia (Oka *et al.*, 1994) and formalin-induced hyperalgesia (Oliva *et al.*, 2006). A major role for EP_3 (and IP) receptors has been claimed in endotoxin-induced enhancement of pain perception (Ueno *et al.*, 2001). Intravenous DG-041 also suppressed the visceromotor reflex to bladder distension in the rat (Su *et al.*, 2008a); the authors defined this antagonist as brain non-penetrant, but no pharmacokinetic evidence was presented. Intrathecal application of L-798106 and DG-041 also produced a long-lasting suppression of the visceromotor reflex, while intracerebroventricular administration produced only a transient reduction (Su *et al.*, 2008b). However, EP_3 agonists are known to exert gastrointestinal cytoprotection and attenuate gastric acid secretion in animal models (Bunce *et al.*, 1990; Kunikata *et al.*, 2002). Although it is widely assumed that there is a similar involvement of EP_3 receptors in man, an extensive search of the literature provides only circumstantial evidence for this. For example, while misoprostol is clinically useful in suppressing gastroduodenal erosion (see Hawkey, 2000), its selectivity is not high enough to infer the involvement of EP_3 receptors ($EP_3 \sim EP_2 \sim EP_4 >> EP_1$ for misoprostol-free acid; Abramovitz *et al.*, 2000). A reduction in myocardial ischaemic damage was also achieved with EP_3 agonist treatment in the rat (Zacharowski *et al.*, 1999). Thus, it is feasible that the therapeutic use of EP_3 antagonists will result in a side effect profile at least comparable to that of COX inhibitors. This possibility does not seem to have hindered development work on other uses of EP_3 antagonists as discussed below.

It has been suggested that endogenous PGE_2 activates EP_3 receptors on stromal cells surrounding a tumour causing the release of vascular endothelial growth factor, which then promotes angiogenesis and tumour growth (Amano *et al.*, 2003). Injection of the EP_3 antagonist ONO-AE3-240 around sarcoma-180 tumours in the mouse markedly suppressed these effects in a manner similar to EP_3 receptor gene-deletion; ONO-8711 (EP_1 antagonist) and ONO-AE3-208 (EP_4 antagonist) were ineffective. In contrast, EP_3 agonists acting on all three mouse EP_3 receptor isoforms expressed in HEK-293 cells caused cell clustering and inhibited their proliferation via a G_{12}-RhoA pathway (Macias-Perez *et al.*, 2008).

A further possible therapeutic application of an EP_3 antagonist is the treatment of pre-term labour. Based on the known activity of misoprostol (Sanchez-Ramoz *et al.*, 1997) and sulprostone (Fruzzetti *et al.*, 1988), it appears that EP_3 receptor stimulation produces cervical ripening, a critical event that precedes parturition. An EP_3 antagonist could be effectively combined with a tocolytic, such as an EP_2 agonist (Senior *et al.*, 1993), to provide therapy for pre-term labour. The EP_3 receptor has been uniquely associated with febrile responses (Ushikubi *et al.*, 1998). However, development of an EP_3 antagonist for treating fever seems unlikely, given that low-cost COX inhibitors are highly effective in reducing body temperature.

Finally, DG-041 has shown promise in the treatment of peripheral cardiovascular disease. Activation of EP_3 receptors on human platelets enhances aggregation induced by a variety of agents (in the presence of a TP antagonist); inhibition of adenylyl cyclase/priming of protein kinase C is thought to be the mechanism (Matthews and Jones, 1993; Vezza *et al.*, 1993). In addition, there is increased bleeding tendency and increased susceptibility to thromboembolism in the EP_3 receptor knock-out mouse (Ma *et al.*, 2001). DG-041 at 0.03–3 μM inhibited the enhancement by sulprostone of ADP- or collagen-induced aggregation in human platelet-rich plasma (PRP) (Heptinstall *et al.*, 2008; Singh *et al.*, 2009). The pA_2 value of 8.3 (our calculation from Heptinstall *et al.*, 2008 data) is probably an underestimate of the affinity owing to plasma protein binding of DG-041. In the rat, DG-041 at 5 or 60 $mg \cdot kg^{-1}$ (by gavage; co-administration with clopidogrel) inhibited enhancement of platelet aggregation induced by PGE_2 *ex vivo*; there was no increase in bleeding time compared with clopidogrel alone (Singh *et al.*, 2009). A similar profile was obtained with DG-041 in Phase I trials in healthy subjects (reported in Heptinstall *et al.*, 2008).

EP_4-receptor antagonists

Development

The first EP_4 antagonist to be reported was AH-23848 (Coleman *et al.*, 1994a), a close relative of the selective TP antagonist GR-32191 (Figure 8). Ligand binding studies on human rc-receptors indicated low EP selectivity for AH-23848, with *K*i values for EP_1, EP_2, EP_3, EP_4 and IP receptors being 45, 50, 4.4, 14 and >100 μM respectively (Abramovitz *et al.*, 2000). However, its selectivity in functional assays appears to be higher and it has been of considerable utility in differentiating the EP_2, EP_4 and IP agonist activities of prostanoid ligands. For example, Jones and Chan (2001) used AH-23848 at 30 μM to demonstrate that the PGI_2 analogues cicaprost and AFP-07 relax certain vascular preparations by activating both EP_4 and IP receptors. Moreover, Lai *et al.* (2008a) showed that pulmonary artery smooth muscle cells from monocrotaline-treated rats have a reduced IP receptor density and that iloprost-induced cAMP elevation is blocked by AH-23848, and therefore likely to be due to activation of EP_4 receptors.

AH-23848 has since been overtaken by more potent agents. Antagonists in the major group contain an acyl-sulphonamide unit (Figure 5) and show some similarity to the ene-acyl-sulphonamide EP_3 antagonists. Indeed, L-161982 is a methyl analogue of the lead molecule **16** for the Merck EP_3

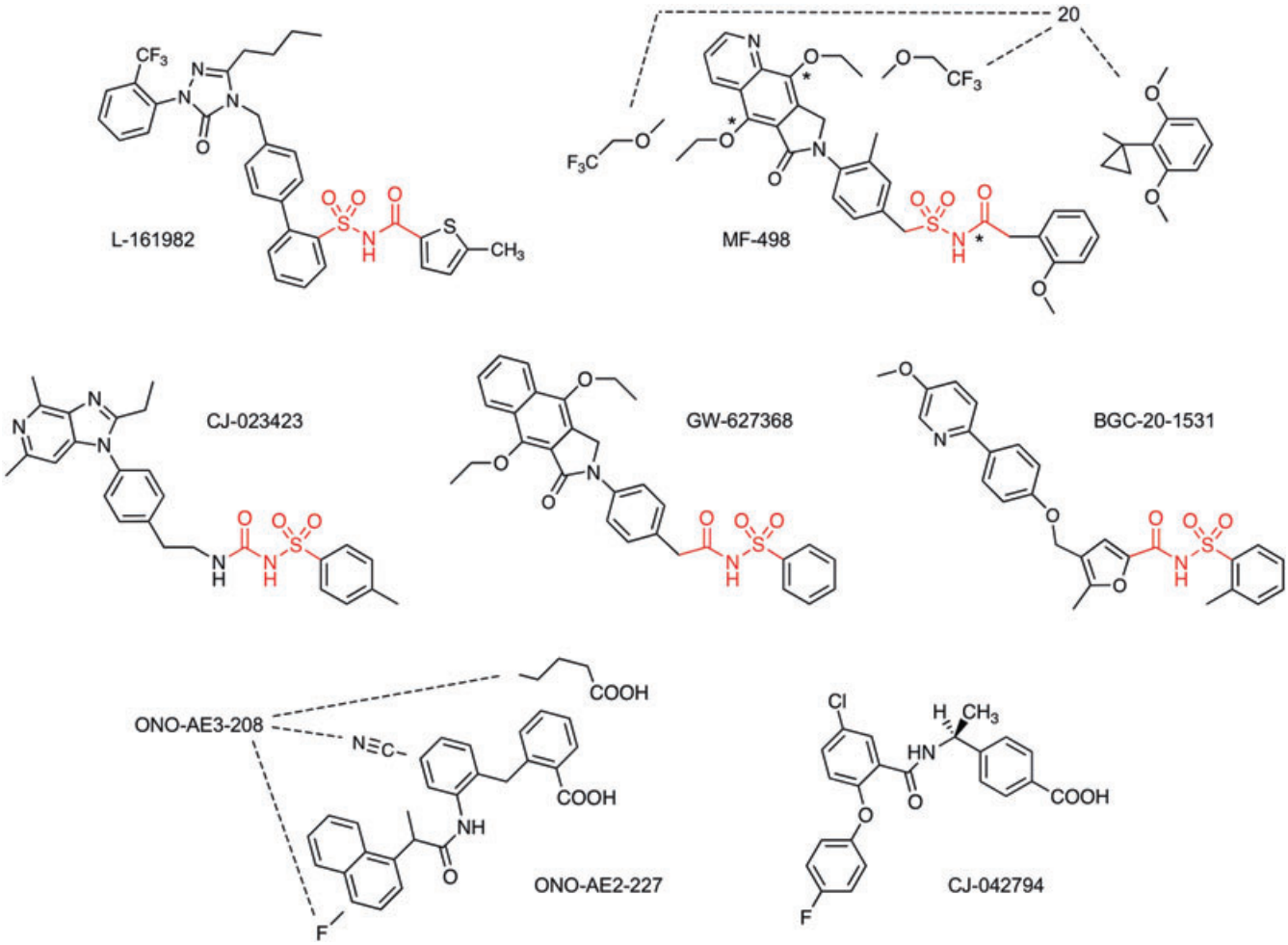

Figure 5 EP_4 receptor antagonists. L-161982 is a methyl analogue of compound **16** in Figure 4. Acyl-sulphonamido residues are shown in red. The bonds indicated by asterisks in MF-498 are subject to oxidative/hydrolytic attack *in vivo*; the corresponding substituents in compound **20** prevent these transformations.

antagonists. L-161982 was reported to have a pA_2 of about 8.5 in rc-EP_4 receptor – adenylyl cyclase assays (Machwate *et al.*, 2001). In a rat native EP_4 assay, L-161982 inhibited PGE_2-induced cAMP accumulation with an IC_{50} of about 30 nM, while forskolin-induced cAMP accumulation was unaffected by 10 μM L-161982. In similar functional assays, CJ-023423 afforded pA_2 values of 8.3 and 8.2 for human and rat rc-EP_4 receptors using the Schild protocol (Nakao *et al.*, 2007). In binding assays, CJ-023423 showed weak affinity for human EP_2 receptors and PAF receptors, while interactions with other prostanoid receptors and a range of non-prostanoid receptors were minimal.

GW-627368 has p*K*i values for binding of 7.0 and 6.8 for human rc-EP_4 and TP receptors respectively; binding to other prostanoid receptors is minimal (Wilson *et al.*, 2006). Correspondingly, a pA_2 of 7.9 was obtained for GW-627368 in a human rc-EP_4 receptor – adenylyl cyclase assay, with good evidence for competition. On human pulmonary vein, GW-627368 had a pA_2 of 7.06 against ONO-AE1-329 (Table 2; Foudi *et al.*, 2008); its higher affinity against PGE_2 may have been due to opposing contractile activity. GW-627368 had a higher affinity on piglet saphenous vein (pA_2 = 9.2) with a linear Schild plot up to a concentration ratio of about 60; further rightward shift of the agonist curve was insignificant owing to PGE_2 activating a less sensitive EP_2 relaxant system (Wilson *et al.*, 2006). GW-627368 at 10 μM did not affect TP receptor-induced contraction under the same conditions. Results for rabbit saphenous vein were discrepant: Wilson *et al.* (2006) showed that 10 μM GW-627368 did not affect PGE_2-induced relaxation implying the presence of an EP_2 system only, while Jones and Chan (2005) found a right-shift of about 1 log unit with 1 μM GW-627368, consistent with the presence of EP_2 and EP_4 systems; GW-627368 did not antagonize relaxation induced by either the selective EP_2 agonist ONO-AEI-259 or the PGI_2 analogue, taprostene in the latter experiments. MF-498 (Clark *et al.*, 2008), which is quite similar in structure to GW-627368, undergoes oxidative/hydrolytic metabolism at the three regions indicated in Figure 5; compound **20** was considerably more resistant to attack while retaining high EP_4 antagonist affinity (Burch *et al.*, 2008). The most recent addition to this group is BGC-20-1531 (Maubach *et al.*, 2009). It exhibits surmountable antagonism of PGE_2-induced relaxation of human cerebral and middle meningeal and dog carotid and middle meningeal

arteries *in vitro*, while having no effect on PGE_2 (probably EP_3)-induced contraction of human coronary, pulmonary and renal arteries.

A second looser group of amide-containing EP_4 antagonists is represented by ONO-AE2-227, ONO-AE3-208 and CJ-042794 (Figure 5). Binding studies indicate that ONO-AE2-227 and ONO-AE3-208 retain considerable affinity for EP_3 receptors (*K*i = 21 and 30 nM; Mutoh *et al*. 2002; Kabashima *et al*. 2003) and this must be borne in mind when interpreting *in vivo* data (see later). CJ-042794 behaved competitively in functional assays (Schild protocol) involving human and rat rc-EP_4 receptors (Murase *et al*., 2008a,b). Its EP_4/EP_3 selectivity ratio is very high, but it still retains measurable affinity for human EP_2 receptors (pK_i = 6.2).

Therapeutic applications

Recent studies involving parenteral administration of several EP_4 antagonists (AH-23848, CJ-023423, CJ-042794, MF-498, ONO-AE3-208) have clearly demonstrated a major involvement of EP_4 receptors in small-animal models of inflammation (Table 3). Joint pain, mechanical and thermal hyperalgesia and oedema were markedly suppressed, often equivalent to the efficacy of selective COX-2 inhibitors such as rofecoxib. A peripheral site of action seems likely given that intraplantar administration of L-161982 suppressed carrageenan-induced mechanical allodynia in the mouse (Kassuya *et al*., 2007). Whether block of the EP_4 receptor alone produces an effective anti-inflammatory drug in man remains to be seen. Moreover, PGE_2 may have a protective role in inflammation. Takayama *et al*. (2002) showed that PGE_2 suppressed chemokine production stimulated by lipopolysaccharide in human macrophages; L-161982 at 100 nM blocked this action.

Prostanoid EP_4 receptors, indeed nearly all EP receptor subtypes, have been implicated as contributors to colon tumorigenesis caused by excessive production of PGE_2 (Fujino and Regan, 2003; Majima *et al*., 2003; Masataka *et al*., 2003; Mutoh *et al*., 2006). The selective EP_4 agonist ONO-AE1-329 (0.1–1 μM) increased colony formation in the human colon cancer cell line HCA-7 (Mutoh *et al*., 2002) and L-161982 blocked PGE_2-induced proliferation of HCA-7 cells (Cherukuri *et al*., 2007). In both the oxymetazoline model of aberrant crypt foci (putative preneoplastic lesions) and the Min mouse model of intestinal polyp development, the EP_4 antagonist ONO-AE2-227 produced about a 67% reduction in the appropriate scores (Mutoh *et al*., 2002). Aberrant crypt foci were similarly reduced in $EP_4^{-/-}$ mice. In the APC1309 mouse, ONO-AE2-227 had a preferential effect on polyp size, while the EP_1 antagonist ONO-8711 had a more pronounced effect on polyp number, and a combination of antagonists behaved additively (Kitamura *et al*., 2003b). In the context of (tumour) vascularity, ONO-AE3-208 reduced IL-1β-induced angiogenesis in the mouse cornea at an oral dose of 1 $mg \cdot kg^{-1}$ o.d. (Kuwano *et al*., 2004). Finally, pretreatment of mouse mammary tumour cells with AH-23848 and ONO-AE3-208 followed by washing and immediate injection into immunologically compatible mice reduced pulmonary tumour score (Fulton *et al*., 2006). These comprehensive studies provide encouragement that EP_4 receptor antagonists may provide a safer replacement for COX-2 inhibitors in treating colon cancer. As, however, all EP receptor subtypes have been implicated in colon cancer, a pan-PGE synthase inhibitor may be more effective in preventing the key cell proliferative and angiogenic events. Arguably, and somewhat paradoxically, the EP_4 receptor has also been shown to participate in the maintenance of intestinal homeostasis by preserving mucosal integrity. In both $EP_4^{-/-}$ mice and wild-type mice treated with an EP_4 antagonist (ONO-AE3-208), susceptibility to the development of colitis was reported in a model of inflammatory bowel disease (Kabashima *et al*., 2003; Narumiya, 2003).

There has been interest in using EP_4 agonists for their anabolic effects on bone (Raisz, 2006). The EP_4 antagonist L-161982 at a dose of 10 $mg \cdot kg^{-1} \cdot day^{-1}$ reversed bone formation induced by PGE_2 in the rat, without affecting its diarrhoeal action (Machwate *et al*., 2001). Also, human mesenchymal stem cells in culture secreted PGE_2 via COX-2, which was associated with production of bone morphogenetic protein-2 (BMP-2), a factor that stimulates differentiation of precursor mesenchymal cells into mature bone. The selective COX-2 inhibitor, NS-398, and the EP_4 antagonist, ONO-AE3-208, (concentration not specified) suppressed BMP-2 expression (Arikawa *et al*., 2004). These findings identify bone loss as a potential side effect of EP_4 antagonists.

In vitro, EP_4 vasodilator systems typically exhibit high sensitivity to PGE_2 and this property is reflected in the role of PGE_2 in maintaining the open state of the ductus arteriosus during gestation in human and animal species. Strong expression of EP_4 (and IP) receptors is found in ductus tissue from the newborn infant and child (Leonhardt *et al*., 2003). Towards the end of gestation, smooth muscle cells within the ductus migrate to the endothelial lining where they form intimal cushions. Declining PG levels at birth result in ductus constriction, thereby bringing the intimal cushions into close contact and effecting permanent closure. Intimal cushion formation is also driven by EP_4 receptor activation (Yokoyama *et al*., 2006). The COX inhibitor, indomethacin, is commonly used to treat failure of ductus closure, but its efficacy is poor in a substantial proportion of cases, perhaps related to suppression of intimal cushion development (see Ivey and Srivastava, 2006). AH-23848 blocked PGE_2-induced relaxation of the rabbit and sheep isolated ductus arteriosus preparations (Smith *et al*., 1994; Bouayad *et al*., 2001), while ONO-AE3-208 constricted the ductus of fetal and neonatal rats *in vivo* (Momma *et al*., 2005). Whether an EP_4 antagonist would be better than a COX inhibitor in treating patent ductus in premature infants is not yet clear.

A second potential role for an EP_4 vasodilator system is the genesis of vascular headache in migraine. EP_4 antagonists block PGE_2-induced relaxation of human-isolated middle cerebral artery (Davis *et al*., 2004; Maubach *et al*., 2009) and the picture has been enlarged to include the interaction of endogenous PGE_2 with calcitonin gene-related peptide release from trigeminal nerves (Maubach *et al*., 2009). The use of AH-23848 and L-161982 also has provided evidence for a role of EP_4 receptors in substance P release by stretching of the renal pelvic wall (volume expansion) leading to activation of renal sensory afferents and subsequent diuresis/natriuresis (reno-renal reflex) (Kopp *et al*., 2004).

FP receptor and prostamide receptor antagonists

Development

A variety of FP receptor antagonists have been reported in the past, but none has stood up to rigorous analysis. Thus, $PGF_{2\alpha}$-1-dimethylamine and $PGF_{2\alpha}$-1-dimethylamide (Maddox *et al.*, 1978; Stinger *et al.*, 1982) exhibited no meaningful FP antagonist or agonist activities (Sharif *et al.*, 2000; Woodward *et al.*, 2008). Phloretin (Kitanaka *et al.*, 1993) was non-selective and very weak in blocking fluprostenol-mediated phosphoinoside turnover in rat A7r5 vascular smooth muscle cells (Sharif *et al.*, 2000). Similarly glibenclamide (Delaey and Van de Voorde, 1995) was a weak, non-selective prostanoid antagonist (Sharif *et al.*, 2000).

AL-3138 and AL-8810, both $PGF_{2\alpha}$ analogues (Figure 6), have received a degree of acceptance as FP antagonists (Griffin *et al.*, 1999; Sharif *et al.*, 2000). AL-8810 proved useful in studying $PGF_{2\alpha}$-mediated up-regulation of the orphan nuclear receptor Nur 77 (Liang *et al.*, 2004). However, further studies reveal that they are neither potent nor selective. For example, AL-8810 appears to block TP receptors (Hutchinson *et al.*, 2003), an effect that has been confirmed in human rc-TP receptor stable transfectants (A.H. Krauss and D.F. Woodward, unpublished). In addition, both agents are FP partial agonists. This can be seen in the original report of AL-8810 on phosphoinositide turnover (Griffin *et al.*, 1999) and is corroborated by the finding of myogenic activity in the mouse uterus (Hutchinson *et al.*, 2003). AL-8810 induced a more pronounced Ca^{2+} signal in human rc-FP stable transfectants (Y. Liang and D.F. Woodward, unpublished) and was a full agonist in the cat isolated iris preparation (Woodward *et al.*, 2007); the latter action was not blocked by a prostamide antagonist (Woodward *et al.*, 2007; see later), so it presumably reflects FP receptor agonism.

A series of octapeptides (THG-131 derivatives, Figure 6) have been claimed to possess selective FP antagonist activity (Chemtob and Peri, 2006; Peri *et al.*, 2006). In particular, THG-113.31 at 1 μM markedly inhibited $PGF_{2\alpha}$-induced contraction of pig retinal blood vessels, while having minimal effect on contraction to 17-phenyl PGE_2, U-46619, phenylephrine, Ang II and endothelin-1; the inhibition of $PGF_{2\alpha}$

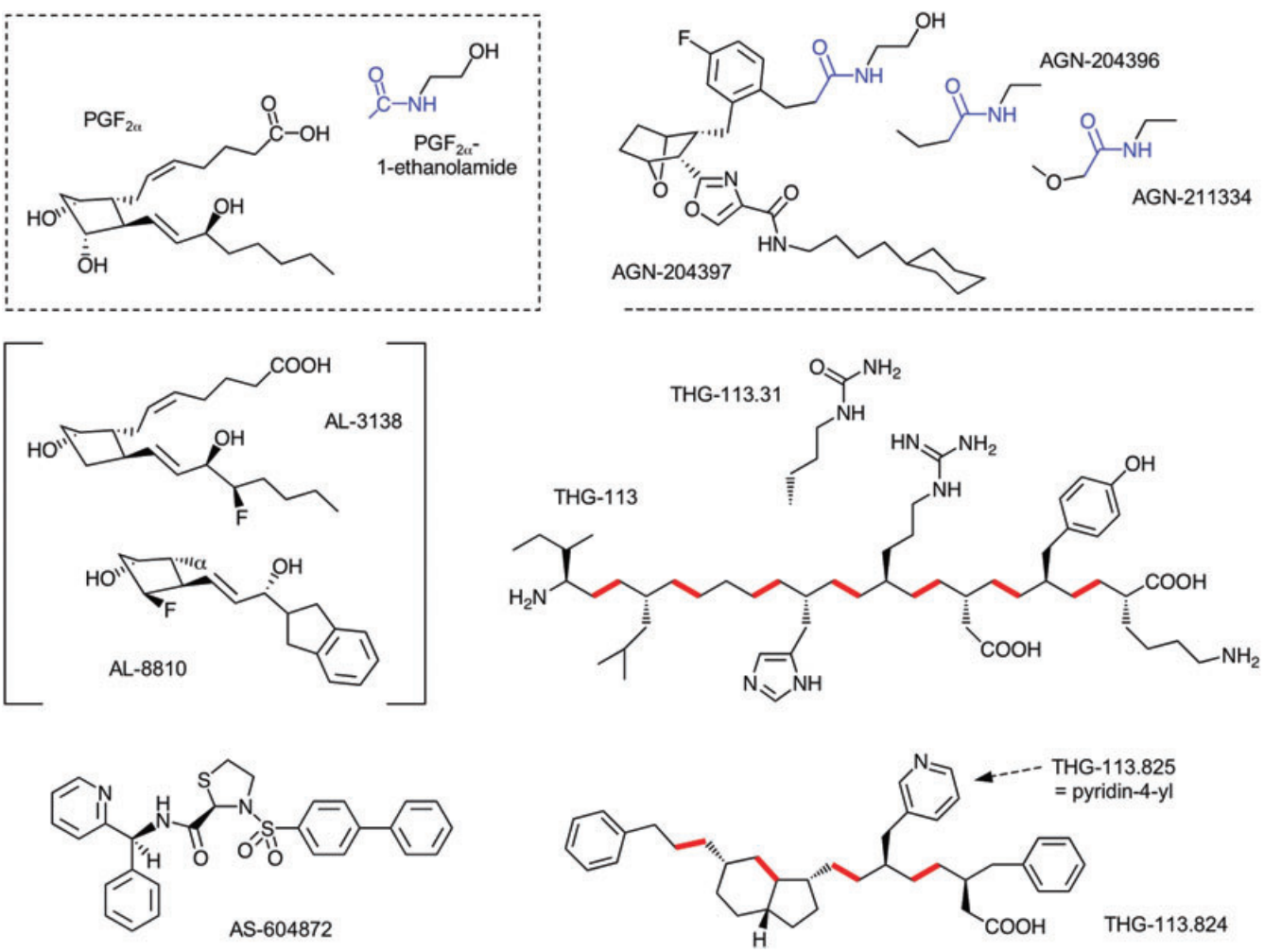

Figure 6 FP receptor and prostamide receptor antagonists. The natural ligand $PGF_{2\alpha}$ and its 1-ethanolamide derivative, prostamide $F_{2\alpha}$, are shown in the box. AL-3138 and AL-8810 are FP partial agonists in many systems; α = corresponding side-chain in $PGF_{2\alpha}$. The THG analogues are peptides: amide (CO-NH) residues are shown as red bars. The AGN analogues (upper right) are prostamide receptor antagonists; C1-amide residues are shown in blue.

contraction was insurmountable. In addition, THG-131.31 inhibited the associated phosphoinositide hydrolysis with an IC_{50} of about 30 nM. However, THG-131.31 competed poorly with [^{3}H]-$PGF_{2\alpha}$ for binding to human rc-FP receptors (~13% at 10 μM). Several other studies have cast doubt on the utility of THG-131.31. At 10 μM, THG-113.31 showed fairly weak antagonism of the contractility of longitudinal and circular strips of sheep myometrium induced by $PGF_{2\alpha}$; there was no effect on PGE_2 contractions (Hirst *et al.*, 2005). Also, 10 μM THG-113.31 had no effect on $PGF_{2\alpha}$-induced contraction of human pregnant myometrium, while inhibiting spontaneous and oxytocin-induced contractions at much lower concentrations (Friel *et al.*, 2005). Finally, Doheny *et al.* (2007) showed that THG-113.31 at 10–50 μM enhanced BK_{Ca} channel opening in isolated myocytes from human uterus, an effect that was reversed by iberiotoxin. Several chemically simpler peptidomimetics in the THG series showed more potent block of $PGF_{2\alpha}$-induced contraction than THG-113.31 (Figure 6, Table 2; Peri *et al.*, 2006); it would be of interest to have full pharmacological profiles.

Currently, the most convincing FP antagonist is the non-prostanoid AS-604872 (Cirillo *et al.*, 2007). It has K_i values of 35, 158 and 323 nM for human, rat and mouse rc-FP receptors and its selectivity was 20-fold for EP_2 receptors and greater than 300-fold for other prostanoid receptors. AS-604872 showed no agonist activity in a human FP receptor – inositol phosphate assay and had an IC_{50} of 47 nM against $PGF_{2\alpha}$. *In vivo*, AS-604872 (1–30 mg·kg^{-1}, i.v.) inhibited $PGF_{2\alpha}$-induced uterine contraction in the non-pregnant rat; inhibition of oxytocin-induced contraction was slight.

Prostamide research originated from studies on neutral $PGF_{2\alpha}$ analogues and notably bimatoprost (17-phenyl $PGF_{2\alpha}$-1-ethylamide), an effective anti-glaucoma drug (Woodward *et al.*, 2003; 2004). As previously mentioned, neutral $PGF_{2\alpha}$ analogues show only weak affinity for FP receptors (Maddox *et al.*, 1978; Schaaf and Hess, 1979), a profile subsequently confirmed for –OH, $–OCH_3$ and $–CON(R)_2$ C1-substitutes (Woodward *et al.*, 2000; 2008; Matias *et al.*, 2004). The pharmacology of bimatoprost was similar, with no meaningful activity at FP receptors but pronounced activity in certain preparations such as cat lung strip, cat iris, rabbit uterus, and human ciliary smooth muscle cells (Liang *et al.*, 2003; Woodward *et al.*, 2003b; Matias *et al.* 2004; Chen *et al.* 2005). The pharmacology of bimatoprost appeared indistinguishable from that of $PGF_{2\alpha}$-1-amides, but this could not be rationalized until the discovery that anandamide (arachidonic acid-1-ethanolamide) was a substrate for COX-2 (Yu *et al.*, 1997).

PGE_2-1-ethanolamide (prostamide E_2) was the first prostamide to be discovered and was identified as the major product following addition of anandamide to rc-COX-2 or cells expressing COX-2, but not COX-1 (Yu *et al.*, 1997). Subsequently, more extensive studies demonstrated that COX-2 oxidizes anandamide to endoperoxide intermediates, which are converted by specific PG synthases to the various prostamides (Kozak *et al.*, 2002; Koda *et al.*, 2004; Yang *et al.*, 2005; Moriuchi *et al.*, 2008). Despite being different terminal biosynthetic products, the evidence to date suggests that prostamides D_2, E_2 and $F_{2\alpha}$ interact with a single receptor to exert their effects. Prostamide $F_{2\alpha}$ and its analogues are, however, about 10 times more potent than prostamides D_2 and E_2 (Woodward *et al.*, 2007).

Initial pharmacological characterization of the prostamides, for example prostamide E_2 (Ross *et al.*, 2002), relied on agonist studies. In the context of PGF analogues, FP agonists (17-phenyl $PGF_{2\alpha}$ and $PGF_{2\alpha}$) and bimatoprost produced Ca^{2+} signals in entirely different cells in a cat iris smooth muscle cell preparation (Spada *et al.*, 2005), suggesting the existence of a receptor with a distinct preference for prostamide F agonists. The receptor structure appears to involve heterodimerization of the wild-type and alternative mRNA splicing variants of the FP receptor, both encoded by *PTGFR*, the FP receptor gene (Liang *et al.*, 2008). This FP/alt-FP co-expression is analogous to the isoprostane binding site which is formed as a result of IP/TP receptor heterodimerization (Wilson *et al.*, 2004).

The strategy for discovering a prostamide antagonist was to identify antagonists in cluster 2 of the prostanoid receptor evolutionary tree (Narumiya *et al.*, 1999) and then form corresponding C1-amides. Cluster 2 contains the TP receptor, whose stable agonists have been a starting point for antagonists at other receptors (DP_1, see Figure 1; EP_1, see Figure 3). Based on the oxabicycloheptane analogue BMS-180291 (Figure 8) (Webb *et al.*, 1993), two prototype prostamide antagonists AGN-204396 (Figure 6) and AGN-204397 were identified (Woodward *et al.*, 2007; 2008). These agents showed good prostamide F/FP selectivity, but were of low affinity (pA_2 ~5.5) and also blocked TP receptors. Substituting oxygen at C3 dramatically enhanced prostamide affinity, by as much as 100-fold for AGN-211334 and AGN-211335 (Wan *et al.*, 2007; Liang *et al.*, 2008; Woodward *et al.*, 2008). AGN-211334 and AGN-211335 potently inhibited prostamide $F_{2\alpha}$ and bimatoprost responses in cat iris preparations, but did not alter responses to FP agonists (Wan *et al.*, 2007; Liang *et al.*, 2008). AGN-211334 blocked the increase in conventional aqueous humour outflow produced by bimatoprost in the human perfused anterior segment preparation (Wan *et al.*, 2007), thereby demonstrating that the effects of bimatoprost in the human eye are prostamide receptor-mediated; previously, it has been suggested that bimatoprost's activity is dependent on deamidation to the FP-active free acid in ocular tissue (Camras *et al.*, 2004). AGN-211335 blocked the secondary Ca^{2+} wave, myosin light chain phosphorylation, and Cyr 61 up-regulation induced by bimatoprost in the FP/alt-FP prostamide system (Liang *et al.*, 2008). These second-generation prostamide antagonists are likely to be sufficiently potent for *in vivo* studies.

Therapeutic applications

Prevention of pre-term labour (tocolysis) is probably the only therapeutic modality where a FP antagonist may be of value. This is a serious and unmet medical need given that premature birth accounts for 60–80% of perinatal deaths (Goldenberg, 2002). Parturition is prevented in $FP^{-/-}$ mice (Sugimoto *et al.*, 1997) confirming a significant role for $PGF_{2\alpha}$ (see Challis *et al.*, 2002 for a review of PG involvement). THG-113.31 is tocolytic in the pregnant sheep (Hirst *et al.*, 2005) and may be useful for delaying pre-term birth (Olson, 2005). AS-604872 suppressed spontaneous uterine contractions in late-term pregnant rats and delayed preterm birth caused by

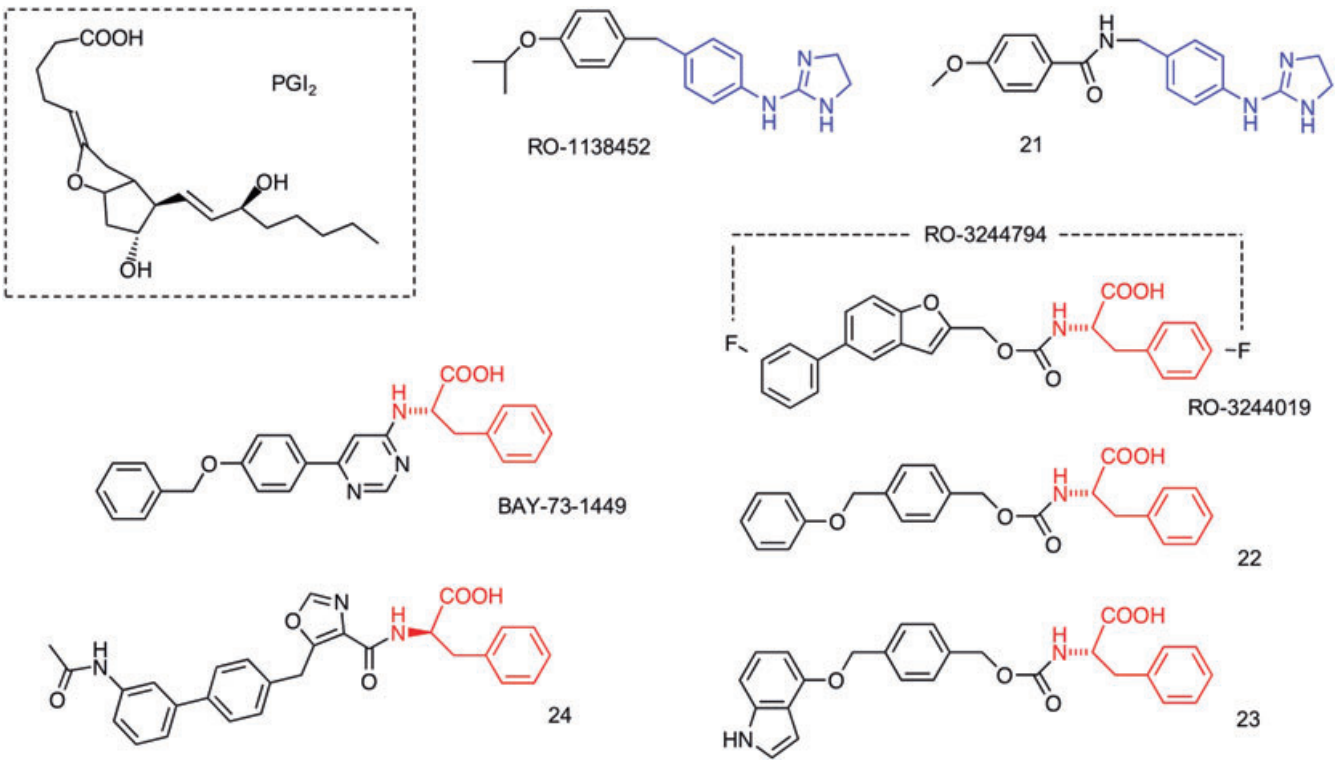

Figure 7 IP receptor antagonists. The natural ligand PGI_2 (prostacyclin) is shown in the box. 2-(Phenylamino)-imidazoline moieties are shown in blue and phenylalanine residues in red (*S*-configuration in compound **24**). RO-3244794 is a difluoro analogue of RO-3244019.

mefipristone in pregnant mice; AS-604872 appeared to be more effective than the β_2-adrenoceptor agonist ritodrine (Chollet *et al.*, 2007). It is not yet clear whether these findings will translate into an effective drug in human reproduction.

Prostanoid FP receptors have been implicated in cardiomyocyte hypertrophy (Pönicke *et al.*, 2000; Xu *et al.*, 2008) and cancer (Fujino and Regan, 2001) and may play a role in regulating water and solute transport based on *in situ* hybridization studies in the mouse kidney (Saito *et al.*, 2003). The utility of a selective FP antagonist is, however, unclear.

The therapeutic utility of prostamide antagonists is similarly uncertain. Increased anandamide levels may result in the formation of prostamides as major products in inflammation and infection (Glass *et al.*, 2005). Testing of AGN 211334, or a close congener, in relevant animal models should provide evidence for or against a functional role of prostamides.

IP receptor antagonists

Development

IP receptor antagonists are a recent development based on the potential role of PGI_2 in mediating pain (Bley *et al.*, 1998). In an extensive series of studies, two structurally distinct classes of selective IP antagonist emerged from focused chemical library screening and synthetic chemistry (Bley *et al.*, 2006) (Figure 7). The 2-(phenylamino)-imidazoline series is represented by RO-1138452 and compound **21** (Clark *et al.*, 2004; Keitz *et al.*, 2004), while the N-substituted phenylalanine series has a 'traditional' carboxylate in the phenylalanine residue and is typified by RO-3244019 (Fitch *et al.*, 2004) and its difluoro analogue RO-3244794 (Bley *et al.*, 2006).

RO-1138452 has high affinity for human native (platelet) and rc-IP receptors, with pK_i values of 9.3 and 8.7 respectively in studies utilizing [^{3}H]-iloprost, although it also displays considerable affinity for PAF (7.9) and imidazoline (8.3) receptors (Bley *et al.*, 2006). In functional studies in platelets (Jones *et al.*, 2006), pA_2 values were lower than K_i values obtained in radioligand competition studies with platelet membrane preparations and for inhibition of carbacyclin-induced cAMP formation in cells over-expressing rc-IP receptors (Bley *et al.*, 2006); the difference was attributed to protein binding in studies involving PRP. The pA_2 values obtained in isolated blood vessel preparations (human pulmonary artery 8.20, guinea pig aorta 8.39 and rabbit mesenteric artery 8.12) were intermediate between values obtained in PRP and plasma membranes/cells in buffer (Jones *et al.*, 2006). The slight suppression of the cicaprost maximum response seen with higher concentrations of RO-1138452 was attributed to functional antagonism emanating from the (albeit weak) EP_3 agonist action of cicaprost. However, RO-1138452 displayed an insurmountable antagonist profile in studies of chemokine release from human airway epithelial cells using taprostene (see Table 1) as IP agonist (Ayer *et al.*, 2008). Moreover, RO-1138452 inhibition of taprostene-induced cAMP response element-dependent transcription was not reversed over a 20-h 'washout' period. These data could not be ascribed to covalent receptor inactivation, allosterism or a state of antagonist hemi-equilibrium and may be due to a pseudo-irreversible interaction with the IP receptor (Ayer *et al.*, 2008).

The N-substituted phenylalanines **22** and **23** (Nakae *et al.*, 2005) are weak IP antagonists, while the greater potency of **24** is associated with as *S*-configuration in the phenylalanine residue (Brescia *et al.*, 2007). RO-3244794 has no meaningful activity at EP_1, EP_3, EP_4 and TP receptors; no data were provided for DP_1, DP_2, EP_2 or FP receptors (Bley *et al.*, 2006). While RO-1138452 did not block EP_2 receptor-mediated relaxation in guinea pig aorta (pA_2 < 6.0; Jones *et al.*, 2006), RO-3244794 had a pA_2 of 6.92 for the human rc-EP_2 receptor expressed in HEK-293 cells (S.M. Hill and M.A. Giembycz, unpubl. data). Compound **24** did not bind to human EP_2 and EP_4 receptors (Brescia *et al.*, 2007). Strictly speaking, the missing data related to these compounds (e.g. RO-1138452 at DP_2 and FP receptors; Jones *et al.*, 2006) need to be addressed. In the context of systemic drug design, RO-3244794 has much greater oral bioavailability than RO-1138452 (51% vs. 0.7% in the rat) (Bley *et al.*, 2006).

Therapeutic applications

Pain has been the initial focus for IP antagonists based on IP receptor agonist effects and IP receptor distribution (reviewed by Bley *et al.*, 1998). Altered pain perception and inflammation were observed in $IP^{-/-}$ mice (Murata *et al.*, 1997). Subsequently, IP antagonists were shown to reduce pain responses in models where prostanoids have been implicated. These include acetic acid-induced abdominal constriction, mechanical hyperalgesia produced by carrageenan and pain associated with models of osteoarthritis and inflammatory arthritis (Bley *et al.*, 2006; Pulichino *et al.*, 2006). Such analgesic effects are accompanied by anti-inflammatory properties, which is invariably the case. In a collagen-induced arthritis model in mice, **21** produced effective inhibition when administered as a pretreatment, but was inactive when given after the initiation of the arthritis (Pulichino *et al.*, 2006). RO-3244794 and indomethacin were equi-effective in reducing carrageenan-induced rat paw oedema and more effective than rofecoxib in inhibiting the foot weight distribution change associated with intra-articular injection of monoiodoacetate (Bley *et al.*, 2006). Ostensibly, these results imply that IP receptors provide a singular target that would result in drugs that are at least as effective as NSAIDs and COX-2 inhibitors. The role of EP receptors in pain/inflammation has already been discussed. Despite a satisfactory preclinical profile, strong circumstantial evidence suggests that EP_1 antagonists were a failure in clinical trials. This, in turn, tends to cast doubt over the clinical prognosis for IP antagonists. Given the number of prostanoids that may be released locally and the even greater number of target receptors available, it may be regarded as counter-intuitive to propose that inflammation can be attributed to only one type of prostanoid receptor.

A role for PGI_2 in mediating the sensitized release of substance P from rat dorsal root ganglion neurons has been suggested based on studies with **23** (Nakae *et al.*, 2005). Related to this is the potential use of IP antagonists for bladder disorders. In rat models of bladder function, RO-3244019 dose-dependently decreased bladder contraction frequency and increased micturition threshold and voiding interval (Cefalu *et al.*, 2007). RO-3244019 was also effective in treating neurogenic detrusor overactivity arising from spinal cord injury in the rat (Khera *et al.*, 2007). Again, these results are of uncertain predictive value for clinical success, because indomethacin exhibited good activity in these models. The IP antagonist, BAY-73-1449 (Figure 7), was effective in acutely reducing shunt vessel blood flow in a rat model of portal hypertension (Bexis *et al.*, 2008); full details of its pharmacology are unavailable.

Finally, side effects originating from perturbation of the TXA_2/PGI_2 balance could be greater with IP antagonists than with COX-2 inhibitors, including hypertension, stroke, myocardial infarction and atherosclerosis. Nephrotoxicity and K^+ and Na^+ retention also may occur (Nasrallah and Hébert, 2005), because prostacyclin synthase (PGIS) and COX-2 deficiencies produce similar renal toxicity. This suggests a protective role for PGI_2, but it is important to note that no marked renal phenotype occurs in $TP^{-/-}$ or $IP^{-/-}$ mice (Breyer and Breyer, 2000; Yokoyama *et al.*, 2002; Nasrallah and Hébert, 2005).

TP receptor antagonists

Development

Elucidation of the structures of PGH_2 and TXA_2 (Figure 8) was soon followed by a range of carba/thia bicyclic analogues (see Wilson and Jones, 1985). One aim was to produce a chemically stable TP agonist (e.g. U-46619, STA_2); another was to investigate the potential for TP receptor antagonism. Thus, pinane-TXA_2 (PTA_2) was reported to block both constriction of cat coronary artery and aggregation of human platelets elicited by U-46619 (Nicolaou *et al.*, 1979). However, this analogue often behaves as a partial agonist, inducing a 60% maximal activation in some TP systems (Jones *et al.*, 1982; Tymkewycz *et al.*, 1991). More useful TP antagonists emerged from additional modification of the ω-chain. The pinane analogue ONO-11120 (Figure 8) is a true antagonist (Katsura *et al.*, 1983), while EP-045 (Jones *et al.*, 1982) and EP-092 (Armstrong *et al.*, 1985) were developed from the PG endoperoxide analogue, 9,11-etheno PGH_2, also a partial agonist (Jones *et al.*, 1982). SQ-29548 (Ogletree *et al.*, 1985) and BMS-180291 (Ogletree *et al.*, 1993) have a hybrid bicyclic system (oxabicyclo[2.2.1]heptane) and differ from the natural agonists in the *cis*-orientation of the α and ω-chains. While GR-32191 (Lumley *et al.*, 1989) and ICI-192605 (Brewster *et al.*, 1988) still retain a prostanoid skeleton, other TP antagonists do not, including L-655240 (related to indomethacin; Hall *et al.*, 1987), BM-13505 (daltroban; Yanagisawa *et al.*, 1987), KW-3635 (Karasawa *et al.*, 1991a,b), AA-2414 (seratrodast; Ashida *et al.*, 1989) and even the K_{ATP}-channel blocker glibenclamide (Cocks *et al.*, 1990). The attractively simple chemistry involved in the synthesis of daltroban has spurred the development of higher-potency, non-prostanoid antagonists containing a (*p*-halo)-benzenesulphonylaminomethyl residue at a critical distance from the carboxylate, for example, Z-335 (Tanaka *et al.*, 1998) and S-18886 (terutroban; Cimetière *et al.*, 1998). A similar residue in prostanoid molecules such as S-145 (domitroban; Mihara *et al.*, 1989), I-SAP (Naka *et al.*, 1992) (Figure 8), ONO-NT-126 (*p*-bromo;

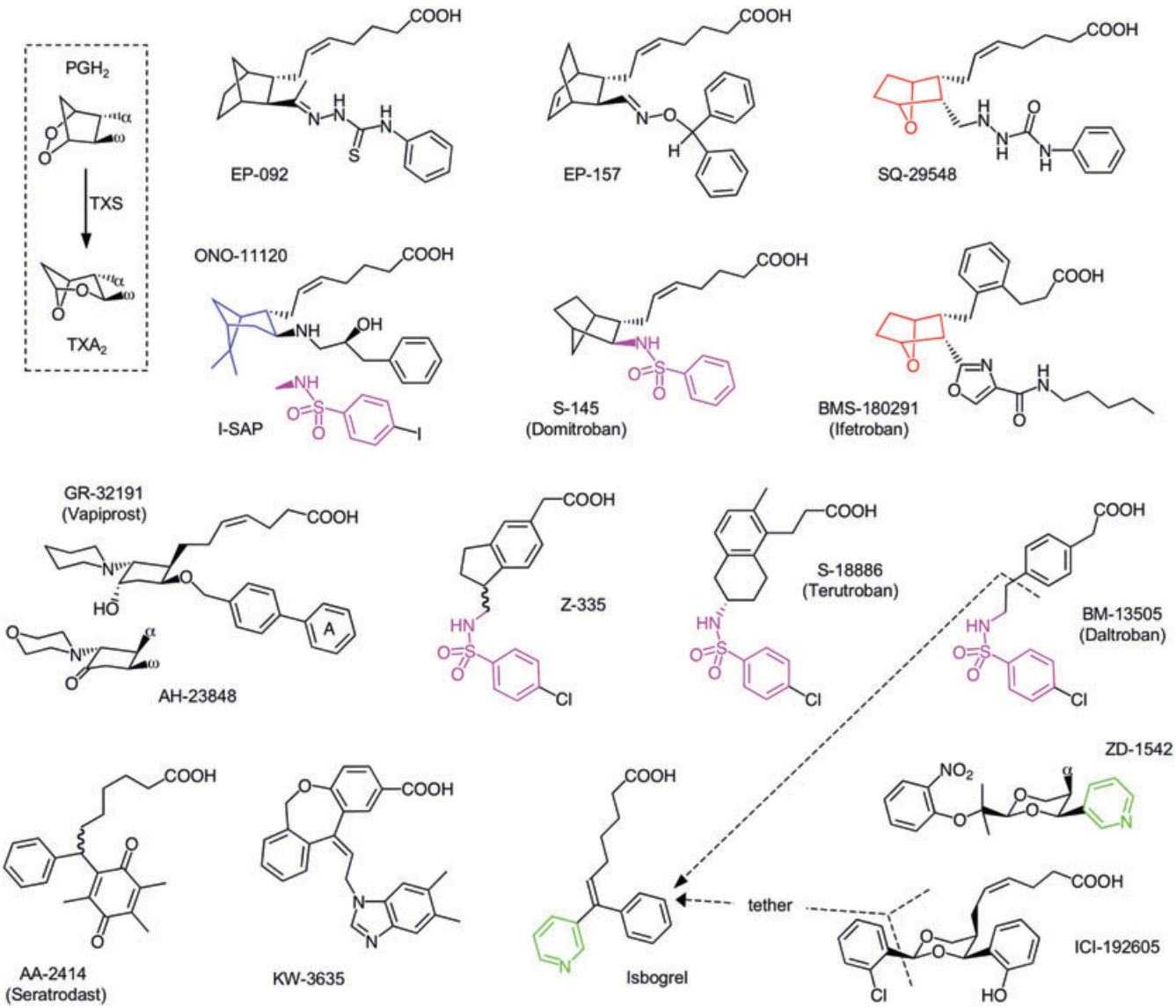

Figure 8 TP receptor antagonists. Conversion of PGH_2 to TXA_2 by thromboxane synthase (TXS) is shown in the box; α and ω represent natural 2-series side-chains. The pinane-thromboxane residue (related to 1(*S*)-α-pinene) is shown in blue; the 6-oxabicyclo(2.2.1)heptane system is in red. AH-23848 has the same α- and ω-chains as GR-32191. Benzenesulphonamide residues present in both prostanoid and non-prostanoid antagonists are shown in cerise. TP antagonists with two types of additional activity are presented. (A) IP agonism is conferred by the diphenylmethyl-heteroatomic unit in the bicyclo[2.2.2]octene analogue EP-157. (B) TXS inhibitory activity is conferred by the pyridin-3-yl residue (green) in isbogrel and ZD-1542 and by a similar replacement for ring A in relatives of GR-32191 (e.g. GR-83783; see text). Additionally, the broken arrows (lower right) typically indicate attachment of part of a TP antagonist to isbogrel (or ridogrel) to generate novel combined TP antagonist/TXS inhibitors; the tether (0–11 carbon units) has also been attached to the left-hand phenyl ring of ICI-192605 (2–8 carbon units) (Ackerley *et al.*, 1995).

Nakahata *et al.*, 1990) and oxa- and thia-bicyclo[3.1.0]hexane derivatives (Kamata *et al.*, 1990) also confers high TP affinity.

Differences in antagonist affinity constants between platelet and vascular smooth systems have stimulated much debate about the existence of TP receptor subtypes (Mais *et al.*, 1985; 1988; Swayne *et al.*, 1988; Morinelli *et al.*, 1989; Masuda *et al.*, 1991; Tymkewycz *et al.*, 1991; Folger *et al.*, 1992). Species heterology, the difficulty of distinguishing partial agonism from functional antagonism, and incomplete equilibration of high-affinity antagonists have been confounding factors. For example, antagonism of U-46619 by BMS-180291 on human platelets was surmountable for the shape-change response, but insurmountable for aggregation; the rate of aggregation was also slowed (Ogletree *et al.*, 1993). Similar profiles had been reported previously for EP-092 (Armstrong *et al.*, 1985) and GR-32191 (Lumley *et al.*, 1989) on human platelets, and for EP-169 and AH-23848 on human and rat platelets, but not on rabbit platelets where their affinities are lower (Tymkewycz *et al.*, 1991). However, against the slow-acting high-affinity TP agonist EP-171 (Jones *et al.*, 1989), GR-32191 did not alter the aggregation rate (Lumley *et al.*, 1989). It is likely that slow dissociation of a high-affinity antagonist from the TP receptor retards U-46619 occupancy

in the early stage of the aggregation response thereby favouring the disaggregation process and insurmountability; in contrast, shape change, which does not fade, allows a true measure of the equilibrium state. In pig platelets, longer preincubation times were required for S-145 than SQ-29548 owing to the smaller association rate constant of the former antagonist (Mihara *et al.*, 1989). BMS-180291 also had a Schild slope greater than unity on guinea pig aorta (Zhang *et al.*, 1996). However, in the low nanomolar range BMS-180291 requires up to 2 h to reach steady state on the aorta (Jones *et al.*, 2008) and the non-ideality may simply reflect retarded diffusion of a high-affinity ligand ($pA_2 = 9.8$, Table 2) through the extracellular space.

Radioligand binding studies of TP receptors coincided with these functional measurements. Two saturable binding sites were identified on human platelets using [^{3}H]-9,11-epoxymethano PGH_2, the first radioligand developed for the TP receptor (Armstrong *et al.*, 1983; Pollock *et al.*, 1984). Binding to the more abundant site was displaced by EP-045 at concentrations similar to those required to inhibit [^{3}H]-phosphatidate formation, Ca^{2+} elevation and aggregation induced by the TP agonist. A less abundant higher-affinity binding site was also identified, but it did not show the characteristic preference for a 15(*S*) configuration in the natural prostanoid ω-chain. The presence of high- and low-affinity binding sites for agonist (but not antagonist) ligands was also evident using [^{3}H]-trimetoquinol (Ahn *et al.*, 1988) and [^{3}H]-SQ-29548 (Hedberg *et al.*, 1988). Further studies showed that the high-affinity site was associated with the platelet shape change (and increase in cytosolic Ca^{2+}), while the lower-affinity site was associated with aggregation [and activation of phospholipase C (PLC)] (Dorn, 1989; Takahara *et al.*, 1990). [^{3}H]-GR-32191 played an important role in the elucidation of these relationships by binding reversibly to the 'shape change site' and irreversibly to the 'aggregation site' (Takahara *et al.*, 1990). It is difficult to explain this irreversibility given that GR-32191 does not obviously contain a chemically reactive group (Figure 8). Detailed studies have shown that 30-min exposure of human platelets to GR-32191 resulted in about 50% loss of binding sites for either [^{3}H]-GR-32191 or [^{3}H]-SQ-29548, while neither SQ-29548 nor BM-13177 affected B_{max}. It was speculated that GR-32191 binds to internalized TP receptors (Armstrong *et al.*, 1993); the zwitterionic nature of GR-32191 at neutral pH may be relevant. A light-activated, covalent-bonding TP antagonist, azido-BSP, also discriminated these platelet sites by blocking aggregation but not shape change induced by U-46619 (Zehender *et al.*, 1988). The subsequent identification of a second TP receptor isoform (TPβ) from a human umbilical vein endothelial cDNA library (Raychowdhury *et al.*, 1994) and the detection of mRNA for the α and β isoforms in human platelets (Hirata *et al.*, 1996) would appear to complete the argument. However, these isoforms, which arise by alternative gene splicing and differ only in their cytoplasmic tails, do not show the ligand discrimination typical of the high- and low-affinity binding sites. Finally, significant expression of the TPα isoform only was found in human platelets (Habib *et al.*, 1999).

The seventh transmembrane domain (TM-7), which is strictly conserved in all the TP receptors characterized to date, is critical to TP agonist and antagonist function. Point mutations in this domain in the human TP receptor severely suppressed the binding of SQ-29548 (Funk *et al.*, 1993b). Chimeric substitutions of the human TP receptor with the corresponding TM 1, 2 and 4 from rat resulted in modest suppression of SQ-29548 binding, lesser suppression of I-BOP (TP agonist) binding, and a poor correlation between the data sets (Dorn *et al.*, 1997).

Several different inhibitory properties have been combined with specific TP antagonism, either by chance or deliberately. For example, TP antagonists based on PGH_2 with diphenylmethyl-oxime (e.g. EP-157, Figure 8) or diphenylmethyl-azine residues in the ω-chain were found to activate IP receptors in platelet and vascular systems (Armstrong *et al.*, 1986; 1989; Jones *et al.*, 1993). A diaryl-hetero(cyclic) moiety is critical to the IP agonism (Jones *et al.*, 1993). Related compounds lacking a prostanoid ring system (e.g. octimibate) showed similar profiles (Merritt *et al.*, 1991a,b), with BMY-45778 being the most potent of a large series of non-prostanoid prostacyclin mimetics synthesized by Bristol-Myers Squibb (Meanwell *et al.*, 1994; Seiler *et al.*, 1997). Accurate estimation of the TP antagonist affinities of many of these compounds is difficult owing to their high lipophilicity, slow onset/offset (Jones *et al.*, 1997) and, in certain instances, an ability to inhibit (non-prostanoid) Gq-PLC-driven responses (Chow *et al.*, 2001).

Combining TP receptor antagonism with thromboxane synthase (TXS) inhibition has been an extensively investigated strategy, with the aim of balancing antagonistic/inhibitory activities several hours after dosing. TXS inhibition is expected to divert PGH_2 to PGD_2 and PGI_2 (Vermylen *et al.*, 1981; Smith, 1982), which both inhibit human platelet activation, and usually requires the presence of either a (*N*)-imidazole as found in dazoxiben (Randall *et al.*, 1981) or a pyridin-3-yl group as in ridogrel (Hoet *et al.*, 1990) and the related CV-4151 (isbogrel, Figure 8, Imura *et al.*, 1988). ZD-1542 (Brownlie *et al.*, 1993) is a pyridin-3-yl derivative related to ICI-192605, while GR-83783 (Campbell *et al.*, 1991a), a relative of GR-32191, has a 4-(pyridin-3-yl)-phenyl moiety (Figure 8). The (*N*)-benzimidazole KW-3635 did not inhibit cow platelet TXS at 100 μM (Miki *et al.*, 1992). Several groups have successfully expanded the biaryl region of ridogrel (Cozzi *et al.*, 1994) or combined sulotroban/daltroban moieties with ridogrel/isbogrel moieties (Figure 8), resulting in GR-108774 (Campbell *et al.*, 1991b), CGS-22652 (Bhagwat *et al.*, 1993) and compound **36** in Soyka *et al.* (1993). In addition, Zeneca have used various tethers to connect the whole or part of the ICI-192605 nucleus to either a dazoxiben or an isbogrel nucleus (Figure 8) (Ackerley *et al.*, 1995). BM-531 and BM-573 are combined TP antagonist/TXS inhibitors lacking a carboxylic acid group (Dogné *et al.*, 2001; Rolin *et al.*, 2001).

Finally, YM-158 has similar high affinity for TP and cys-LT receptors in guinea pig trachea (Arakida *et al.*, 1998) (see later).

Therapeutic applications

The discovery of thromboxanes was Nobel prize-winning research. Thromboxane A_2 is undoubtedly important in

regulating cardiovascular homeostasis. Its biosynthesis by platelets and other tissues and highly potent actions on platelets and blood vessels provided a strong impetus for the development of TP antagonists (Patrono, 1990; Patscheke, 1990; Davis-Bruno and Halushka, 1994). However, these agents have not been a success to date. Clear evidence of benefit was not established in early studies (Misra, 1994), but economics was also a major factor. Low-dose aspirin, which targets platelet TXA_2 synthesis by irreversibly acetylating COX-1, has proven benefits (Patrignani *et al.*, 1982; Fitzgerald *et al.*, 1983; Reilly and Fitzgerald, 1987). More potent TP antagonists, with superior pharmacokinetic profiles, were developed, but these also do not appear successful at the commercial level. The reasons are not entirely clear. At the pharmacological level, a TP antagonist would appear preferable to low-dose aspirin, especially in the light of recent events surrounding COX-2 inhibitors.

1. The clinical side effects associated with COX-2 inhibitor therapy reveal the TXA_2/PGI_2 balance as more delicate to perturbation and more important than was previously believed.
2. Low-dose aspirin does not always have the required TXS/PGIS selectivity (Knapp *et al.*, 1988; Patscheke, 1990).
3. Isoprostanes are alternative endogenous human TP receptor agonists and hence blockable by TP antagonists: their non-enzymatic formation would not be affected by aspirin, other COX inhibitors or TXS inhibitors (Kawikova *et al.*, 1996; Gardan *et al.*, 2000; Janssen *et al.*, 2001).
4. TP antagonists exhibit cardio-protective effects that are not shared by aspirin (Gomoll and Ogletree, 1994; Grover *et al.*, 1994).

Given these considerations, a potent, highly selective TP antagonist may be worth revisiting in large-scale clinical trials on cardiovascular disease. As oxidative stress and resultant formation of isoprostanes is now linked to atherogenesis (Dogné *et al.*, 2005), an initially unappreciated dimension to the TXA_2/PGI_2 balance and cardiovascular risk is made manifest. Specifically, the clinical outcomes would be related to stroke, heart attack and angina. Thromboxane A_2 has also been implicated in hypertension occurring in pregnancy and TP antagonists (/TXS inhibitors) have been proposed as treatments for pre-eclampsia (Keith *et al.*, 1993; Dogné *et al.*, 2006).

In addition to the vasculature, TXA_2 potently stimulates other smooth muscles to contract. Thus, TP antagonists have been proposed as potential therapeutic modalities for asthma. Seratrodast (Figure 8) has received marketing approval for treatment of asthma in Japan (see Rolin *et al.*, 2006). Ramatroban and seratrodast have also been evaluated in Phase III clinical trials in the USA (Dogné *et al.*, 2002). The therapeutic rationale is to ameliorate the marked bronchoconstriction produced by TXA_2 and its involvement in bronchial hyperesponsiveness. However, TXA_2 and other prostanoids that activate TP receptors (e.g. PGD_2) are not the only powerful bronchoconstrictors generated in asthma and it is unlikely that a TP antagonist alone would be adequate therapy in most patients. In this context, SQ-29548 and the cys-LT antagonist montelukast acting alone inconsistently inhibited contraction of human lung slices in culture challenged with antigen; a combination of the antagonists was much more effective, while an H_1 antagonist was ineffective (Wohlsen *et al.*, 2003). A combined TP/cys-LT antagonist (e.g. YM-158) may have greater clinical efficacy.

Activation of EP_3, FP, TP and possibly EP_1 receptors causes contraction of the pregnant human myometrium, while DP_1, EP_2 and IP receptors mediate relaxation (Senior *et al.*, 1993). Given this scenario, the potential of selective TP antagonists for treating labour-associated disorders, such as pre-term labour, would seem low. Recent studies on human myometrial specimens obtained at parturition provide a new perspective. It appears that there is a marked alteration in prostanoid receptor functional dynamics at term, prior to and following the onset of labour (Fischer *et al.*, 2008). Comparing the effects of U-46619, PGE_2 and $PGF_{2\alpha}$, a loss of FP but not TP responsiveness was apparent following the onset of labour (Fischer *et al.*, 2008). As PGE_2 produces a net inhibition of myogenic activity, this leaves TXA_2 as the only COX-derived product capable of exerting a contractile effect during labour. The role of TXA_2 in human parturition may be greater than previously envisaged.

The involvement of TXA_2 in inflammatory bowel disease may be significant (Rampton and Collins, 1993). The aetiology of ulcerative colitis and Crohns' disease is not fully understood, but initiating factors in pre-disposed individuals elicit severe and prolonged inflammation of the gut mucosa. Early studies with ridogrel and picotamide, combined TP antagonist/TXS inhibitors, claimed some clinical improvement associated with reduced TXA_2 (TXB_2) release (Rampton and Collins, 1993). Given the multifactorial nature of these diseases and the number of other eicosanoids purported to be involved, pronounced beneficial effects of TP antagonists alone seem unlikely.

Evidence is available that activation of the TP receptor is implicated in chronic persistent cough (CPC). This is a detrimental and debilitating condition that serves no obvious function (French *et al.*, 2002), afflicts 9–33% of the population in Europe and North America and for which no satisfactory treatment is currently available (see Chung and Pavord, 2008). Thus, the mechanisms underlying CPC and the identification of new anti-tussive agents present a grossly neglected and unmet clinical need.

In humans, PGs have high tussive potency suggesting that they may be released locally in a variety of respiratory diseases where cough is a characteristic symptom. In subjects with asthma, both indomethacin and ozagrel (OKY-046; a TXS inhibitor) increase the threshold for cough when compared with placebo, suggesting that TXA_2 may be one of the cyclooxygenase products that sensitize the cough reflex (Fujimura *et al.*, 1995). Indomethacin and ozagrel also attenuate cough in subjects with hypertension treated with angiotensin-converting enzyme inhibitors (Fogari *et al.*, 1992; Malini *et al.*, 1997; Umemura *et al.*, 1997). Thus, the potential application for TP antagonists in alleviating CPC is clear. Indeed, in an animal model of asthma-related cough, the TP antagonist, seratrodast (Figure 8) significantly suppressed cough induced by capsaicin (Xiang *et al.*, 2002). Moreover, in 16 patients with stable chronic bronchitis, seratrodast (80 mg b.i.d. for 28 days) significantly increased the threshold for capsaicin-

induced cough (Ishiura *et al.*, 2003). Finally, ramatroban is reported to attenuate cough in subjects with cough variant asthma (Kitamura *et al.*, 2003), which could be due to blockade of both TP and DP_2 receptors (Gardiner and Browne, 1984).

A role for TXA_2 in cancer of the female breast and genital tract has also been suggested. Findings are reminiscent of the TXA_2/PGI_2 balance in the cardiovascular system: TXA_2 promotes tumour growth and metastasis, while PGI_2 exerts a protective effect by maintaining vascular and platelet homeostasis (Honn and Meyer, 1981; Nigam *et al.*, 1992). A significant role in ovarian, cervical and other gynaecological cancers may exist (Nigam *et al.*, 1992), but there remains insufficient evidence to assign a pivotal role.

Thromboxanes have also been implicated in a number of other conditions, including glomerulonephritis (Patrono, 1990; Wardle, 1999), allergic conjunctivitis (Woodward *et al.*, 1990b), allergic rhinitis (Misra, 1994), diabetes (Dogné *et al.*, 2006), septic shock and pulmonary embolism (Ghuysen *et al.*, 2005). In summary, while numerous potential uses have been proposed over several years, clinical and economic success has been modest to date despite the availability of extremely potent and long-acting TP antagonists. What are the future therapeutic prospects? An argument could be made for superiority over low-dose aspirin. In order to establish this, extensive clinical trials would be essential. The bigger concern for the pharmaceutical industry would be 'how much better would a TP antagonist perform compared with low-dose aspirin?' If the difference is small, economics will dictate the fate of excellent TP antagonists. Perhaps the brightest new star in the galaxy of therapeutic options is the treatment of pre-term labour, given that TXA_2 is now known to be the major prostanoid involved in parturition. The only potential side effect that may occur with TP antagonist therapy is possible enhancement of hypersensitivity reactions (Narumiya, 2003). This would not be a major burden under most circumstances.

Therapeutic future of prostanoid receptor antagonists

The future for selective prostanoid antagonists does not appear to provide an encouraging scenario. Potent and selective antagonists for every prostanoid receptor are now available, with the exception of the EP_2 subtype. Some selective antagonists have received regulatory approval as drugs but these instances tend to be few and not worldwide. In this category are DP_2 and TP antagonists. Drug approval filings/development have been discontinued on numerous TP antagonists, EP_1 antagonists and, most recently, an EP_4 antagonist. Arguments in favour of more potent and irreversible antagonists have been put forward but, in the global scheme of drug research, the current drugs seem adequate at the very least.

DP_2 antagonists apart, of those drugs designed as selective for a single prostanoid receptor, the TP blockers still appear to be the best prospect. This would require revisiting cardiovascular studies and daring to directly compare with low-dose aspirin. The use of the TP antagonists for treating pre-term labour and even perhaps primary and secondary dysmenorrhoea should be contemplated in the light recent evidence (Fischer *et al.*, 2008). This is, however, a rather narrow spectrum of utility and does not fulfil three decades of high expectation.

Straightforward consideration of the current situation does not favour the widespread therapeutic utility of highly selective prostanoid receptor antagonists. Stated simply, where COX inhibitors are clinically effective, selective antagonists are not. This is not really astonishing. There are five major prostanoids biosynthesized by COX and additional active metabolites (e.g. 13,14-dihydro-15-oxo PGD_2, 19(R)-OH PGE_2). The isoprostanes are formed by non-enzymatic oxidation of arachidonic acid (Morrow *et al.*, 1990; 1994). Finally, there are the PG-ethanolamides (prostamides) and PG-glyceryl esters, which are COX-2 products of the endocannabinoids anandamide and 2-arachidonyl glycerol. Expectations that one prostanoid and one dedicated receptor play a dominant, all-important role in most disease processes are lofty. It is unlikely that this all distils down to one important receptor activity in most diseases: a receptor widely controlling synergy or solely mediating a critical pathophysiological event.

Individual PGs and their receptors may play compensatory, fail-safe roles. Individual prostanoids may act sequentially to initiate and sustain disease states. They may subserve complementary roles. The role of DP_1 and DP_2 receptors in allergy, notably allergic rhinitis, provides an excellent example of two receptors behaving in a complementary manner to initiate and maintain the disease state. The combination of DP_1/DP_2 antagonism in a single molecule appears a very promising therapeutic approach (Pettipher, 2008) and one that appears feasible. Equally so, combining TP and EP_3 antagonistic properties to prevent both platelet activation and vasoconstriction in cardiovascular disease states appears to be an achievable goal. Prostanoid-based drugs for pre-term labour may require even more versatility, with ideal therapy perhaps embodying. the following attributes in a single molecule: (i) TP antagonism, (ii) EP_3 antagonism to block cervical ripening and (iii) EP_2 agonism to provide a tocolytic effect. Such a molecule would be a formidable medicinal chemistry challenge. Further challenges relate to diseases where COX inhibitors are widely and successfully used, because the ideal spectrum of antagonist properties may not be entirely clear. If the promise of prostanoid-based therapeutics is ever to be fulfilled, the role of prostaglandins may need to be carefully thought-out on a disease-by-disease basis.

Conflicts of interest

The authors affirm no conflicts of interest.

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