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Teaser The structural requirements for novel highly potent and selective agonists and antagonists of prostaglandin EP1 to EP4 receptors and their therapeutic potential are presented in this original review.

Structural features of subtype-selective EP receptor modulators

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Prostaglandin E2 is a potent endogenous molecule that binds to four different G-protein-coupled receptors: EP1–4. Each of these receptors is a valuable drug target, with distinct tissue localisation and signalling pathways. We review the structural features of EP modulators required for subtype-selective activity, as well as the structural requirements for improved pharmacokinetic parameters. Novel EP receptor subtype selective agonists and antagonists appear to be valuable drug candidates in the therapy of many pathophysiological states, including ulcerative colitis, glaucoma, bone healing, B cell lymphoma, neurological diseases, among others, which have been studied *in vitro*, *in vivo* and in early phase clinical trials.

Introduction

Prostaglandin E2 (PGE2) is the most widely produced prostanoid in the human body. It signals via four specific G-protein-coupled receptors: EP1–4, responsible for distinct biological outcomes [1]. The engineering of mice deficient in each respective EP receptor enabled the elucidation of PGE2-mediated processes including inflammation, bone healing, regulation of gastrointestinal mucosa, embryo implantation, induction of labour and vasodilatation [2–4]. The understanding of the role of PGE2 in human physiological and pathophysiological conditions has also been facilitated by the development of highly selective agonists and antagonists of these receptors [2–5]. Manipulation of PGE2-mediated processes would be of great importance, in particular if the enormous therapeutic potential of selective EP receptor modulation could be exploited.

PGE2 is a very unstable molecule with an extremely short half-life. It rapidly undergoes terminal- and beta-oxidation *in vivo* and is of limited clinical use. PGE2 is approved for human use to induce childbirth and as a vasodilator in severe ischemia or pulmonary hypertension. Its other therapeutic potentialities are not exploited owing to its systemic effects, including side-effects such as lethargy, headache, diarrhoea and flushing [2,3]. To overcome these unwanted effects of PGE2, novel potent and specific agonists and antagonists of distinct EP receptors have been developed, especially in the past decade, exploring a vast diversity of scaffolds. The timeline of pivotal discoveries concerning EP receptors and their modulators is depicted in Fig. 1. We

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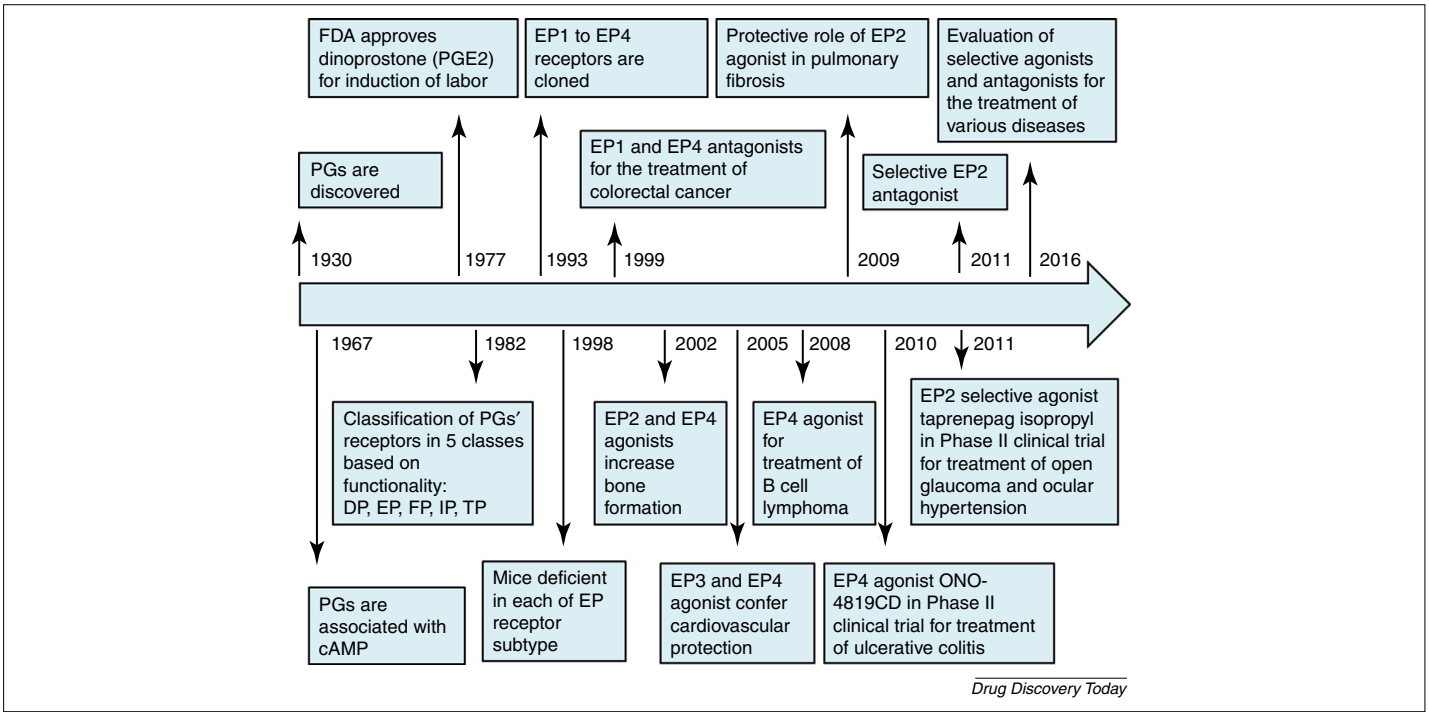

FIGURE 1

Chronological overview of key discoveries in the field of prostaglandin E2 (PGE2) receptor modulators [4,5,14,16,31,32,67–69,78–80,85–103]. *Abbreviations:* DP, prostaglandin D2 receptor; EP, prostaglsndin E2 receptor; FP, prostaglandin F2α receptor; IP, prostacyclin receptor; TP, thromboxane A2 receptor.

review novel potent and selective ligands of EP receptors reported in the past decade with the emphasis on the diversity of reported scaffolds, followed by the reported therapeutic indications and an overview of relevant clinical trials.

PGE2 receptor subtypes

PGE2 is a potent endogenous molecule, synthesised by constitutive (COX-1) and inducible (COX-2) cyclooxygenases and the cytosolic (cPGES-1) and microsomal (mPGES-1 and mPGES-2) prostaglandin E synthases (Fig. 2) [1]. It binds to prostaglandin receptors EP1–4. All four EP receptor subtypes respond to PGE2, despite their relatively low amino acid sequence similarities. The percentages of sequence similarities of EP1 to EP2, EP3 and EP4 are 30%, 33% and 28%, respectively. The EP2 and EP4 receptors, which have similar signalling pathways via activation of adenylate cyclase, share only 31% homology [4,6].

Each EP receptor subtype is specifically distributed in the human body; EP1: myometrium, pulmonary veins, colon, skin, mast cells; EP2: leukocytes, smooth muscle, central nervous system (CNS), reproductive system, bones; EP3: CNS, cardiovascular system, reproductive system, kidney, urinary bladder; EP4: leukocytes, smooth muscle, cardiovascular system and bones [4,5]. Among the four PGE2 receptors, EP3 and EP4 are the most widely expressed. By contrast, the distribution of the EP1 receptor is restricted to a few organs in humans whereas it is more widely distributed in rodents [5]. All EP receptor subtypes are present on the plasma membrane; EP3 and EP4 are also expressed on the cell nuclei membranes [7]. In addition to the differences in tissue localisation, EP receptor subtypes also differ in their expression regulation mechanisms and signal transduction pathways [4].

Signal transduction pathways

The PGE2 receptors EP1–4 as well as other prostanoid receptors DP, FP, IP and TP are G-protein-coupled receptors [5]. The EP receptors are composed of seven transmembrane segments with each receptor subtype being coupled to a different subunit of heterotrimeric G proteins. Ligand binding to different EP receptors leads to the activation of distinct downstream signalling pathways (Fig. 2) [6].

The EP1 receptor is somewhat unique among the PGE2 receptors in that it is coupled to Gq protein and belongs to a 'contractile' receptor group. The binding of PGE2 to the EP1 receptor activates phospholipase C (PLC), which mediates the activation of protein kinase C (PKC), in turn resulting in increased phosphatidylinositol hydrolysis and the elevation of the intracellular calcium concentration [8]. The activation of the EP3 receptor results in the inhibition of adenylate cyclase via Gi protein, which lowers cyclic adenosine monophosphate (cAMP) levels; EP3 has therefore been classified as an 'inhibitory' receptor. Three isoforms of mouse EP3 and eight of human EP3 have been identified [9,10]. The EP2 and EP4 receptors activate adenylate cyclase and increase cAMP levels by coupling to Gs proteins and have been classified as 'relaxant' receptors. Interestingly, in many processes EP2 and EP4 play different parts. Obviously, EP receptors do not couple exclusively to the pathways described, but often to more than one G protein and the underlying signal transduction pathway [11,12] (Fig. 2). For instance, EP4 receptor signals via Gi, phosphatidylinositol 3-kinase (PI3K), β-arrestin or β-catenin [13,14]. The co-localisation of the EP4 receptor with an EP4-receptor-associated protein (EPRAP) has revealed an interaction with the transcription factor nuclear factor (NF)-κB that causes a decrease in the secretion of proinflammatory cytokines [6,15,16].

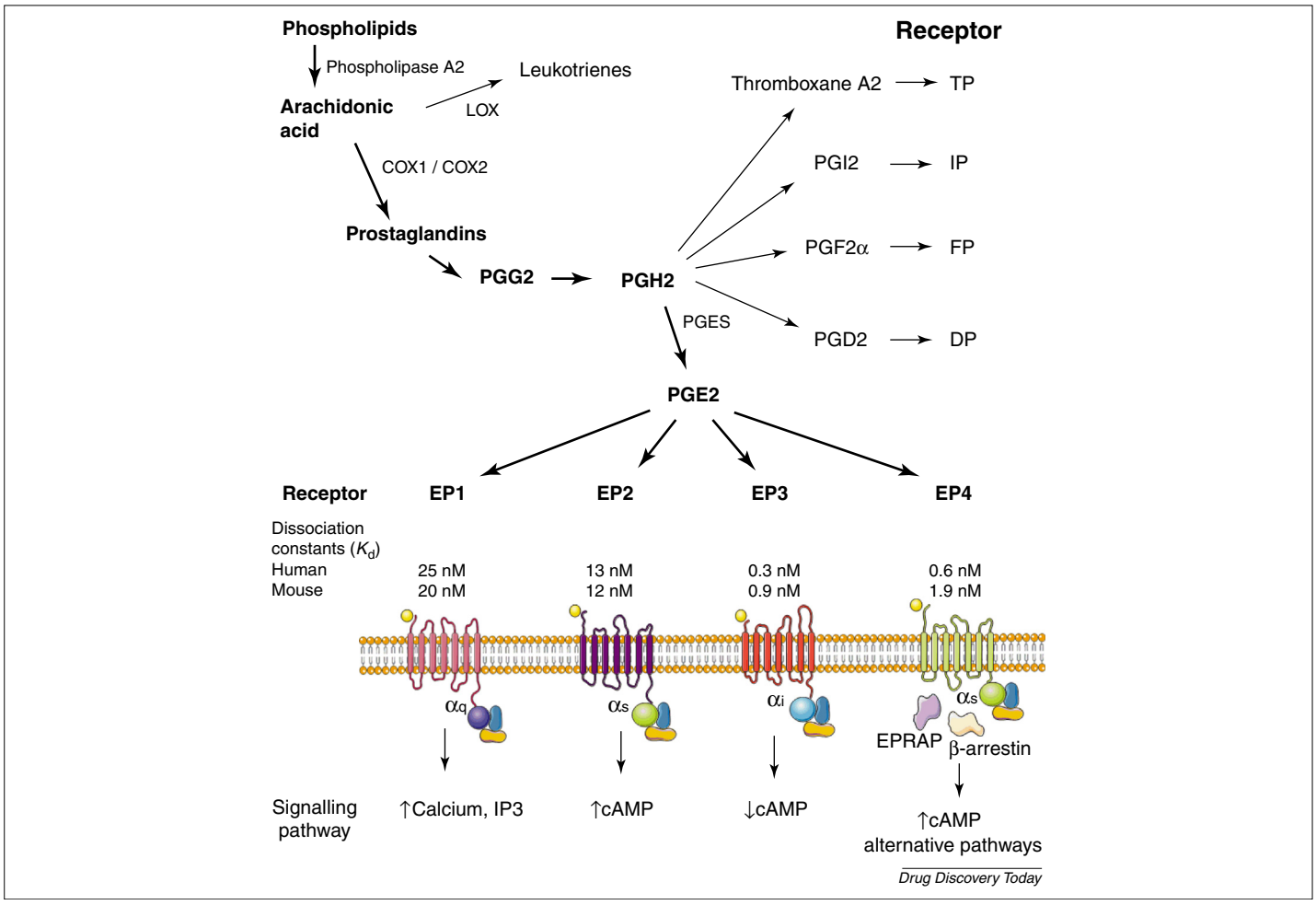

FIGURE 2

Prostaglandin E2 (PGE2) signalling. Overview of the arachidonic acid cascade, PGE2 synthesis and its binding affinities to EP receptor subtypes involving their major signalling pathways. PGE2 is one of the prostanoids involved in the arachidonic acid cascade. The latter is initiated by various physiological and pathophysiological stimuli, which, in turn, result in the hydrolysis of the ester linkage that binds arachidonic acid to glycerol in phospholipids by phospholipase A2. Next, arachidonic acid undergoes oxidation to eicosanoids, a process catalysed predominantly by two groups of enzymes, cyclooxygenases (COX) and lipoxygenases (LOX), which produce prostanoids and leukotrienes, respectively. COX enzymes catalyse the formation of prostaglandin PGH2 in the sequential step of PG synthesis involving the formation of the intermediate PGG2. PGH2 then serves as a key substrate for the conversion to prostaglandins PGD2, PGE2, PGF2, PGI2 and thromboxane A2 by cell-specific synthases or isomerases. These potent prostanoid signalling molecules exert their effects through autocrine and paracrine stimulation of eight specific G-protein-coupled receptors (GPCRs), designated DP, EP1 to EP4, FP, IP and TP. PGE2 binds to the prostaglandin receptors EP1–4. Dissociation constants (K_d) of PGE2 are as follows: EP1: 25 nM (human); 20 nM (mouse); EP2: 13 nM (human); 12 nM (mouse); EP3: 0.3 nM (human); 0.9 nM (mouse); EP4: 0.6 nM (human); 1.9 nM (mouse). Major signalling pathways involve activation of adenylate cyclase (EP2 and EP4) and consequent increase in cAMP, inhibition of adenylate cyclase (EP3), decrease in cAMP and activation of phospholipase C, resulting in the increase in intracellular calcium (EP1). Furthermore, binding of PGE2 to EP4 receptor results in activation of alternative pathways. *Abbreviations*: COX, cyclooxygenase; LOX, lipoxigenase; PGG2, prostaglandin G2; PGH2, prostaglandin H2; PGES, prostaglandin E synthases; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PGF2α, prostaglandin F2α; PGI2, prostaglandin I2; EPRAP, EP4 receptor-associated protein; IP3, inositol trisphosphate; cAMP, cyclic adenosine monophosphate. This figure was produced using Servier Medical Art. Adapted, from [6,11,12,17,18,104].

The signalling pathways activated through distinct PGE2 receptor subtypes are dependent on the concentration and the conformation of the cognate ligand of the subtype [6]. PGE2 binding affinities for each EP receptor subtype were determined for human and mouse prostanoid receptors [17,18] (Fig. 2). Equilibrium dissociation constants (K_d) of the radiolabelled ligand PGE2 were determined for each receptor subtype. PGE2 retains agonist activity in the nanomolar range at all four EP receptor subtypes, although they only share 20–30% of their structural homology. In brief, PGE2 has been shown to bind to EP3 and EP4 receptors with higher affinities when compared with the affinities towards EP1 and EP2 receptors, with K_d values ranging from 0.3 to 25 nM [17,18] (Fig. 2).

In summary, the interactions between PGE2 and the EP receptors depend on tissue or cell type, specific receptor expression and differences in binding affinities, leading to unique EP receptor activation. Hence, PGE2 has the ability to cause diverse effects in many different tissues, thus playing distinct parts in different physiological and pathophysiological conditions.

EP receptor modulators

PGE2 is composed of a cyclopentanone core with appended α- and ω-lipophilic side-chains at position-8 and -12. The α-chain incorporates a 5,6-double-bond element and is terminated by a carboxylic moiety. The ω-chain ends with a methyl group and it also

carries a 13,14-unsaturated-alkene moiety. Further, PGE2 features two hydroxyl groups, one attached to position-11 of the cyclopentanone scaffold and the other to position-15 of the ω-chain. It is worth noting that the natural stereochemistry of PGE2 corresponds to the 8-(*R*), 12-(*R*), 15-(*S*) configuration. The prostaglandin numbering and all structural features of PGE2 (**1**) are depicted in detail in Fig. 3a.

Owing to the pleiotropic effects of PGE2, mediated by EP1–4, EP receptor subtype specific ligands constitute a hot topic in medicinal chemistry. The well characterised structure of prostanoid receptors enables a systematic analysis of the binding characteristics of a therapeutic and the determination of the selectivity for those receptors. Several recently patented ligands and general structures have been reviewed [19]. Because very little bioactivity and biologically oriented data are included in the patent literature, we focused our attention predominantly on published papers containing complete affinity and selectivity data. The affinity data of presented ligands of EP receptors (expressed as K_i or pK_i) and their potency and activity data (expressed as EC_{50}, IC_{50} or K_b values) are listed in Table S1 (see supplementary material online). We discuss these ligands in terms of their oral bioavailability and CNS penetration – their ability to cross the blood–brain barrier (pharmacokinetic data of selected ligands are listed in Table S2, see supplementary material online). This ability determines whether, and to what extent, these ligands will mediate central versus peripheral EP-mediated activity.

EP1 receptor modulators

Despite the well-established key pharmacophores required for EP1 receptor agonist activity of prostaglandin derivatives [20], only one EP1-selective agonist: ONO-D1-004 (**2**) (K_i = 150 nM), has been identified to date (Fig. 3b) [21]. By contrast, several dual EP1/EP3 agonists with nanomolar affinities to both receptors have been reported, including 17-phenyltrinor PGE2 and carbacyclin, to name but two [18,20]. Most marketed prostanoid agonists, although fairly potent, exhibit poor selectivity.

The majority of EP1 ligands were designed as antagonists of this receptor to be used as potential treatment for hyperalgesia (shown in Fig. 3c). Surprisingly, none of these ligands are structurally similar to PGE2. The tricyclic EP1 antagonist SC51322 (**3**) displays a low nanomolar affinity for the EP1 receptor (K_i = 13.8 nM) [17]. Compound **4** belongs the biphenylene dibenzazocinone class of compounds, and is proven to be a selective and potent EP1 receptor antagonist (K_i = 17 nM) and structurally resembling SC51322 [22]. Ono Pharmaceuticals disclosed a set of *N*-phenylheteroarylsulfonamides, exemplified by compound **5**, which have inhibitory activities in the low nanomolar range and high selectivity for the EP1 receptor [23]. Unfortunately, these compounds contain a highly lipophilic arylsulfonyl moiety that precluded them from entering clinical studies. Compound **6**, developed by Astellas Pharma, was based on a scaffold related to compound **5** and was also found to exhibit subnanomolar affinity with a K_i value of 0.33 nM [19].

Compound **7**, which belongs to a structurally different class of substituted indazoles developed by Asahi Kasei Pharma, has an IC_{50} value of <20 nM [19]. Initial research at GlaxoSmithKline (GSK) has demonstrated that GW-848687X (**8**), which incorporates a 1,2-biaryl-cyclopentene pharmacophore, possesses nanomolar affinity for the EP1 receptor (pK_i = 8.6) while also displaying high selectivity and, most importantly, *in vivo* efficacy in inflammatory pain preclinical models, owing to good CNS penetration [24]. Because GW-848687X (**8**) might undergo oxidation at the allylic position of the cyclopentene ring under *in vivo* conditions, a potential backup compound, GSK-345931A (**9**), has been developed that carries an *ortho*-substituted aryl moiety in place of the cyclopentene ring. Although it possesses a slightly lower binding affinity (pK_i = 7.8), compound **9** displays improved pharmacokinetic parameters, including good metabolic stability, as well as good CNS penetration and, above all, excellent *in vivo* efficacy [25]. Further efforts led to the discovery of a methylene-linked picolinic acid derivative GSK-269984A (**10**) that overcame the major development issues encountered with previous compounds; consequently, it was selected as a development candidate for treatment of inflammatory pain [25]. In addition, the researchers at GSK identified the first nonacidic benzofuran-based EP1 antagonist (**11**) (pK_i = 8.0) with good CNS penetration and promising efficacy [26]. Several other EP1 antagonists exhibiting affinity in the nanomolar range have been described [19]; however, they have not been included in this review owing to a lack of selectivity data.

EP2 receptor modulators

Increased EP2 transduced signalling is implicated in seizures and epilepsy, whereas its decrease is implicated in glaucoma, bone healing and pulmonary fibrosis. Thus, agonists and antagonists are of potential clinical use. However, in contrast to the EP1 modulators, the field of EP2 modulators has been focused on the development of selective agonists, whereas little effort has been made to discover selective antagonists. As a result, three classes of EP2 receptor agonists have been reported to date, and EP2-selective antagonists have emerged only recently (shown in Fig. 3d and e, respectively).

The first class of agonists comprises ligands that structurally resemble the endogenous ligand PGE2 but incorporate major modifications in the ω-lipophilic chain that contribute to enhanced potency and selectivity. The free acid metabolite of butaprost (**12**) was one of the first selective EP2 agonists reported [27]; however, its clinical usefulness is limited because of its chemical instability and weak potency relative to PGE2. More specifically, the hydroxy-cyclopentanone ring of butaprost is prone to dehydration, which renders the compound inactive. Nevertheless, its uniquely designed ω-chain provided a template for the development of several more-potent and more-selective EP2 agonists of the prostanoid type, 16-hydroxy-17,12-trimethylene PGF derivatives. By virtue of a minor chemical modification, namely the introduction of a 9β-chloro group in place of the C9-carbonyl moiety, compounds **13** (EC_{50} = 2.8 nM; K_i = 2.2 nM) and **14** (EC_{50} = 1.8 nM; K_i = 1.7 nM) have been obtained. These compounds are much more resistant to degradation and have a promising selectivity and agonist activity profile [27,28]. Compound **13** has been additionally stabilised as a lysine salt. The second class of agonists is a series of pyridyl-sulfonamide derivatives developed by Pfizer, the most potent being taprenepag isopropyl [also designated PF-04217329 (**15**)], the prodrug of active acid metabolite CP-544326 (**16**) (K_i = 10 nM; EC_{50} = 2.8 nM) [29].

Compound **17** belongs to the third class, a set of pyridylaminoacetic acids disclosed by Ube Industries. It is the most potent

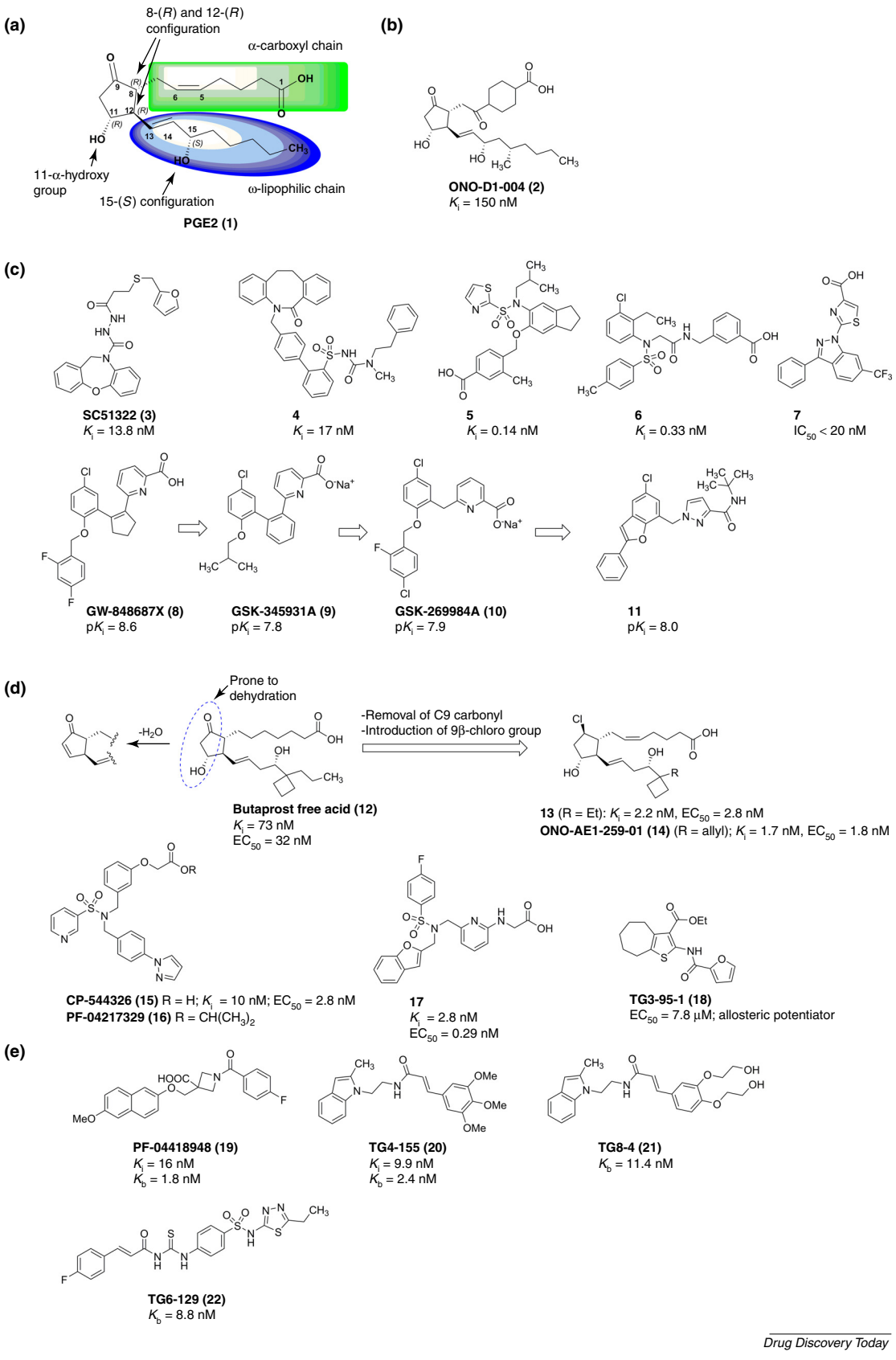

FIGURE 3

Key structural features of prostaglandin E2 (PGE2) and subtype-selective modulators of EP1 and EP2 receptor. **(a)** Key structural features of PGE2. **(b)** Selective agonists of EP1 receptor. **(c)** Selective antagonists of EP1 receptor. **(d)** Selective agonists of EP2 receptor. **(e)** Selective antagonists of EP2 receptor.

EP2 agonist to date, with regard to its EC_{50} value of 0.28 nM and also in terms of binding affinity (K_i = 2.8 nM) [30]. It should be noted that the report lacked data regarding the selectivity of the compound. Finally, TG3-95-1 (**18**), although only weakly active (EC_{50} = 7.8 μM), is worth mentioning because it represents the only identified EP2-selective class of allosteric potentiators [29].

Only a few selective EP2 antagonists have been reported (Fig. 3e). PF-04418948 (**19**), an azetidine-3-carboxylic acid derivative, was the first selective EP2 antagonist, it has an IC_{50} of 16 nM (K_b = 1.8 nM), exhibiting >10 000-fold increase in selectivity for the EP2 receptor relative to other prostanoid receptors [29,31]. In addition, cinnamic amides have been identified as a new class of selective EP2 antagonists. Two representatives of this class are shown in Fig. 3e: compound TG4-155 (**20**) and compound TG8-4 (**21**), with K_b values of 2.4 nM and 11.4 nM, respectively. Despite the structural similarity between these two compounds, the introduction of two pendant hydroxyethyl ether moieties in the structure of TG8-4 brought about a significantly enhanced aqueous solubility.

TG8-4 also displays improved EP2 selectivity over prostanoid receptors relative to that of TG4-155. Recently, another chemical class of carbamothioacrylamide antagonists has been discovered by the Ganesh group, as exemplified by TG6-129 (**22**), which displays potency at nanomolar concentrations (K_b = 8.8 nM) and also possesses high selectivity [32]. In contrast to TG4-155 and TG8-4, TG6-129 cannot cross the blood–brain barrier, with the consequence that it might only be useful as a probe for peripheral disease models. Other EP2 agonists and antagonists of potential importance reported in the patent literature will not be discussed here because they have been highlighted in recent reviews [19,29].

EP3 receptor modulators

In the field of EP3 receptor modulators, the main focus has been on the discovery of novel selective antagonists of the EP3 receptor (examples are shown in Fig. 4a) as potential antithrombotics. Most selective antagonists of the EP3 receptor consist of an acidic proton and a lipophilic tail. Initially, Merck reported a series of biaryl-ene-acylsulfonamides exemplified by compound L798,106 (**23**) with a K_i value of 0.3 nM [33]. Afterwards, two additional classes of selective EP3 antagonists were developed based on this series, differing in the nature of the acidic moiety. It is worth noting that the acylsulfonamide moiety undergoes rapid hydrolysis to the corresponding sulfonamide *in vivo*. To counteract this negative characteristic, structurally related *ortho*-substituted cinnamic acid derivatives [exemplified by compound **24** (K_i = 3 nM)], in which the acylsulfonamide moiety was replaced by a carboxy group, were developed as potent and selective antagonists of the EP3 receptor [34]. Later on, yet another class of *N*-acylsulfonamides **25** (K_i = 0.086 nM) and **26** (K_i = 0.16 nM), developed on the basis of L798,106 (**23**), represent the second class of highly potent and selective EP3 antagonists that carry a novel structural feature: the *N*-(3,4-difluorobenzene)sulfonyl moiety. Incorporation of this moiety rendered the compounds active *in vivo* [35]. Additionally, a series of 1,7-disubstituted indole scaffold-based EP3 antagonists have been reported by DeCode Genetics, the most prominent compound DG-041 (**27**) possesses an IC_{50} value of 4.6 nM [36–38]. Surprisingly, DG-041 displays good plasma and metabolic stability in spite of the presence of an acylsulfonamide group. A different approach was employed by Gallant *et al.* who discovered that the introduction of a propenoic acid group on a lipophilic triaryl scaffold results in a potent EP3 antagonist. The ensuing optimisation of this hit compound included the introduction of an *ortho*-substituted lipophilic tail and the incorporation of a constrained cyclopropan tether, ultimately resulting in compound **28**, possessing a K_i value of 3 nM and an acceptable selectivity profile over other prostanoid receptors [39].

Ono Pharmaceuticals, a dominant force in the research and development of novel EP modulators, developed several EP3-selective antagonists. Notably, ONO-AE3-240 (**29**) and compound **30** display extremely high binding affinity to the EP3 receptor, with K_i values of 0.23 nM and 0.068 nM, respectively, as well as high selectivity compared with other prostanoid receptors [40,41]. In recent years, other pharmacophores for potent EP3 antagonists have emerged, taking the form of the 3-oxazolidinone-substituted pyridinone **31** [42] and the aminothiadiazole **32** [43] which, structurally speaking, differ significantly from all previously reported EP3 antagonists. Both exhibit potent and selective EP3-antagonistic functional activities in addition to excellent pharmacokinetic properties.

Despite the industry focus on EP3 antagonists, several EP3-selective agonists have also been identified in the past decade as potential antiulcer agents (Fig. 4b). Sulprostone (**33**), with an EC_{50} value of 0.42 nM, has been identified as one of the first potent and selective EP3 agonists [17]. Further, Savage *et al.* reported the identification of SC-46275 (**34**) as the most potent and highly selective agonist of the EP3 subtype receptor discovered thus far (EC_{50} = 0.04 nM) [44]. Similarly, compound MB-28767 (**35**) displays potency in the subnanomolar range (EC_{50} = 0.55 nM); however, it also displayed significant TP agonism. Researchers at Ono Pharmaceuticals identified ONO-AE-248 (**36**) as one of the most selective and potent EP3 agonists [21]. It is unique in its structure because 11- and 15-OH groups benefit from protection in the form of methyl ethers. Further, Shimazaki *et al.* reported the discovery of a new class of selective EP3 agonists based on a 13,14-dehydro-16-phenoxy PGE scaffold [45]. The introduction of the phenoxy group to the ω-side-chain resulted in enhanced selectivity for EP3, as exemplified by compound **37**. However, it proved to be inactive in terms of pharmacologic activity owing to its rapid hydrolysis in *in vivo* conditions. To overcome this issue, the methyl ester of **37** was (i) replaced by a bulky ester moiety (compound **38**) or (ii) reduced to its alcohol congener (compound **39**), resulting in more-stable derivatives [45]. Although most of the reported agonists are based on a prostanoid scaffold, TEI-3356 (**40**) is a unique ligand in that it is a prostacyclin analogue [46]. It displays high binding affinity – with an IC_{50} value of 10 pM – and high potency, as displayed by its EC_{50} of 10 nM.

EP4 receptor modulators

Papers and patents describing the discovery of novel EP4 receptor modulators represent the majority of publications describing EP modulators. The first reported EP4 antagonist, AH-23848, proved nonselective and has since been overtaken by more-potent and -selective ligands. Various companies, such as Merck Frosst, GSK and Ono Pharmaceuticals have put significant effort into generating novel agonists and antagonists of this receptor [19]. In particular, agonists belonging to distinct structural classes

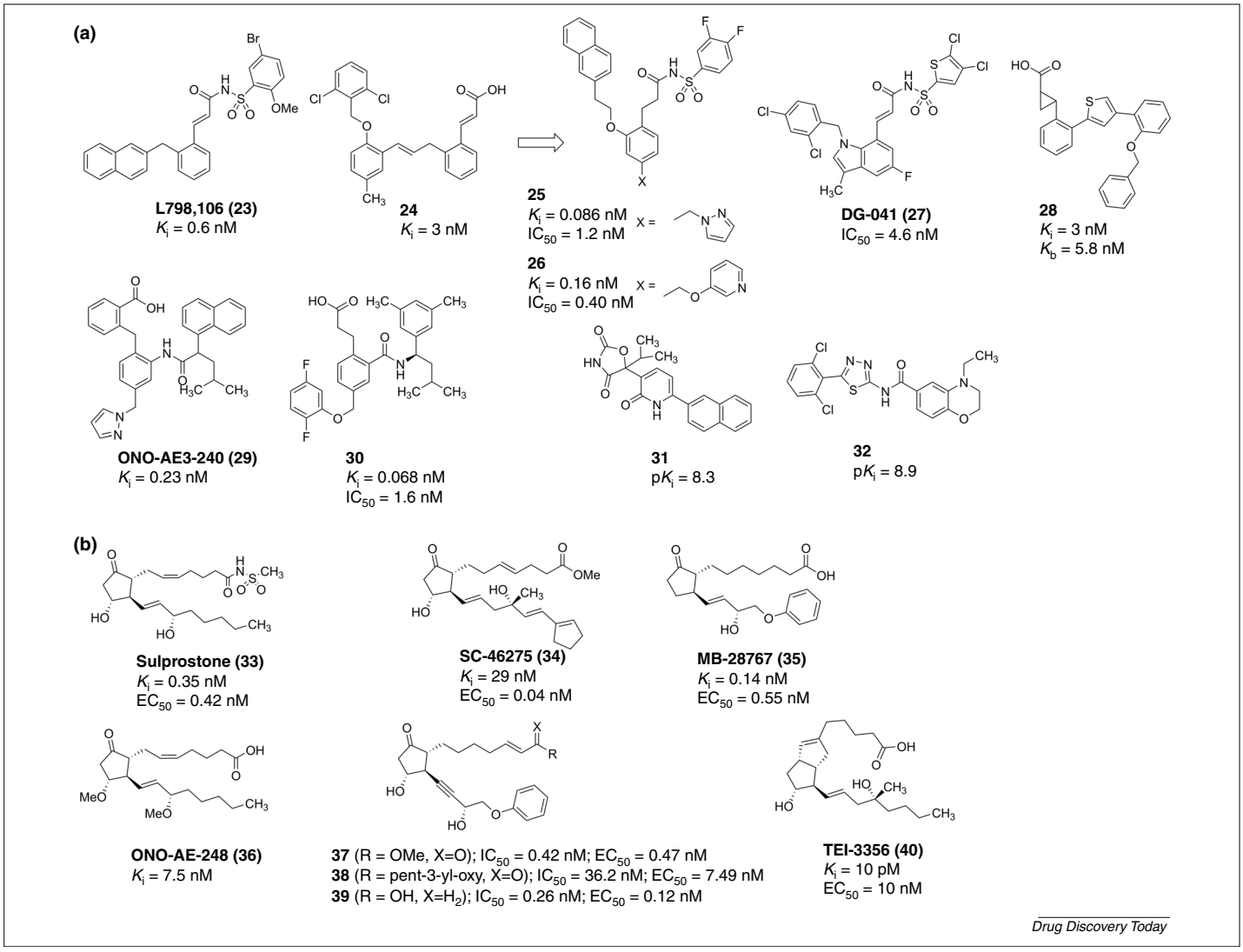

FIGURE 4

Selective modulators of EP3 receptor. **(a)** Selective antagonists of EP3 receptor. **(b)** Selective agonists of EP3 receptor.

(depicted in Fig. 5) were developed as potential drug candidates for the treatment of osteoporosis and ulcerative colitis.

The first class of EP4-selective agonists is based on the cyclopentanone core (shown in Fig. 5a), exemplified by a series of 3,7-dithiaPGE1 derivatives, including compound ONO-AE1-329 (**41**) (EC_{50} = 3.1 nM). Introduction of thioether features into the α-chain achieved a marked enhancement of activity and selectivity; however, this type of compound proved to be unstable [47]. It has been observed that these compounds undergo not only self-degradation but also epimerization at position-8 (according to prostaglandin numbering), this being a result of rapid enolization (Fig. 5a) [48]. To pursue the goal of developing more chemically stable EP4 agonists, further chemical modifications were made to the molecule. A combination of the removal of the 7-thia moiety, the shift of sulfur to the 5-position of the α-chain and the introduction of the 16-(*m*-methoxylmethyl)phenyl moiety as the ω-chain resulted in the discovery of compound ONO-AE1-734 (**42**), optimised in terms of stability and agonist activity (EC_{50} = 1.6 nM) [48]. Further optimisations resulted in the 9β-chloro derivatives, exemplified by compound **43**, which were designed to prevent the well-known self-degradation process and dehydration [48]. The obtained compounds **41**–**43** also retained high subtype selectivities, but exhibited poor pharmacokinetics. Owing to the poor pharmacokinetics of this class of compounds, researchers turned their attention towards the development of orally available drug candidates. The focus was shifted towards the derivatives belonging to the lactam structural class encompassing pyrrolidinones, piperidones and pyrazolidinones (Fig. 5b), because the preliminary findings had revealed 8-aza-11-deoxyPGE1 (**44**), bearing the 12(*R*),15(*S*)-configuration and corresponding to the natural stereochemistry of PGE2, to be a highly selective agonist of the EP4 receptor [49]. Subsequently, it became apparent that the replacement of the hydroxy-cyclopentanone ring with its 2-pyrrolidinone counterpart was itself sufficient for achieving subtype selectivity. More importantly, the 2-pyrrolidinone heterocycle also imparts chemical as well as metabolic stability to the molecules. The absence of the C11-OH group of the original PGE2 prevents β-elimination, but showed no detrimental effect on

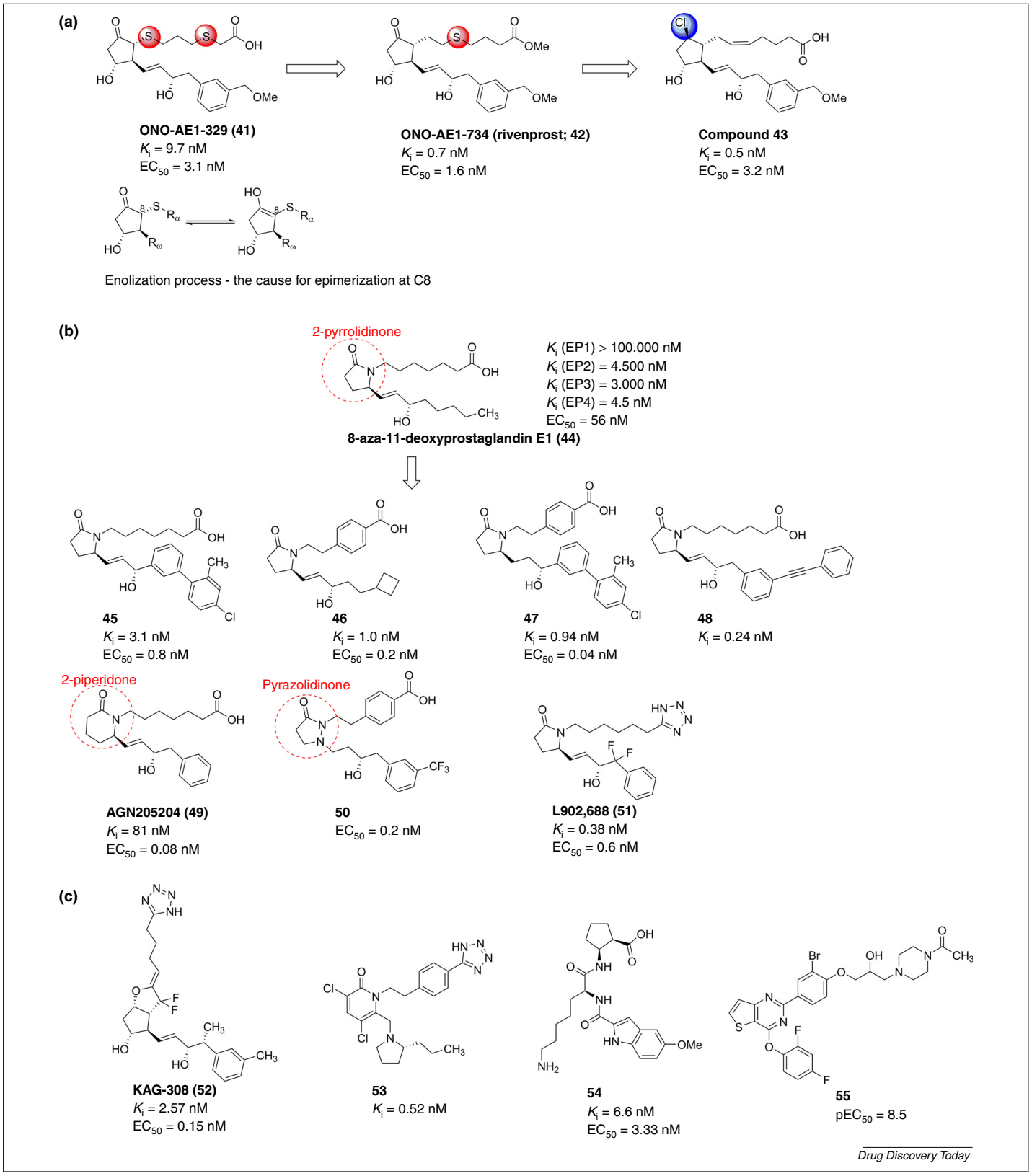

FIGURE 5

Selective agonists of the EP4 receptor. **(a)** Derivatives based on a functionalised cyclopentane core. **(b)** Derivatives carrying a lactam counterpart of the hydroxycyclopentanone core. **(c)** Structurally diverse EP4 agonists.

either binding or agonism. In addition, the 2-pyrrolidinones are a simplified chemical class therefore numerous derivatives were synthesised. A structural analysis of compound **45** shows that the α-chain of the parent compound is retained, and a biphenyl fragment has been incorporated into the ω-chain, resulting in a high-affinity compound possessing potent agonistic activity (K_i = 3.1 nM; EC_{50} = 0.8 nM) [49]. Furthermore, the interphenylene analogues **46** (EC_{50} = 0.04 nM) and **47** (EC_{50} = 0.2 nM), which incorporate aromatic acids in their α-chain, represent the most potent and EP4-subtype-selective compounds of the series [50]. Their ω-chains are terminated by either a cyclobutyl (compound **46**) or an appropriately substituted biphenyl (compound **47**) moiety, which confer high subtype selectivity and retain potency. The biphenyl subclass also enables the removal of the metabolically labile 13,14-unsaturated double bond structural feature. It is worth noting, too, that Ono Pharmaceuticals also identified an 8-azaprostaglandin derivative **48** as a subnanomolar agonist (K_i = 0.24 nM) of the EP4 receptor [19]. Further insight into the SAR was obtained with the identification of 2-piperidones, exemplified by compound AGN205204 (**49**) (EC_{50} = 0.08 nM), the active metabolite of methyl ester AGN205203. The replacement of the 2-pyrrolidinone core with its ring homologue and a minor modification of the ω-chain of compound **45** resulted in a subnanomolar agonist of the EP4 receptor [51]. However, it should be noted that minor loss in terms of affinity was observed relative to its 2-pyrrolidinone counterpart. A similar replacement was employed by researchers of Applied Research Systems, who replaced the 2-pyrrolidinone heterocycle with its pyrazolidinone counterpart, identifying compound **50** (K_i = 0.2 nM) as one of the most potent binders to EP4 reported so far [19]. Researchers at Merck Frosst kept the established lactam scaffold and optimised the molecule by employing a bioisosteric replacement approach. The tetrazole feature was introduced into the α-chain in place of the terminal carboxylic acid functionality, with the intention of improving bioavailability, which led to the discovery of L902,688 (**51**), a subnanomolar agonist of the EP4 receptor (EC_{50} = 0.2 nM) [52]. The third group of EP4 agonists encompassing structurally diverse scaffolds is presented in Fig. 5c. The structure of KAG-308 (**52**), a low nanomolar EP4-agonist, is somewhat unique in the field of EP4 agonists because it is the only one based on a 7,7-difluoroprostacyclin scaffold. Applying a similar strategy and introducing the tetrazole moiety in place of the carboxylic acid rendered the compound chemically and metabolically stable, thus resulting in high oral bioavailability in addition to *in vivo* efficacy [53]. By contrast, compounds **53**, **54** and **55** are representatives of the non-prostanoid EP4 agonists possessing (sub)nanomolar affinities for EP4. The pyridone-based compound **53**, which bears a vague structural resemblance to the lactam series of EP4 agonists, showed an affinity of 0.52 nM for EP4 [19]. Moreover, researchers from Astellas disclosed a series of ornithin homologues, as exemplified by compound **54,** which exerts a K_i value of 6.6 nM. Finally, compound **55,** incorporating a pyrimidinothiophene scaffold, was identified as one of the most potent compounds (EC_{50} = 3.2 nM) developed at Janssen Pharmaceuticals [19]. It is worth noting that none of the reports describing these non-prostanoid EP4 agonists included the selectivity data relative to other prostanoid receptors and, as a consequence, the former cannot be regarded as EP4-selective.

By contrast, significant efforts have also been made in the development of selective and potent EP4 antagonists (presented in Fig. 6a) as potential drug candidates for the treatment of diverse groups of diseases, including chronic inflammation, pain, solid tumours and migraine. Burch *et al.* recently investigated a series of quinoline acylsulfonamides designed on the basis of the known EP4 antagonist GW-627368X and identified MF-498 (**56**), which possesses subnanomolar binding potency towards EP4 (K_i = 0.74 nM; IC_{50} = 1.1 nM) while also exhibiting excellent selectivity for EP4 relative to other prostanoid receptors [54]. To solve the problem of metabolic instability of compound **56**, more oxidatively stable trifluoroethyl moieties were incorporated into the molecule, and the introduction of steric hindrances, such as the replacement of a methylene linker by a bulkier cyclopropyl linker adjacent to the carbonyl group, resulted in MF-310 (**57**) (K_i = 0.79 nM; IC_{50} = 3.2 nM), which showed a more favourable pharmacokinetic profile but displayed less selectivity [54]. Unfortunately, MF-310 has also been shown to undergo hydrolysis, giving rise to the corresponding sulfonamide metabolite in *in vivo* conditions. The thiophene-based MK-2894 (**58**) (K_i = 0.56 nM) and nonacylsulfonamide analogue indole MF-766 (**59**) (K_i = 0.23 nM) represent the second generation developed at Merck Frosst and are highly potent and selective EP4 antagonists in which the hydrolytically unstable acylsulfonamide moiety is replaced by a carboxylic acid [55,56].

Pfizer's diaryl ether CJ-42794 (**60**) represents yet another chemical class of EP4 antagonists, showing high affinity (K_i = 3.16 nM) as well as displaying a good selectivity profile [57]. Furthermore, Schiffler *et al.* have described another interesting class of potent and highly bioavailable EP4 antagonists, exemplified by compound **61** [58]. Their structure is unique in that they are amphoteric and thus contain an acidic carboxylic acid functionality as well as a basic (phenoxyethyl)piperidine functionality; this provides diverse formulation options to facilitate absorption [58]. Low nanomolar affinity has also been reported for the naphthyl derivative ONO-AE3-208 (**62**) (K_i = 1.3 nM) developed by Ono Pharmaceuticals [59]. Researchers at Pfizer identified another potent and highly selective EP4 antagonist, grapiprant (CJ-023,423; compound **63**), which showed efficacy in animal models of inflammatory pain. It is based on an imidazopyridine scaffold with a pendant sulfonylurea acidic centre [60]. Interestingly, compound L161,982 (**64**) also proved to be a selective EP4 antagonist despite its structural resemblance to the previously reported Merck EP3 antagonists [61]. Compound ER819762 (**65**) is atypical from a structural point of view because it is the only reported selective EP4 antagonist lacking an acidic centre in its structure [62]. In an effort to discover alternative scaffolds for EP4 antagonists, researchers at Fujisawa employed a random screening approach so as to explore the chemical space fully. This exploration led to the identification of diphenyloxazole **66** and a substituted *Z*-ornithin derivative **67** as potent antagonists with K_i values of 0.3 nM and 0.91 nM, respectively [63]. Finally, scientists at Pharmagene implemented an extensive virtual screening procedure followed by several rounds of optimisation that led to the discovery of a trisubstituted furan derivative PG-1531 (**68**); this subsequently proved to be a nanomolar EP4 antagonist with an excellent selectivity profile and enhanced aqueous solubility [64]. It has recently been demonstrated that, through the

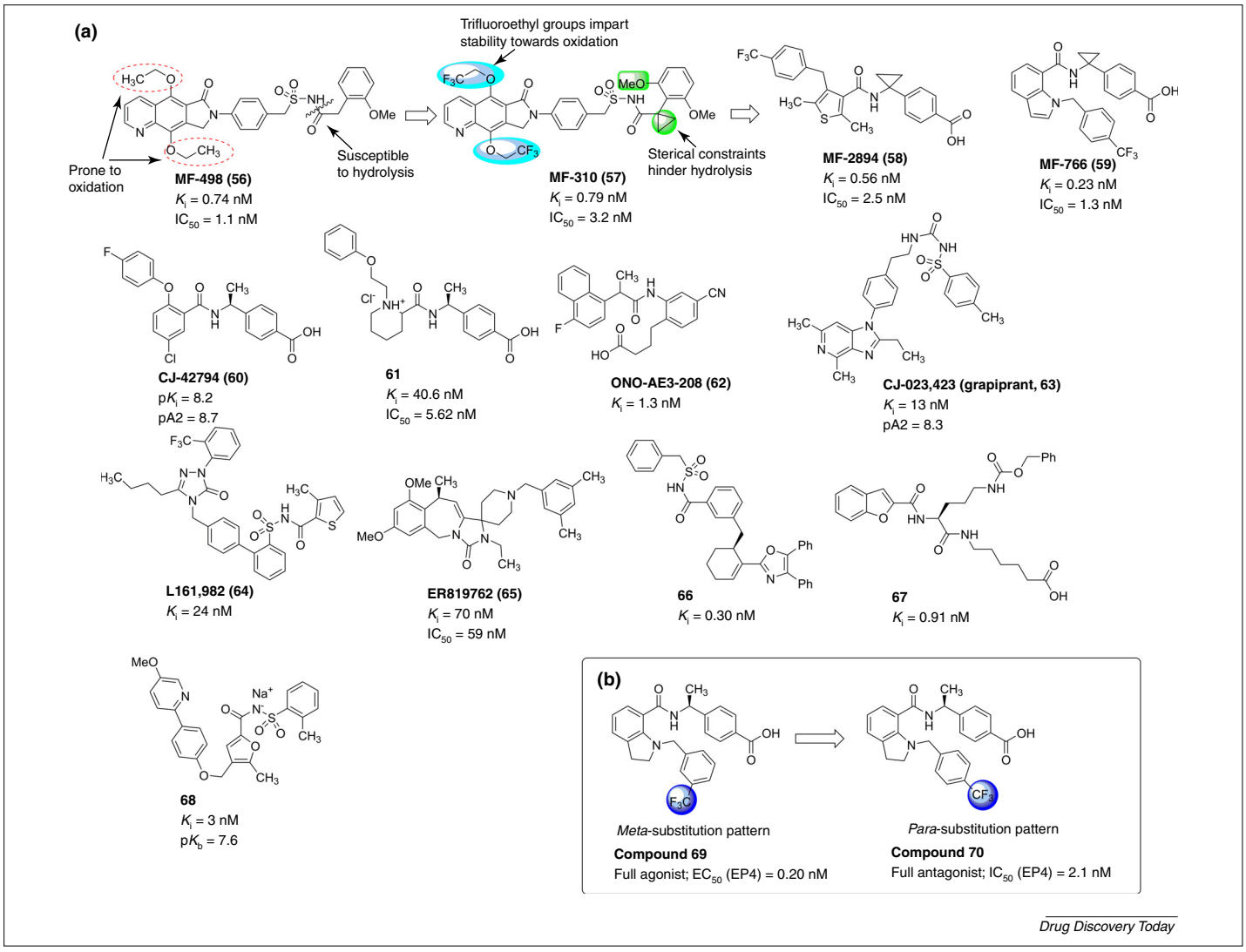

FIGURE 6

Selective antagonists of the EP4 receptor and switching in the functional response as a result of minimal structural variation. **(a)** Selective antagonists of the EP4 receptor. **(b)** Switch of agonism and antagonism at the EP4 receptor.

introduction of minor modifications into the molecule, it is possible to fine-tune the intrinsic activity of the latter at the EP4 receptor (an example is shown in Fig. 6b). For example, the intrinsic activity (agonism vs antagonism) has been shown to depend solely on the substitution pattern of the trifluoromethyl substituent on the benzylic group of compounds **69** and **70** [65]. A dramatic change of function can be achieved with minimal variation of ligand structure.

Potential therapeutic applications of selective EP receptor modulators

Selective EP1–EP4 receptor agonists and antagonists were evaluated as drug candidates for the treatment of various diseases. We overview the therapeutic potential of EP modulators by first presenting those that are or have been evaluated in clinical studies, and then proceed with the most promising therapeutic applications of EP modulators demonstrated in preclinical studies. The potential therapeutic applications of EP modulators are reviewed in Table 1.

Potential therapeutic applications of EP4 modulators evaluated in clinical trials

Use of EP4-selective agonists in the treatment of ulcerative colitis

Ulcerative colitis manifests as impaired mucosal barrier function and as elevated levels of proinflammatory and immunoregulatory cytokines. The role of PGE2 in prevention of disease initiation and progression is demonstrated in murine models of disease, indicating a pivotal role of the EP4 receptor in this process. Hence, dextran sodium sulphate (DSS) only induced experimental colitis in the severe form in $EP4^{-/-}$ mice, but not in mice deficient in other types of prostanoid receptors. The same phenotype was reproduced in wild-type mice treated with EP4-selective antagonists. Induced EP4 deficiency resulted in reduced mucosal barrier function, epithelial loss and accumulation of neutrophils and $CD4^+$ T cells in the colon [59]. This led to the evaluation of EP4-selective agonists (ONO-AE1-734 and AGN205203) in wild-type mice, resulting in prevention of DSS-induced colitis upon their administration [51,66]. These discoveries implicated a major

TABLE 1

Potential therapeutic applications of selective EP1–4 agonists and antagonists as drug candidates

Potential therapeutic application	Drug candidate	Refs
Ulcerative colitis	EP4 agonists: rivenprost (ONO-4819), KAG-308 ONO-AE1-329, AGN205203	[51,53,59,66,67]
Glaucoma and intraocular hypertension	EP2 agonist: taprenepag isopropyl (PF-04217329)	[68,69]
Solid tumours (prostate, breast, lung)	EP4 antagonists: grapiprant (CJ-023,423) ONO-AE3-208, GW627368X, AH23848	[70–75]
Bone formation	EP2 agonist: CP-533,536 EP4 agonists: ONO-4819, CP-734,432, AE1-329	[78,79]
Neurologic diseases **Alzheimer's disease**	EP2 agonist: butaprost EP4 agonist: 1-hydroxy-PGE_1	[81–84]
Parkinson's disease	EP1 antagonists: SC-19220, SC-51089	
B cell lymphoma	EP4 agonist: 1-hydroxy-PGE_1	[16,85–88]
Pulmonary fibrosis	EP2 agonist: butaprost	[89,90]
Colorectal cancer	EP1 antagonist: ONO-8711 EP4 antagonist: ONO-AE2-227	[92–94]
Cardiovascular disease	EP3 agonists: GR 63799X, MB-28767, ONO-AE-248, TEI-3356 EP4 agonist: EP4RAG	[95–98]

role of EP4 receptor in preserving mucosal integrity and down-regulating immune response, hence maintaining intestinal homeostasis.

These findings laid the groundwork for use of EP4-selective agonist rivenprost (ONO-4819) in a randomised, double-blinded placebo-controlled Phase II clinical trial including patients with mild-to-moderate ulcerative colitis [67]. An improvement in the ulcerative colitis condition was observed in three out of four patients: one patient achieved complete remission. Histological assessments of patients suffering from ulcerative colitis further imply the clinical benefits of rivenprost [67]. The novel EP4-selective agonist KAG-308 is advantageous when compared with rivenprost owing to its oral bioavailability. When tested in a DSS-induced mouse colitis model, KAG-308 was shown to inhibit tumour necrosis factor (TNF)-α secretion in whole-blood peripheral cells, whereas it also suppressed the onset of DSS-induced colitis and promoted mucosal healing [53]. Furthermore, it showed potential in the risk prevention of colorectal carcinogenesis. The safety of KAG-308 was evaluated in a Phase I clinical trial (http://adisinsight.springer.com/drugs/800039010).

Phase II clinical trial of taprenepag isopropyl, an EP2-selective agonist, in the treatment of glaucoma and ocular hypertension

Involvement of the EP2 receptor in intraocular pressure regulation was confirmed in several *in vivo* studies wherein an EP2 receptor agonist exhibited ocular hypotensive effects. Taprenepag isopropyl (PF-04217329) is a prodrug of the active acid metabolite CP-544326, which is a potent and selective EP2 receptor agonist [68]. Taprenepag isopropyl was evaluated in a Phase II controlled randomised trial, in the form of a topical ophthalmic solution in patients with primary open-angle glaucoma or ocular hypertension [69]. Taprenepag isopropyl monotherapy significantly reduced intraocular pressure and ocular hypertension and was comparable to the conventional drug latanoprost. In addition, the simultaneous use of taprenepag isopropyl and latanoprost displayed additive effects, suggesting that combination therapy in the treatment of open glaucoma and ocular hypertension has some benefit [69].

EP4 receptor antagonists in the treatment of solid tumours

The effects of blocking EP4 receptor activation for the prevention of solid tumours have been confirmed in several studies on mice deficient in EP4 receptor, and by means of a pharmacological blockade with EP4-selective receptor antagonists. Furthermore, the EP4-selective antagonist grapiprant (CJ-023,423) is presently entering Phase II clinical trials to evaluate its potential in the treatment of advanced solid tumours, namely those of the prostate and breast as well as non-small-cell lung cancer (NSCLC) (http://www.clinicaltrials.gov).

The role of the EP4 receptor in prostate cancer has been studied in detail. First, the overexpression of this receptor led to the progression of prostate cancer in a mouse xenograft disease model and the treatment with EP4 receptor antagonist ONO-AE3-208 suppressed castration-resistant prostate cancer progression [70]. Consistent with these findings, the EP4 receptor expression was upregulated in prostate cancer patients [71,72].

PGE2 has an important role in lung cancer. PGE2 is abundantly expressed in lung tissue and the inhibition of its signalling suppressed tumorigenic effects in animal models of lung cancer. These observations have further been corroborated by subsequent findings that show that: (i) PGE2 promotes human NSCLC growth *in vitro* and its tumorigenic effects are successfully inhibited by pharmacologically blocking EP4 receptor with its antagonist AH-23848 and siRNA approaches [73]; and (ii) pulmonary metastasis of lung carcinoma cells injected intravenously in mice was facilitated via the EP4 receptor. In agreement with the role of EP4 receptor in prostate and lung cancer, studies evaluating the role of EP4 receptor in breast cancer show EP4 receptor activation mediated regulation of the proliferation and invasion of inflammatory breast cancer cells using EP4 antagonist GW-627368X and shRNA EP4 knockdown approaches [74]. Moreover, EP4 receptor

antagonist AH-23848 and ONO-AE3-208 reduced metastasis of murine mammary tumour cells [75].

Promising therapeutic applications of EP modulators evaluated in preclinical studies

EP4 antagonist grapiprant shows great potential in treating pain and inflammation

Evidently, grapiprant has been indicated in the treatment of diverse pathologies as shown by recent studies that demonstrate effectiveness and safety of grapiprant in the treatment of pain and inflammation in dogs with osteoarthritis. First, grapiprant was found to be safe in a long-term (9 months), daily oral administration in dogs [76]. Second, grapiprant proved to be effective in alleviating pain in dogs with osteoarthritis and represents an alternative treatment that might be better tolerated than current therapy with non-steroid anti-inflamamtory drugs [77].

Activation of EP2 and EP4 receptors increases bone formation

The role of PGE2 in bone remodelling is clearly demonstrated by a local increase of endogenous PGE2 levels at the fracture site. The effects of the EP2-selective receptor agonist CP-533,536 on local bone augmentation, repair and healing have been evaluated in numerous animal studies using mice, rats and dogs [78]. The effects of EP4-selective agonists ONO-4819, CP-734,432 and ONO-AE1-329 were evaluated in preclinical studies, which demonstrated stimulated bone formation and increased bone mass and strength [79]. The involvement of EP2 and EP4 receptors in this process is evident by shared signalling pathways. EP2 and EP4 are Gs coupled, act by activating adenylate cyclase and increase cAMP levels, and EP4-receptor-specific signalling via Gi or EPRAP contributes to differential outcomes [13,14]. However, signalling pathways of EP4 and/or EP2 in osteoblasts and osteoclasts have so far not been fully delineated. Selected insight is generated in a study utilising EP-receptor-specific knockouts, demonstrating involvement of osteoblast-specific transcription factor Runx2/Cbfa1 in EP4-transduced PGE2 signalling [79]. A study involving EP4-selective agonist ONO-4819CD in rat models of bone repair detected an increased expression of bone morphogenetic protein (BMP)2 and receptor activator of nuclear factor-kB ligand (RANKL) [80]. These results imply a great potential of EP2 and EP4 agonists for therapeutic use; however, no clinical study has taken place to date.

Protective role of EP1 and EP4 receptor signalling in neurologic disease

The role of PGE2 and EP receptor subtypes has been identified in several neuropathophysiological processes in various diseases of the CNS. First, the EP2 receptor agonist butaprost and the EP4 agonist 1-hydroxy-PGE1 suppressed the neurotoxic effects of the aggregated Aβ peptides *in vitro*, indicating a beneficial modulatory effect in neurodegeneration in Alzheimer's disease [81]. The results obtained in the conditional deletion of microglial EP4 in a transgenic mouse model of Alzheimer's disease have confirmed the protective role of anti-inflammatory EP4 signalling in the early stages of this disease. Furthermore, EP4 receptor levels measured in the human cortex decreased significantly with the progression from normal to Alzheimer's disease states, suggesting that an early loss of the EP4 signalling system in preclinical Alzheimer's disease development could contribute to the subsequent progression of the pathology [82].

The studies on rat primary dopaminergic neurons have shown the protective role of EP1 antagonists SC-51089 and SC-19220 with regard to the 6-hydroxydopamine-mediated toxicity in a model of Parkinson's disease [83,84]. This protective role has been shown in a brain tissue model of transient focal ischemia by the pharmacological inhibition of EP1 activity as well as by genetic ablation of the EP1 receptor [84].

EP4 receptor agonists in the treatment of B cell lymphoma

EP4 receptor agonists exhibit a protective role in B cell lymphoma. EP4 receptor is a negative feedback regulator of proliferation in response to B cell receptor signalling [85–87]. EP4 signalling prevented the proliferative burst of immature B cells triggered by antigen–receptor cross-linking. In agreement with these results, the EP4 antagonist ONO-AE3-208 blocked the effects of PGE2 after targeting the B cell receptor in immature B cell lymphoma [85]. EP4-knockdown mice with B cell lymphoma had accelerated tumour spread and EP4 overexpression had a protective role against tumour progression [86]. Furthermore, the *Ptger4* gene, which codes for the EP4 receptor, has been suggested as a tumour suppressor in mouse B cell lymphoma [86]. Antiapoptotic NF-κB-dependent signalling pathways in B cell lymphoma were inhibited by the EP4 receptor agonist 1-hydroxy-PGE1 [16,88]. In addition, 1-hydroxy-PGE1 increased the sensitivity of cells towards bortezomib- and doxorubicin-induced chemotherapeutic effects. As a consequence, targeting the EP4 receptor could present novel therapeutic approaches in B cell malignancies.

A protective role of EP2 activation in pulmonary fibrosis

PGE2, abundantly expressed in lung tissue, displays suppressive effects on fibrosis by inhibiting fibroblast proliferation, collagen secretion and by inhibiting transforming growth factor (TGF)-β1-induced fibroblast to myoblast differentiation [89]. A protective role of the EP2 agonist butaprost has been observed against the induction of idiopathic pulmonary fibrosis. This protective role is supported by the findings that EP2 receptor$^{-/-}$ mice showed exaggerated fibrotic responses relative to wild-type mice in a pulmonary-fibrosis-induced mouse model [90,91].

EP1 and EP4 receptor antagonists in the prevention of colorectal cancer

The beneficial effects of blocking EP4 receptor signalling in colorectal cancer have been confirmed in several *in vivo* studies as well as by a pharmacological blockade of the EP4 receptor. For example, the EP4$^{-/-}$ mice treated with carcinogen had reduced development of aberrant crypt foci (precursors of colorectal tumours) relative to wild-type mice treated with the same carcinogen [92]. The administration of the EP4 receptor antagonist ONO-AE2-227 reduced the formation of aberrant crypt foci and intestinal polyps in a mouse disease model [93]. Partial resistance to colon carcinogen was also observed in mice with deletions of EP1 receptor and, in agreement with this finding, EP1 receptor antagonist (ONO-8711) decreased the incidence of colorectal cancer markers *in vivo* [94].

EP3 and EP4 receptor agonists confer cardiovascular protection

The role of PGE2 in the pathogenesis of cardiovascular diseases and in cardiovascular inflammation is complex. Cardioprotective effects of EP3- and EP4-receptor-transduced signals have been evaluated in various *in vitro* and *in vivo* models. PGE2 demonstrated

cardioprotective effects via EP3 receptor activation in myocardial ischemia. Thus, ischemic myocardial injury was attenuated in transgenic mice with cardio-specific overexpression of the EP3 receptor [95]. Corroborating these findings, the structurally diverse EP3 agonists GR-63799X, MB-28767, ONO-AE-248 and TEI-3356 reduced the myocardial infarct size in rats. The data suggest that the therapeutic effect is mediated by PKC activation and opening of K_{ATP} channels [96]. The EP4-selective agonist EP4RAG prevented myocardial dysfunction after infarction and reduced infarction size and ischemic myocardium in rat myocardial ischemia/reperfusion injury models. EP4 receptor activation decreased the level of inflammatory cytokines and chemokines by inhibiting the activation of macrophages [97]. Pharmacological activation of EP4 receptor by EP4RAG also exhibited anti-inflammatory effects in the rat experimental autoimmune myocarditis model [98]. Therefore, EP3 and EP4 agonists represent promising cardioprotective compounds.

Concluding remarks

The EP receptors represent interesting targets to medicinal chemists; consequently, numerous possible indications have been suggested for the pharmacological modulation of all EP receptor subtypes. The literature suggests that, although the antagonists of EP receptors have gained the interest of most of the scientific community, some agonists have also been developed, something that holds true especially in the cases of the EP2 and EP4 receptors. Although there is an enormous amount of information regarding the affinity, functional activity and selectivity of EP receptor ligands, very little is known with regard to their true pharmacological value.

Novel EP receptor subtype selective agonists and antagonists appear to be valuable drug candidates in the therapy of many pathophysiological states, including ulcerative colitis, glaucoma, bone healing, B cell lymphoma and Alzheimer's disease. In addition to extensive *in vitro* and *in vivo* studies, clinical trials have been performed to determine the clinical effect of therapeutics that target the EP receptors. EP4 and EP2 have been particularly well investigated in clinical trials, with Phase II clinical trials for the treatment of ulcerative colitis (EP4) and glaucoma/ocular hypertension (EP2). However, further studies are needed to evaluate the efficacy and safety of novel drug candidates targeting EP1–4 receptors. The current state of the literature data suggests enormous promise for the therapeutic potential of subtype-selective EP receptor modulators.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drudis.2016.08.003.

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