Bioorganic & Medicinal Chemistry Letters 22 (2012) 396–401

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a novel EP2/EP4 dual agonist with high subtype-selectivity

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ARTICLE INFO

Article history:
Received 12 September 2011
Revised 26 October 2011
Accepted 31 October 2011
Available online 7 November 2011

Keywords:
Prostaglandin
Agonist
EP2 receptor
EP4 receptor

ABSTRACT

A series of γ-lactam prostaglandin E_1 analogs bearing a 16-phenyl moiety in the ω-chain and aryl moiety in the α-chain were synthesized and biologically evaluated. Among the tested compounds, γ-lactam PGE analog **3** designed as a structural hybrid of **1** and **2** was discovered as the most optimized EP2/EP4 dual agonist with excellent subtype-selectivity (K_i values: mEP2 = 9.3 nM, mEP4 = 0.41 nM). A structure–activity relationship study is presented.

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Receptors for prostaglandin E_2 (PGE_2) can be classified into four subtypes, EP1, EP2, EP3 and EP4, each of which mediates different effects in various tissues and cells.[1] The EP4 receptor is distributed in thymus, lung, heart, kidney, bone, womb and other organs, and mediates an increase of the intracellular c-AMP concentration. Various biological actions of PGE_2, including a cytoprotective action, improvement of blood flow, regulation of inflammatory cytokine production and bone resorption/formation, are thought to mediate the EP4 subtype. A recent report suggests that an EP4 agonist is capable of restoring bone mass and strength normally lost in rats subjected to ovariectomy or immobilization.[2] EP2 receptor agonists have been shown to have an anabolic effect on bone formation in various animal models.[3] These findings led us to development of a novel EP2/EP4 dual agonist which could prove to be a more beneficial agent for the treatment of bone diseases such as osteoporosis and fracture healing in humans. Several research groups have been investigating the improvement of pharmacological properties of PGE_2.[4–7] Efforts to improve the selectivity and chemical stability of PGE_2 have been focused mainly on two general chemical modifications: replacement of the α-alkenyl side chain with the phenylethyl group and replacement of the γ-hydroxycyclopentanone moiety with 2-pyrrolidinone.

Our purpose was to develop PGE_2 analogs with subtype-selectivity and high potency for both receptor subtypes EP2 and EP4 because of their presumed therapeutic potential for the treatment of bone diseases.

In our previous reports, we described 8-aza-5-thia PGE_1 analog **1** (Fig. 1), an EP4 receptor agonist with high subtype-selectivity and high potency which showed potent inhibitory activity of LPS-induced production of TNF-α in rats.[8] Compound **2** was also reported to be an EP4 receptor agonist.[4] We focused on the activity profiles of **2** because it displayed weak to moderate binding affinity (K_i = 340 nM for mEP2, in-house data) for the EP2 subtype in addition to potent EP4 subtype affinity and moderate EP3 subtype affinity (Table 1). Based on the information described above, more detailed chemical modification of the benzoic acid moiety of **2** was predicted to lead us to the discovery of a new structure which possesses a desirable activity profile as an EP2/EP4 dual agonist with subtype-selectivity and high potency.

Herein we report on the discovery of a novel PGE analog of structure **3** (Fig. 1) consisting of a structurally novel α-chain beneficial for EP2/EP4 receptor affinity, a ω-chain beneficial for EP4 subtype-selectivity and a γ-lactam ring as a replacement for the chemically unstable γ-hydroxycyclopentanone.

Synthesis of test compounds listed in Tables 1–3 is described in Schemes 1–6. Synthesis of **3** and **10**–**12** are presented in Scheme 1a. Ethanolysis of the *S*-acetyl group of **16**[9] followed by *S*-arylation with ethyl 2-bromothiazole-4-carboxylate and ethyl 2-bromothiazole-5-carboxylate resulted in **17** and **22**, respectively. Deprotection of the *tert*-butyldimethylsilyl (TBS) group with tetrabutylammonium fluoride (TBAF) afforded **18a** and **23**, respectively. Compound **18a** and **23** were transformed to the enones **20a**–**c** and **24**, respectively by DMSO oxidation followed by Horner–Emmons reaction using an optional phosphonate chosen from among **26a**–**c** (Scheme 1c). Stereoselective reduction of **20a**–**c** and **24** yielded **21a**–**c** and **25**, respectively. Alkaline hydrolysis of **21a** and **25** afforded **3** and **10**, respectively. Alkaline hydrolysis of **21b** and **21c** afforded **11** and **12**, respectively.

Synthesis of **13**–**15** is outlined in Scheme 1b. The ethyl ester **17** was transformed to the corresponding butyl ester **18b**, which was

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doi:10.1016/j.bmcl.2011.10.109

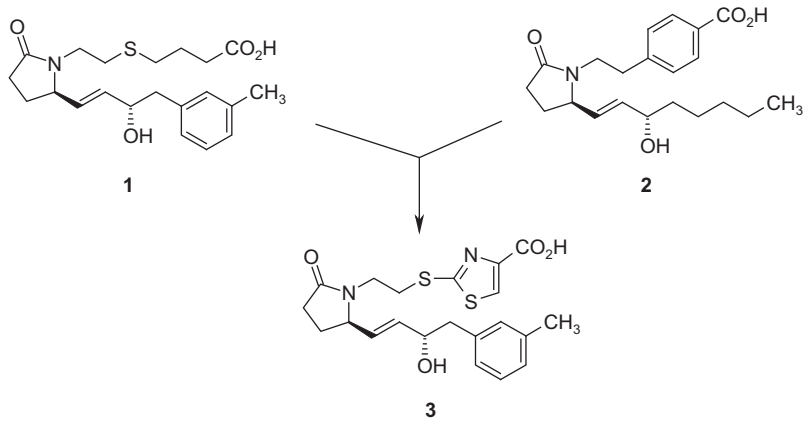
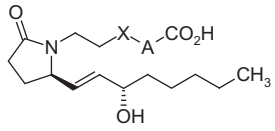


Figure 1. Molecular design of γ-lactam PGE analog **3** bearing the structurally novel ω-chain beneficial for EP2/EP4 dual selectivity.

Table 1
Effect of the α-chain structure of the γ-lactam PGE framework bearing the natural ω-chain on the activity profiles



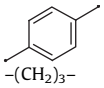
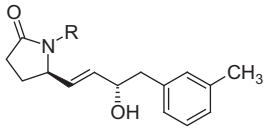
Compound	X	A	Binding assay (K_i, nM)			
			mEP1	mEP2	mEP3	mEP4
2	Bond		>10^4	340	86	1.7
4	CH_2	–$(CH_2)_3$–	>10^4	>10^4	39	9.2
5	S	–$(CH_2)_3$–	>10^4	2500	26	2.0
PGE_2			6.0	22	5.0	3.1

Table 2
Effect of the α-chain structure of the γ-lactam PGE framework bearing the 16-(3-methylphenyl) ω-chain on the activity profiles



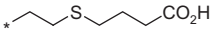
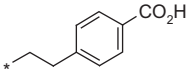
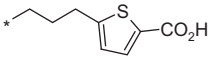
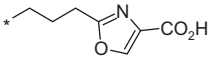
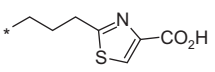
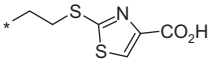
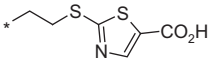
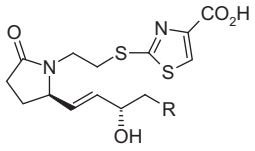
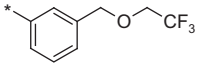
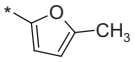
Compound	R	Binding assay[a] (K_i, nM)			
		mEP1	mEP2	mEP3	mEP4
1		>10^4	>10^4	5800	1.8
6		>10^4	410	>10^4	0.9
7		>10^4	127	1600	0.6
8		>10^4	240	>10^4	35
9		>10^4	84	>10^4	12
3		>10^4	9.3	540	0.41
10		>10^4	320	>10^4	5.5

Table 3
Activity profiles of γ-lactam PGE analogs bearing the 2-mercaptothiazole-4-carboxylic acid α-chain and miscellaneous ω-chain

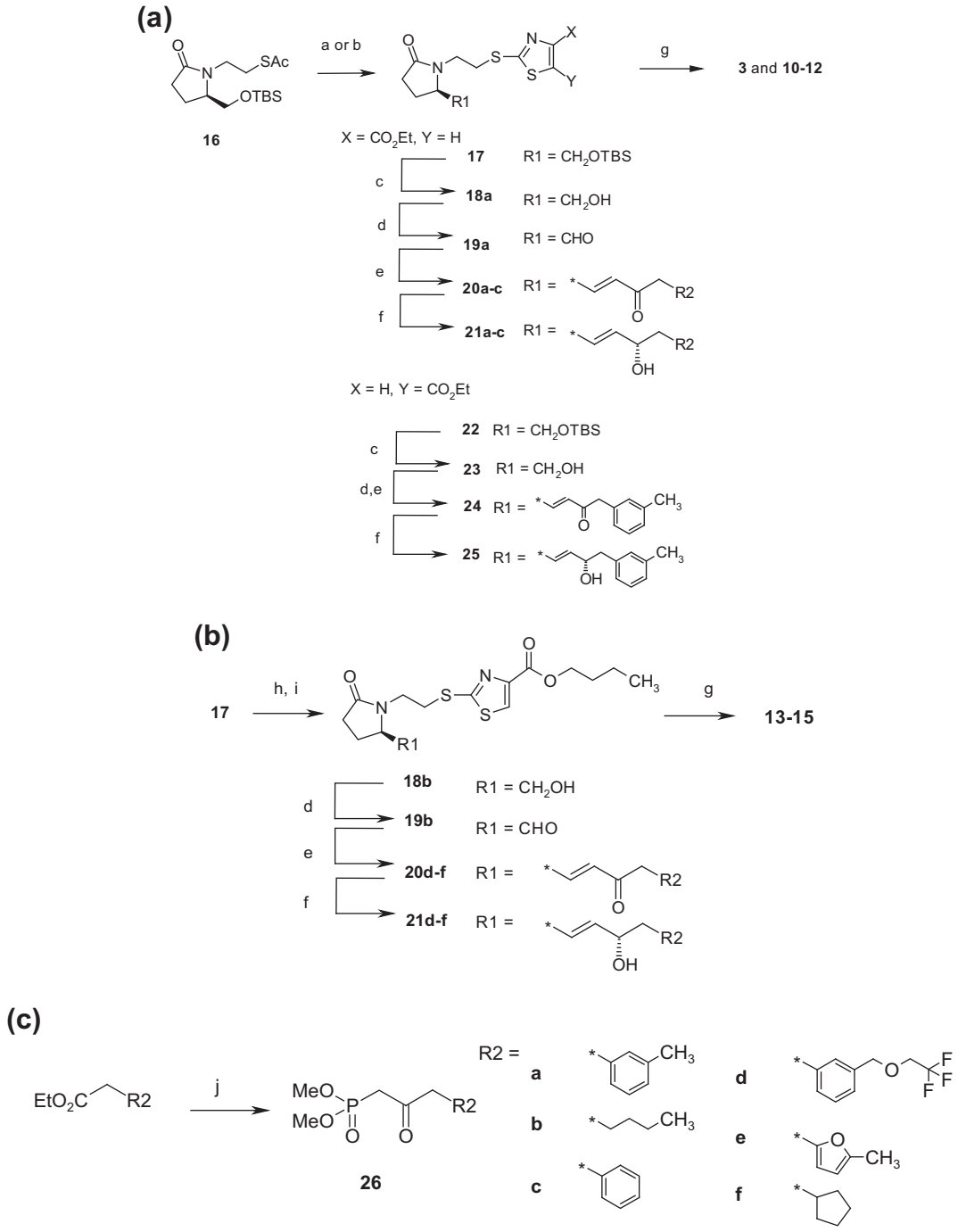


Compound	R	Binding assay (K_i, nM)			
		mEP1	mEP2	mEP3	mEP4
11	–$(CH_2)_3CH_3$	3400	3.0	15	0.5
12		>10^4	19	770	1.5
13		1900	15	1200	1.4
14		>10^4	42	49	0.77
15		>10^4	13	85	43

converted to the enones **20d–f** by DMSO oxidation followed by Horner–Emmons reaction using an optional phosphonate chosen from among **26d–f** (Scheme 1c). Alkaline hydrolysis of enones **20d–f** afforded **13–15**, respectively.

To synthesize **6**, we developed an alternative synthetic method of 8-aza PGE analogs starting from *N*-Boc-D-glutamic acid as described in Scheme 2. Esterification of *N*-Boc-D-glutamic acid γ-ethyl ester **27** with *N*-hydroxysuccinimide followed by hydride reduction with lithium borohydride afforded **29**, Swern oxidation of which provided **30**. The aldehyde **30** was converted to allyl alcohol **31** by Horner–Emmons reaction using **26a** as a phosphonate followed by stereoselective reduction of the formed enone carbonyl. Acidic deprotection of **31** afforded **32**. Reductive alkylation of **32** with an appropriate aldehyde and sodium triacetoxyborohydride followed by *O*-protection with TBS chloride resulted in the cyclized product **33**. Palladium-catalyzed carbonyl insertion reaction of **33** followed by treatment with methanol afforded a methyl ester **34**, which was converted to **6** by acidic deprotection followed by alkaline hydrolysis.

Synthesis of **7** is outlined in Scheme 3. *N*-Alkylation of **35**[10] with methyl (5-bromopropyl)thiophene-2-carboxylate afforded **36**.



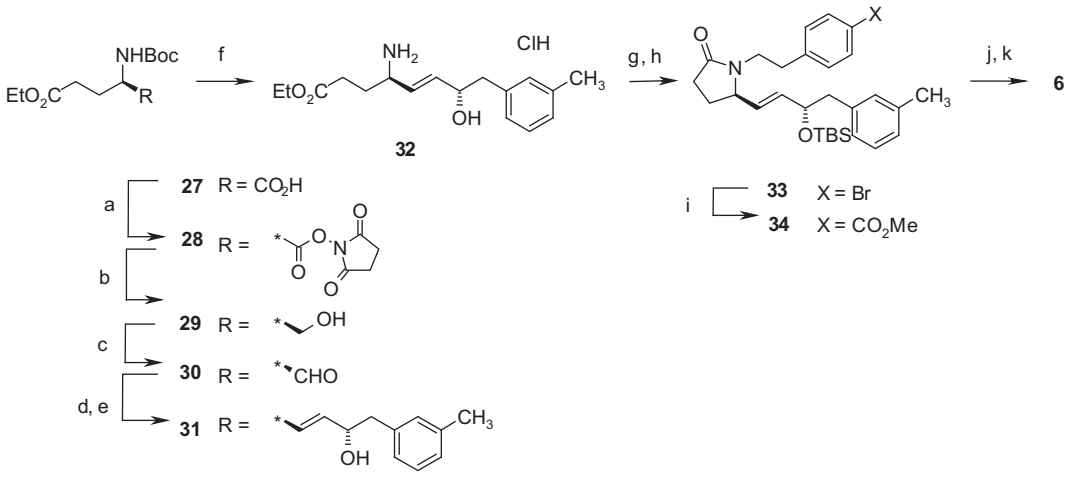
Scheme 1. Synthesis of **3**, **10**–**15** and preparation of phosphonate **26**. Reagents: (a) ethyl 2-bromothiazole-4-carboxylate, K_2CO_3, EtOH; (b) ethyl 2-bromothiazole-5-carboxylate, K_2CO_3, EtOH; (c) TBAF, THF, 63–64% in two steps; (d) SO_3-Py, *i*-Pr_2NEt, DMSO, AcOEt; (e) **26a**–**c**, NaH, THF; (f) (*R*)-Me-CBS, BH_3-THF, THF, 35–56% in three steps; (g) aq NaOH, DME, 80–93%; (h) K_2CO_3, *n*-BuOH; (i) TBAF, THF, 59% in two steps; (j) dimethyl methylphosphonate, *n*-BuLi, toluene, 75%.

Compound **36** was converted to **7** by the following sequential reactions: (1) Swern oxidation; (2) Horner–Emmons reaction using **26a** as a phosphonate; (3) stereoselective reduction of the formed enone carbonyl; (4) alkaline hydrolysis.

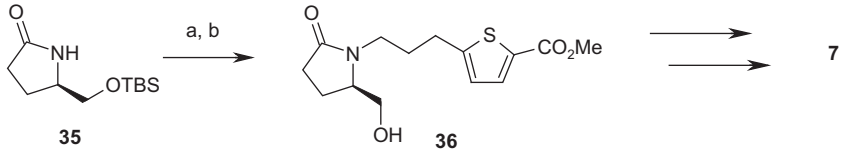
Synthesis of **8** is described in Scheme 4. O-Protection of sodium 4-hydroxybutyrate with the *tert*-butyldiphenylsilyl (TBDPS) group followed by peptide formation with the DL-serine methyl ester afforded **37**. Dehydration of **37** followed by oxidation resulted in an oxazole **38**, deprotection of which afforded **39**.[11] Reductive alkylation of **32** (Scheme 2) with an aldehyde, which was prepared from **39** by the DMSO oxidation, followed by cyclization afforded a γ-lactam framework **40**. Alkaline hydrolysis of **40** produced **8**.

Compound **9** was synthesized as outlined in Scheme 5. 4,4-Dimethoxybutyronitrile was converted to ethyl thiazole-4-carboxylate **41** by the following sequential reactions: (1) thioamide formation with sodium hydrogen sulfide; (2) thiazole formation with ethyl bromopyruvate; (3) acidic deprotection.[12] Compound **41** was transformed to **9** according to the same method as described above.

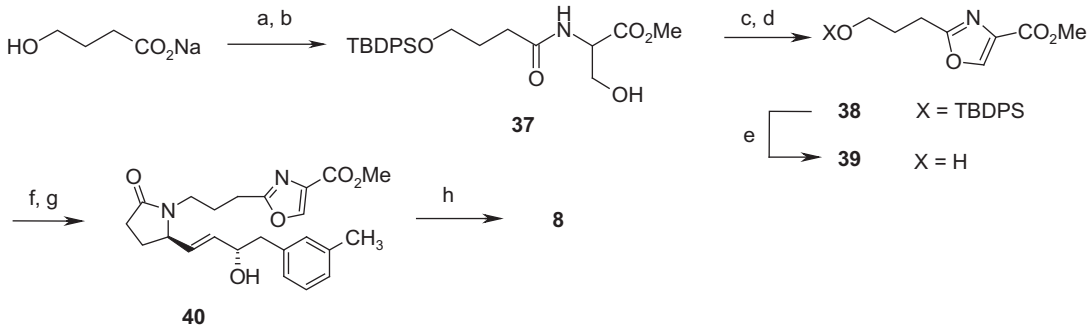
Synthesis of **2** and **5** is described in Scheme 6. Compound **30** was converted to **42** by the usual method. Acidic deprotection of



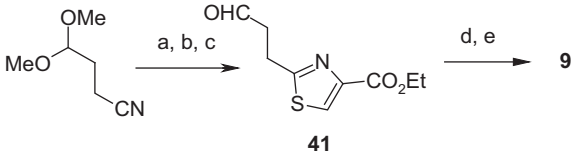
Scheme 2. Synthesis of **6**. Reagents: (a) *N*-hydroxysuccinimide, $SOCl_2$, pyridine, DMF, MeCN, 70%; (b) $LiBH_4$, THF, 66%; (c) $(COCl)_2$, *i*-Pr_2NEt, DMSO, CH_2Cl_2; (d) **26a**, NaH, THF; (e) (*R*)-Me-CBS, BH_3-THF, THF, 51% in three steps; (f) HCl in dioxane, EtOH, 100%; (g) 3-(4-bromophenyl)-propionaldehyde, $NaBH(OAc)_3$, THF; (h) TBSCl, imidazole, DMF, 72% in two steps; (i) CO gas, $PdCl_2(PPh_3)_2$, 1,1-bis(diphenylphosphino)ferrocene, Et_3N, DMSO, MeOH, 94%; (j) 2 N HCl, MeOH, DME; (k) 2 N NaOH, MeOH, DME, 76% in two steps.



Scheme 3. Synthesis of **7**. Reagents: (a) methyl 5-(3-bromopropyl)thiophene-2-carboxylate, NaH, DMF, 32%; (b) TBAF, THF, 100%.



Scheme 4. Synthesis of **8**. Reagents: (a) TBDPSCl, imidazole, DMF; (b) DL-serine methyl ester hydrochloride, EDC-HCl, Et_3N, CH_3CN, 32% in two steps; (c) diethylaminosulfur trifluoride, K_2CO_3, CH_2Cl_2; (d) bromotrichloromethane, DBU, CH_2Cl_2, 54%; (e) TBAF, THF, 79%; (f) SO_3-Py, *i*-Pr_2NEt, DMSO, AcOEt; (g) **32**, $NaBH(OAc)_3$, THF, 74%; (h) aq NaOH, MeOH, DME, 70%.

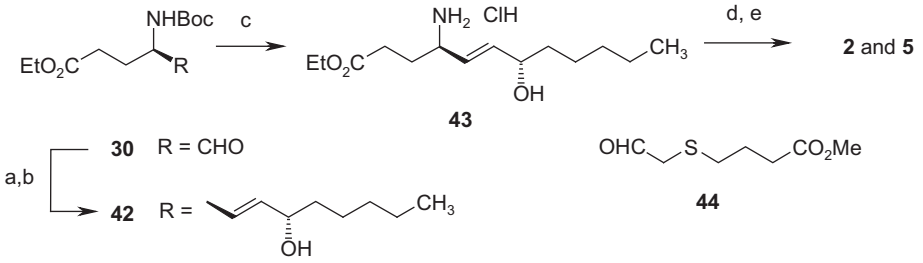


Scheme 5. Synthesis of **9**. Reagents: (a) NaSH, $MgCl_2$–$6H_2O$, DMF; (b) ethyl bromopyruvate, DMF; (c) 2 N HCl, DME, 12% in three steps; (d) **32**, $NaBH(OAc)_3$, THF, 60%; (e) 2 N NaOH, DME, MeOH, 66%.

42 afforded **43**. Reductive alkylation of **43** with 4-carbomethoxy phenylacetaldehyde or an aldehyde **44** followed by alkaline hydrolysis afforded **5** and **2**, respectively.[13,14]

Compounds listed in Tables 1–3 were evaluated for their binding affinity using membrane fractions of CHO cells expressing the mouse EP-receptor. K_i values were determined by a competitive binding assay which was performed according to the method of Kiriyama et al. with some modifications.[9,15]

To investigate the effect of the α-chain structure on the activity profiles, α-chain analogs **2**, **4** and **5** bearing a natural ω-chain were compared for their subtype-selectivity and receptor affinity. Results are summarized in Table 1. Compound **4**, which possesses an *N*-heptanoic acid moiety, exhibited moderate binding affinity and potent binding affinity for the EP3 and EP4 subtypes, respectively while it did not exhibit receptor affinity for both the EP1 and EP2 subtypes up to 10 μM. The corresponding 5-thia analog **5** showed moderate and highly potent receptor affinity for the EP3 and EP4 subtypes, respectively while it showed very weak receptor affinity for the EP2 subtype and no receptor affinity for the EP1 subtype up to 10 μM, respectively. According to our in-house data, compound **2** tended to show increased affinity for the EP2 receptor while retaining its potent EP4 receptor affinity relative to the analog **4** while compound **2** showed moderate receptor affinity for the EP3 subtype.



Scheme 6. Synthesis of **2** and **5**. Reagents: (a) **26b**, NaH, THF; b) (*R*)-Me-CBS, BH_3-THF, THF, 83% in two steps; (c) HCl in dioxane, EtOH, 100%; (d) 4-carbomethoxy phenylacetaldehyde or **44**, $NaBH(OAc)_3$, THF, 50–55%; (e) 2N NaOH, MeOH, DME, 73–82%.

According to one of our previous reports, the 16-(3-methylphenyl) moiety of **1** (Figure 1 and Table 2) was found to be one of the optimized ω-chain moieties which helped to improve EP4 subtype-selectivity especially with reduction of EP3 receptor affinity relative to **5** (Tables 1 and 2).[8] Based on the results described above, structural hybridization of **1** and **2** was thought to be a rational entry toward discovery of an EP2/EP4 dual agonist with high subtype-selectivity and high potency. As shown in Table 2, replacement of the 16-*n*-butyl moiety of **2** with the 16-(3-methylphenyl) moiety afforded **6** with improved EP2/EP4 dual subtype-selectivity due to the remarkable reduction of its EP3 receptor affinity. Replacement of the 1,4-disubstituted phenylene moiety of **6** with 5-methylenethiophene-2-carboxylic acid, which was considered to be a bioisostere of a 1,4-phenylene moiety, afforded **7** with equipotent EP4 receptor affinity and increased receptor affinities for the EP2 and EP3 subtypes.[16] Replacement of the 1,4-disubstituted phenylene moiety of **6** with the 2-methylene-oxazole-4-carboxylic acid and 2-methylene-thiazole-4-carboxylic acid moieties afforded **8** and **9** with a tendency of reduction of EP4 receptor affinity and increase of EP2 receptor affinity, respectively. Replacement of the 1,4-disubstituted phenylene moiety of **6** with the 2-mercaptothiazole-4-carboxylic acid moiety afforded **3** with excellent EP2/EP4 dual subtype-selectivity and potency although it showed increased EP3 receptor affinity. Thus, replacement of the 2-methylene of the thiazole moiety of **9** with a sulfur atom resulted in increased affinities to all the three receptors, EP2, EP3 and EP4, while retaining excellent EP2/EP4 dual subtype-selectivity.

It was of great interest that 2-mercaptothiazole-5-carboxylic acid analog **10**, which has similar activity profiles to the 1,4-disubstituted phenylene analog **6** due to the reduction of its affinities to the three receptor subtypes EP2, EP3 and EP4, exhibited different biological profiles from its isomer **3**. The contrastive biological results of the two isomers **3** and **10** strongly suggested a different mode of their interaction with the receptor subtypes EP2 and EP4. According to our hypothesis, compound **3** was considered to interact strongly with the EP4 receptor through the electrostatic interaction of the carboxylic acid function. Another hydrogen bond through the nitrogen atom of the thiazole moiety of **3** was considered to be beneficial for binding both the receptors EP2 and EP4. The expected hydrogen bond of **10** with the receptors through the nitrogen atom of the thiazole-5-carboxylic acid moiety, if any, was considered not to be supportive as the one of **3** although it was considered not to disturb such an interaction. The sulfur atom of the 2-mercapto moiety of **3** was also considered to have some contribution to the fine tuning of the α-chain length and/or the angle of the sulfide moiety which can influence the three dimensional positions of carboxylic acid and the nitrogen atom functionalities. According to our calculation, p*K*a values of the sulfide analog **3** and the corresponding methylene analog **9** are 3.30 and 3.59, respectively. It may be plausible that the receptor affinity of **3** could be enhanced by its stronger acidity relative to **9**. Also the expected π/π or CH/π interactions of the phenylene moiety and other heterocycles in the α-chain with the receptors may provide additional contributions to the increase of EP2/EP4 dual affinities. Thus, the excellent EP2/EP4 dual selectivity of **3** was thought to be due to the overall effect expressed by the combination of all of the above described factors.

Optimization of the α-chain toward EP2/EP4 dual selectivity was conducted by maintaining the ω-chain once optimized toward EP4 receptor selectivity as shown in Table 2. To reconfirm the rationality of the 16-(3-methylphenyl) moiety as the most optimized ω-chain structure for EP2/EP4 dual selectivity, further chemical modification of the ω-chain was carried out while maintaining the most optimized α-chain structure bearing 2-mercaptothiazole-4-carboxylic acid. Results are summarized in Table 3. Replacement of the 16-(3-methylphenyl) moiety of **3** with a *n*-butyl moiety afforded **11** with loss of EP2/EP4 dual selectivity mainly due to the increased EP3 receptor affinity. Removal of the 3-methyl group of the 16-(3-methylphenyl) moiety of **3** afforded **12** with maintenance of EP2/EP4 dual selectivity while reduction of the affinities for both the EP2 and EP4 receptors was observed. Replacement of the 3-methyl moiety of the 16-(3-methylphenyl) group of **3** with a 2,2,2-trifluoroethoxymethyl moiety afforded **13** also with maintenance of the dual selectivity while the receptor affinities for EP2, EP3 and EP4 were slightly reduced. Replacement of the 16-(3-methylphenyl) moiety of **3** with the 2-methylfuran-5-yl moiety afforded **14** with 4.5-fold less potent EP2 receptor affinity and 11-fold more potent EP3 receptor affinity, respectively with maintenance of the potent EP4 receptor affinity compared with compound **3**. Replacement of the 16-(3-methylphenyl) moiety of **3** with the aliphatic cyclopentyl moiety afforded **15** while retaining EP2 receptor affinity, but possessing 6.4-fold more potent EP3 receptor affinity and 105-fold less potent EP4 receptor affinity. Thus, the terminal 16-phenyl moiety, which is required to reduce EP3 receptor affinity, was found to be one of the important structural requirements for the EP2/EP4 dual selectivity.

In summary, a series of γ-lactam PGE analogs bearing a 16-phenyl ω-chain were synthesized and evaluated. Among the tested compounds, γ-lactam PGE analog **3** bearing a 16-(3-methylphenyl) group in its ω-chain and 2-mercaptothiazole-4-carboxylic acid in its α-chain was discovered as the most optimized EP2/EP4 dual agonist. Compound **3** showed both EP2 and EP4 agonist activity[9] in rat CHO overexpressed cells with 90 and 0.79 nM (EC_{50}s), respectively. Full details including in vivo efficacy in rats will be reported in due course.

References and notes

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