Discovery of Diphenyloxazole and Nδ–Z-Ornithine Derivatives as Highly Potent and Selective EP_4 Receptor Antagonists

Kouji Hattori, Akira Tanaka, Naoaki Fujii, Hisashi Takasugi, Yoshiyuki Tenda, Masayuki Tomita, Shoko Nakazato, Keiko Nakano, Yasuko Kato, Yutaka Kono, Hidetsugu Murai, Kazuo Sakane,

Supporting Information

Experimental method

3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}benzoic acid (3): To a solution of **6** (42g) in dichloromethane (800mL) were added trifluoromethanesulfonic acid anhydride (26mL) and 2,6-lutidine (24mL) at -78□. After being stirred fof 2 h at the same temperature, the solvent was evaporated in vacuo. The residue was diluted with ethyl acetate and the mixture was washed with water, sat. $NaHCO_3$ and brine. The dried solventwas evaporated in vacuo and the residure was solved into a mixtue of methanol(200mL) and DMF(400mL). To the mixture were added 1,3-bis(diphenylphosphino)propane (7.8g), palladium acetate(4.2g), and triethylamine. (40mL) After being stired for 5h at 80□ under CO_2 atmosphere, the mixture was partitioned between ethyl acetate and water and the organic layer was washed with 1N-HCl, sat,$NaHCO_3$, and brine. The dried solvent was evaporated in vacuo and the obtained residure was purified by chromatography on silica gel to give corresponding methyl ester. To a solution of the ester derivative of a mixture of methanol (500mL) and THF (500mL) was added 1N-NaOH(400mL). After being stirred over night, the solvent was removed. The residure was partitioned between ethyl acetate and 1H-HCl and the organic layer was washed with brine. The dried solvent was evaporated in vacuo and the obtained solid was washed with ether to afford 3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}benzoic acid (**3**) (69% from **6**, 31.5g).

1H NMR (200MHz, CDCl3) δ 1.4-1.9(4H, m), 2.2-2.4(2H,m), 2.65(1H, dd, J = 10.0, 13.0Hz), 3.2(1H, m), 3.35(1H, dd, J = 3.0, 13.0Hz), 6.93(1H, t, J = 3.8Hz), 7.2-7.8(12H, m), 7.93(1H, d, J = 8.0Hz), 8.10(1H, s)

API-ESMS: 436 (M^++H).

Anal. Calcd for $C_{29}H_{25}NO_3$+0.76H_2O: C, 77.54; H,5.92; N, 3.12. Found: C, 77.54; H, 6.11; N, 2.97.

N-(benzyloxy)-3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}benzamide (7): To a solution of **3** (100mg, 1eq) in DMF were added TBTU (1.5eq) and benzyloxyamine (2eq). After being stirred for 2h, the mixture was partitioned between ethyl acetate and water and the organic layer was washed with 1N-HCl, sat,$NaHCO_3$, and brine. The dried solvent was evaporated in vacuo and the obtained residure was purified by chromatography on silica gel to afford N-(benzyloxy)-3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}benzamide (**7**) (89%, 110 mg).

1H NMR (200MHz, CDCl3) δ 1.4-1.8(4H, m), 2.2-2.4(2H,m), 2.59(1H, dd, J = 9.8, 12.0Hz), 3.1(1H, m), 3.31(1H, dd, J = 3.8, 12.0Hz), 6.92(1H, t, J = 3.8Hz), 7.2-7.8(14H, m), 8.61(1H, s)

API-ESMS: 541 (M^++H).

Anal. Calcd for $C_{36}H_{32}N_2O_3$+0.99H_2O: C, 79.70; H,6.31; N, 5.16. Found: C, 79.70; H, 6.31; N, 5.16.

N-(benzylsulfonyl)-3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}benzamide (8): To a solution of **3** (15g,) and benzylsulfoneamide (5.9g) in DMF (230mL) were addedethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (13g) and 4-dimethylaminopyridine (6.3g). After being stirred for 16h, the mixture was partitioned between ethyl acetate and water and the organic layer was washed with 1N-HCl, sat,$NaHCO_3$, and brine. The dried solvent was evaporated in vacuo and the obtained residure was

purified by chromatography on silica gel to afford N-(benzylsulfonyl)-3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)- 2-cyclohexen-1-yl]methyl}benzamide (**8**) (89%, 18.1g).

1H NMR (200MHz, CDCl3) δ 1.4-1.9(4H, m), 2.2-2.5(2H,m), 2.61(1H, dd, J = 11.0, 13.0Hz), 3.05(1H, m), 3.28(1H, dd, J = 3.8, 13.0Hz), 4.66(1H, d, J = 14.2Hz), 4.76(1H, d, J = 14.2Hz), 6.92(1H, t, J = 3.8Hz), 7.2-7.8(19H, m), 8.67(1H, s)

API-ESMS: 589 (M^++H).

Anal. Calcd for $C_{36}H_{32}N_2O_4S$+1.98 H_2O: C, 69.25; H,5.60; N, 4.49. Found: C, 69.25; H, 5.26; N, 4.42.

Solid phase synthesis for 11:

step-1. A solution of Fmoc-eAhx-OH(N-Fmoc-6-aminohexanoic acid)(180mg) and diisopropylethylamine (0.12mL) in dichloromethane(3mL) was added to a reactin vessel containing Cl-trytyl resin (200mg, 1.3mmol/g,loading). After the vessel was shaken for 12h at a ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.

step-2 After cleavage Fmoc using 20% piperazine in DMF(5mL), Fmoc-Orn(Z)-OH(254mg) and TBTU(170mg) and HOBT(70mg) and diisopropylethyamine(0.18mL) was added to a solution of the obtained resine in DMF(3mL). After the vessel was shaken for 12h at a ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.

step-3.After cleavage Fmoc using 20% piperazine in DMF(5mL), benzofuran-2-carboxylic acid (210mg) and 1,3-diisopropylcarbodiimide(0.21mL) and diisopropylethyamine(0.23mL) was added to a solution of the obtained resine in dichloromethane(3mL). After the vessel was shaken for 12h at a ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.

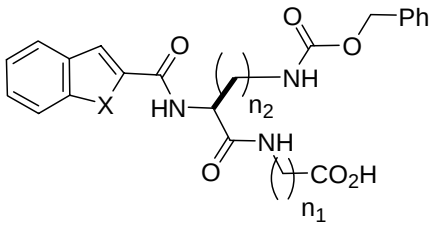
step-4. Cleavage from resin was performed with 1% trifluoromethanesulfonic acid in dichloromethane (5mL) fro 10min at an ambient temperature. After the filtrated solvent was evaporated under pressure, the residue was washed with ether to give 6-[((2S)-2-[(1-benzofuran-2-ylcarbonyl)amino]-5-{[(benzyloxy)carbonyl]amino} pentanoyl)amino]hexanoic acid (**11**) (100mg, 72%).

1H NMR (200MHz,DMSO-d6) δ 1.0-1.8(10H, m), 1.86(2H, t, J = 7.6Hz), 2.9-3.2(4H, m), 4.39(1H, m), 4.98(2H, s), 7.0-7.8(10H, m)

API-ESMS: 546 (M^++H).

Anal. Calcd for $C_{28}H_{32}N_3NaO_7$+4.87H_2O: C,53.11; H,5.93; N, 6.37. Found: C,53.11; H, 6.40; N,6.64.

Table 3. SAR of the dipeptide derivatives[a]



n_1	n_2	X	EP_4: Ki (nM)
1	3	NH	>1000
3	3	NH	41
5	3	NH	0.83
5	3	O	0.91
6	3	O	3.0
5	1	O	>1000
5	2	O	>1000
5	4	O	8.4
5	4(*R*)	O	240
5	3	S	1.8
5	3	C=C	1.2

[a] The results were presented as tas the average of two experiment . Assays were performed by the reported method, see in ref 2 and 4.