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Benzimidazole derivatives as novel nonpeptide luteinizing hormone-releasing hormone (LHRH) antagonists. Part 1: Benzimidazole-5-sulfonamides

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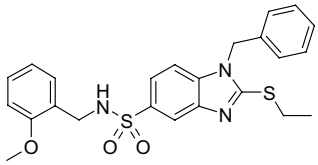
Abstract—A new class of benzimidazole-5-sulfonamides has been identified as nonpeptide luteinizing hormone-releasing hormone (LHRH) antagonists. Initial structure–activity relationships are presented resulting in compounds **19** and **28** with submicromolar dual functional activity on human and rat receptors.

1. Introduction

Luteinizing hormone-releasing hormone (LHRH) is a decapeptide hormone secreted in pulses from hypothalamus.[1] Binding of LHRH to its receptor triggers the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH), which both regulate the gonadal production of sex steroids. Dysfunctional sex steroid production can result in disease states such as benign and malign prostate growth, breast cancer and endometriosis.[2,3]

The treatment with peptidic LHRH agonists is known to desensitize the receptor and results in suppressing serum testosterone to the castration level.[4] This treatment, albeit efficient is associated with adverse effects such as hot flush, flare effects and impotence. Peptidic LHRH receptor antagonists have shown clinical efficacy with less side effects.[5] As peptides however, these agents are not amenable to oral administration. Nonpeptidic LHRH antagonists would therefore be expected to be effective oral treatments of dysfunctional sex steroid production. A plethora of nonpeptidic LHRH antagonists has been described to date.[6] However most of them are primate specific and lack potency on the rodent receptor. This primate specificity is prohibitive for investigating these compounds preclinically, for instance in rodent disease models or toxicology studies. After random screening of approximately one million compounds we have identified 1-benzyl-2-ethylsulfanyl-1*H*-benzimidazole-5-sulfonamide **1** as a functional LHRH antagonist with weak but dual activity on rat and human receptors (Fig. 1).

In this report, we will describe the SAR and optimization of **1** towards submicromolar rat and human LHRH antagonists.



IC_{50}=3.5 μM(rat-lhrh, Ca^{2+})
3.4 μM (human-lhrh, Ca^{2+})

Figure 1. Functional activity of lead compound **1**.

Keywords: LHRH antagonist; Small molecule.
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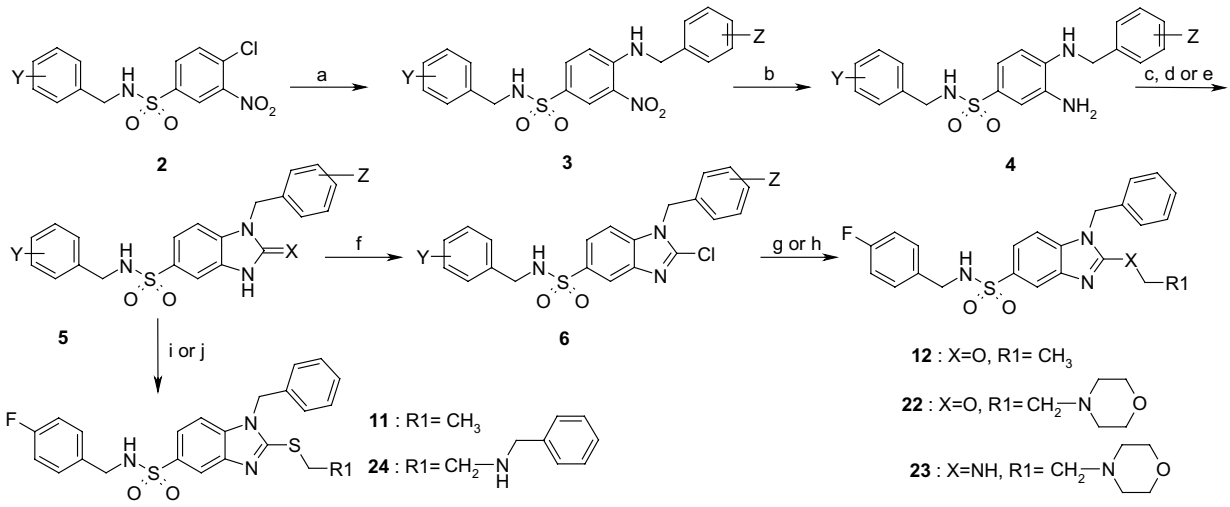


Scheme 1. Reagents and conditions: (a) benzylamine, triethylamine, CH_3CN, 65 °C, 97%; (b) H_2, 10% Pd/C, THF, rt, 99%; (c) X = S, CS_2, DIPEA, EtOH, 50 °C, 78%; (d) X = O, CDI, THF, rt, 43%; (e) $(EtO)_4C$, HOAc, $CHCl_3$, 38%; (f) $POCl_3$, 100 °C, 35%; (g) 4-(2-hydroxyethyl)-morpholine, *t*-BuOK, THF–DMF, 110 °C, 41%; (h) *N*-(2-aminoethyl)morpholine, neat, 90 °C, 47%; (i) EtI, triethylamine, THF, rt, 74%; (j) *N*-benzylethanolamine, PPh_3, di-*t*-butylazodicarboxylate, CH_2Cl_2, rt, 25%.

2. Chemistry

The 1-alkylated benzimidazole-5-sulfonamide analogues were prepared according to the procedure outlined in Scheme 1. Dianilines **4** were prepared as common intermediates by standard methods from sulfonamide **2**.[7] The resulting dianilines **4** were then treated with carbondisulfide, followed by addition of EtI or MeI to form ethyl- (or methyl)-sulfanyl-1*H*-benzimidazoles **7**–**11**, **13**, **16** and **21**.[8] The oxy-linked analogues **12**, **17**–**18** and **20** were obtained from dianilines **4** on heating with tetraethoxymethane in HOAc.[9]

Thiourea **5** (X = S) was subjected to a Mitsunobu reaction with various amino alcohols in order to afford **19**, and **24**–**28**.[10] We did not observe the corresponding *N*-alkylation products. Alternatively, **4** could be converted to the corresponding urea (**5**: X = O) followed by treatment with phosphoryl chloride[11] to yield the corresponding 2-chloro-1*H*-benzoimidazole **6**.

Intermediate **6** could be easily converted with primary alcohols and amines to prepare the corresponding *O*,*N*-linked benzimidazoles **22** and **23** under basic conditions.

3. Results and discussion

The compounds listed in Tables 1–3 have been evaluated as functional antagonists for both rat and human LHRH receptors.[12] All inhibition constants (IC_{50}) are means of at least two experiments each run in triplicates. The substitution pattern of the sulfonamide phenyl ring had a strong effect on potency with electron withdrawing substituents in *para*-position providing first submicromolar examples (**10**, **11**). The corresponding ethyl oxy-ether **12** and the methyl sulfide **13** both showed potencies similar to **11** indicating a high degree of functional tolerance in this structural area. Also, the incorporation of a hydroxyl group or a carboxylic ester group was tolerated in this position (**14**, **15**). In an additional experiment, we confirmed the activity of **13** by determining its binding constant to the human LHRH receptor (IC_{50} = 0.22 μM).[13]

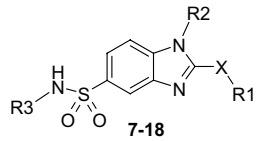
The structure–activity relationship around the benzimidazole *N*-alkyl fragment was much more conservative and even minor variations led to complete loss of activity (**16**–**18**).

Most known LHRH antagonists display a flexible side chain, typically associated with a secondary or tertiary amine.[6] Hoping to improve the potency of our lead, we introduced an ethyl morpholino group as a basic side chain at three different positions (Table 2). In accord with trends observed earlier, basic functionality was tolerated best in 2-position, which led to the discovery of **19**. Interestingly, the presence of a *S*-linker was crucial in the case of **19**, the corresponding *O*- or *N*-linker resulted in loss of potency (**22**, **23**). The morpholino fragment could be replaced by other basic moieties such as benzylamines or pyridine containing compounds (**24**–**28**). A 3-picolyl group appeared to be optimal, furnishing **28**, which is 28-fold more potent than the lead compound **1**.

In summary, the potency of our initial lead **1** was improved by changing the aromatic substitution pattern in the sulfonamide moiety as well as through incorporating a basic functionality at the 2-position of the benzimidazole core. These SAR investigations led to two optimized compounds, **19** and **28**, which show improved potency by 1.5 orders of magnitude. Of note, this optimization was possible on both human and rat receptors.

However, LHRH exhibits picomolar affinities to its receptor and reaches high peak concentrations after its

Table 1. Functional activity of compounds **7**–**18**



7-18

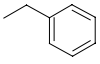
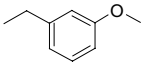
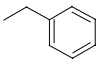
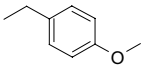
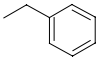
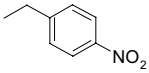
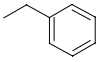
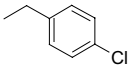
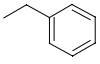
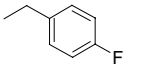
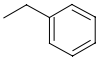
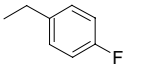
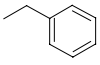
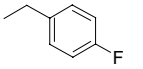
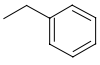
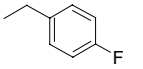
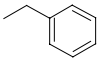
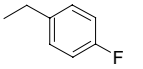
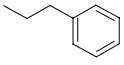
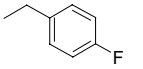
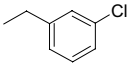
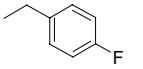
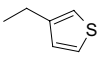
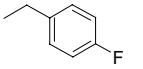
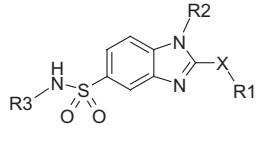
Compd	XR1	R2	R3	IC_{50} (μM)	
				r-LHRH	h-LHRH
7	S–Et			4.0	4.8
8	S–Et			0.94	1.1
9	S–Et			1.2	1.1
10	S–Et			0.33	0.28
11	S–Et			0.20	0.25
12	O–Et			0.47	0.30
13	S–Me			0.21	0.22
14	$S(CH_2)_2OH$			0.60	0.27
15	SCH_2CO_2Et			0.81	0.49
16	S–Et			>10	>10
17	O–Et			1.9	0.88
18	O–Et			2.0	1.4

Table 2. Introduction of basic side chains into structure **11**. Functional activity of compounds **19**–**21**



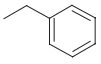
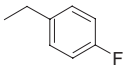
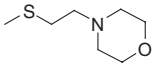
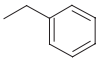
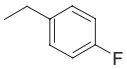
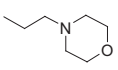
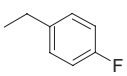
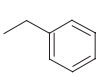
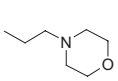
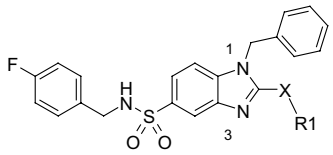
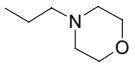
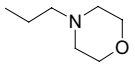
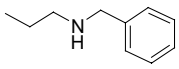
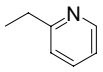
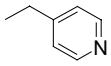
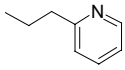
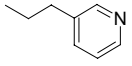
Compd	X-R1	R2	R3	IC_{50} (μM)	
				r-LHRH	h-LHRH
11	–SEt			0.20	0.25
19				0.17	0.16
20	–OEt			7.2	2.1
21	–SEt			>10	>10

Table 3. Functional activity of compounds **22–28**



Compd	X	R1	IC_{50} (μM)	
			r-LHRH	h-LHRH
22	O		3.2	5.8
23	NH		>10	>10
24	S		0.53	0.67
25	S		0.96	0.82
26	S		0.24	0.32
27	S		0.28	0.23
28	S		0.14	0.12

release into the hypothalamus.[14] Therefore, despite the respectable potency of our compounds, we felt that further optimization towards at least single digit nanomolar compounds would be necessary for investigations of our lead structure in vivo.

References and notes

1. (a) Schally, A. V.; Arimura, A.; Kastin, A. J.; Matsuo, H.; Baba, Y.; Redding, T. W.; Nair, R. M. G.; Debeljuk, L.; White, W. F. *Science* **1971**, *173*, 1036; (b) Matsuo, H.; Baba, Y.; Nair, R. M. G.; Arimura, A.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334.
2. Filicori, M.; Flamigni, C. *Drug* **1988**, *35*, 63.
3. (a) Karten, M. J.; Rivier, J. E. *Endocrinol. Rev.* **1986**, *7*, 44; (b) Dutta, A. S. *Drugs Future* **1988**, *13*, 43.
4. Fujino, M.; Fukuda, T.; Shinagawa, S.; Kobayashi, S.; Yamazaki, I.; Nakayama, R.; Seely, J. H.; White, W. F.; Rippel, R. H. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 406.
5. (a) Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropoulos, E. E.; Engel, J. *Angew. Chem., Int. Ed.* **1997**, *36*, 2148; (b) Blithe, D. L. *Trends Endocrinol. Metab.* **2001**, *12*, 238.
6. (a) Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Gao, Y.; Connors, P. J., Jr.; Struthers, R. S.; Xie, Q.; Tucci, F. C.; Reinhart, G. J.; Wu, D.; Saunders, J.; Chen, C. *J. Med. Chem.* **2003**, *46*, 1769; (b) Sasaki, S.; Cho, N.; Nara, Y.; Harada, M.; Endo, S.; Suzuki, N.; Furuya, S.; Fujino, M. *J. Med. Chem.* **2003**, *46*, 113; (c) Young, J. R.; Huang, S. X.; Walsh, T. F.; Wyvratt, M. J., Jr.; Yang, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Cheng, K.; Coulet, M. T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 827; (d) Devita, R. J.; Walsh, T. F.; Young, J. R.; Ujjainwalla, F.; Toupence, R. B.; Parkh, M.; Huang, S. X.; Fair, J. A.; Goulet, M. T.; Wyvratt, M. J.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Cui, J.; Mount, G.; Rohrer, S. P.; Schaeffer, J. M.; Rhodes, L.; Drisko, J. E.; McGowan, E.; MacIntyre, D. E.; Vincent, S.; Carlin, J. R.; Cameron, J.; Smith, R. G. *J. Med. Chem.* **2001**, *44*, 917.
7. Wu, Z.; Rea, P.; Wickham, G. *Tetrahedron Lett.* **2000**, *41*, 9871.
8. Hazelton, J. C.; Iddon, B.; Suschitzky, H.; Woolley, L. H. *Tetrahedron* **1995**, *51*, 10771.
9. Singh, B.; Lesher, G. Y. *J. Heterocycl. Chem.* **1991**, *28*, 933.
10. Typical procedure to prepare 2-substituted benzimidazole (synthesis of compound **28**); To a stirred solution of thiourea **5** (50.0 mg, 0.120 mmol), 3-(2-hydroxyethyl)pyridine (14.4 mg, 0.120 mmol), and polymer supported PPh_3 (1.6 mmol/g, 146 mg, 0.230 mmol) in CH_2Cl_2 was added di-*tert*-butylazodicarboxylate (53.9 mg, 0.230 mmol). The resulting suspension turned to be a clear solution in 5 min. The solution was stirred at room temperature for 2 h. After filtering off the resin, the filtrate was concentrated in vacuo. The crude residue was purified through preparative thin layer chromatography (CH_2Cl_2/MeOH = 20/1) to give desired 1-benzyl-2-(2-pyridin-3-yl-ethylsulfanyl)-1*H*-benzimidazole-5-(4-fluorobenzyl)sulfonamide **28** (19.5 mg, 31% yield) as waxy material. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.14–3.19 (2H, t, J = 7.4 Hz), 3.64–3.69 (2H, t, J = 7.4 Hz), 4.10–4.12 (2H, d, J = 6.2 Hz), 4.87 (1H, br s), 5.29 (2H, s), 6.88–6.93 (2H, t, J = 8.7 Hz), 7.13–7.38 (9H, m), 7.60–7.68 (2H, m), 8.21 (1H, s), 8.46–8.51 (2H, m).
11. James, A. T.; Turner, E. E. *J. Chem. Soc.* **1950**, 1515.
12. Intracellular Ca mobilization data were obtained using recombinant CHO cells transfected with human and rat LHRH receptor cDNA. The cells were preincubated with a variable concentration of test compounds for 20 min at

25 °C. Fluorescence changes indicating mobilization of cytoplasmic calcium (Flu-3 AM, Molecular Probes) were measured on a FDSS-3000 machine (Hamamatsu photonics) after the stimulation with 10 nM LHRH for human receptor expressing cells and 1 nM LHRH for rat receptor expressing cells. The inhibitory effect of the compounds was calculated by comparing the Integral ratio (ratio = actual fluorescence/initial fluorescence).

13. The receptor binding assay was performed according to Ref. 14. Briefly, membrane fractions from recombinant CHO cells[12] were incubated with 150 pM of [125I]-D-Trp6-LHRH (Pharmacia) in the presence of various concentration of compounds in assay buffer. The total binding was defined as that measured in the absence of a competing agent. Nonspecific binding was determined in the presence of 10 μM D-Trp6-LHRH.
14. Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190.