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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5285–5288

Synthesis and structure–activity investigation of iodinated arylhydantoins and arylthiohydantoins for development as androgen receptor radioligands

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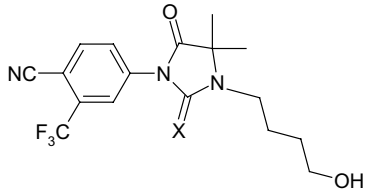
Received 14 July 2004; accepted 13 August 2004
Available online 15 September 2004

Abstract—A series of side-chain derivatives of the arylhydantoin RU 58841 and the arylthiohydantoin RU 59063, wherein the aromatic trifluoromethyl group was replaced with iodine, was synthesized for possible development as radioiodinated androgen receptor (AR) ligands. Derivatives containing the cyanomethyl, methoxyethyl and propenyl side-chains displayed moderately high affinity (K_i = 20–59 nM) towards the rat AR. Side-chains containing bulky lipophilic groups such as, benzyl and phenylpropyl, were poorly tolerated (K_i > 219 nM). Superior AR binding affinities (0.71 nM < K_i < 11 nM) were displayed by arylhydantoins and arylthiohydantoin derivatives containing hydroxybutyl or methyl side-chains. The latter compounds are potential candidates for development as radioiodinated AR ligands.

Prostate cancer (PC) is the most frequently diagnosed cancer and the second leading cause of cancer death in American men. The American Cancer Society estimates that in 2004, 230,110 men will be diagnosed with PC and an estimated 29,900 will die of the disease.[1] The importance of circulating androgens for the growth of prostate tumors is well established and androgen receptor (AR) expression is frequently observed in primary and metastatic PC.[2,3] This finding has presented strong impetus for the development of radiolabeled AR ligands for external diagnostic imaging of tumor sites in PC using positron emission tomography (PET) or single photon emission computed tomography (SPECT).[4] To date, the majority of these studies have focused on steroid-based radioligands and reports of several radiofluorinated androgen derivatives for PET imaging of PC have appeared in the recent literature.[5,6] In contrast, the development of high-affinity, radioiodinated steroid-based AR radioligands for SPECT imaging has had limited success. This may be attributable, in part, to the sensitivity of the AR ligand binding domain to the increased steric bulk of iodinated steroids.[7]

A suitable [^{123}I]-labeled AR radioligand for the early detection and diagnosis of PC with SPECT could have broad clinical utility due to the wide availability of SPECT instrumentation in nuclear medicine clinics. To address this goal, we focused on RU 58841 and RU 59063, prototypes of a new class of selective, high-affinity aryl(thio)hydantoin AR ligands[8,9] (Chart 1), as leads for development of a radioiodinated SPECT



LIGAND	X	K_i (nM)[a]
RU 58841	O	26 ± 5[b]
RU 59063	S	2.23 ± 0.50[c]

[a]Mean ± SEM. [b]reference 16. [c]reference 10.

Chart 1.

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doi:10.1016/j.bmcl.2004.08.031

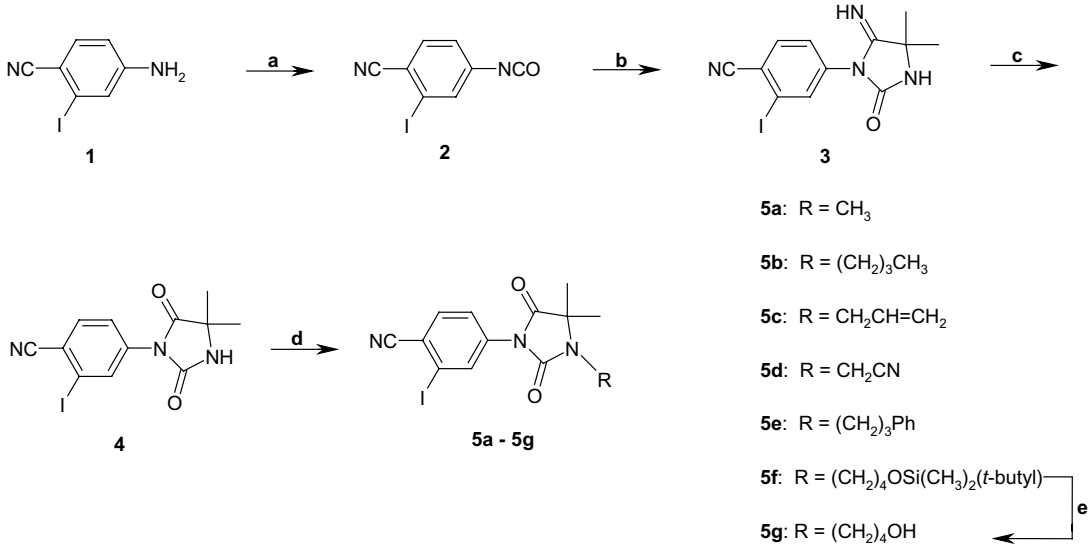
radioligand. We hypothesized that the increased conformational flexibility of these nonsteroidal ligands over steroid-based ligands could favor the design of iodinated ligands that are better accommodated at the AR ligand binding domain leading to improved binding affinity. In the initial phase of this work, we observed that replacement of the trifluoromethyl group in RU 59063 with the similarly hydrophobic iodine atom resulted in a 3-fold higher AR binding affinity (**8e**, $K_i = 0.71$ nM).[10] However, tissue distribution studies conducted in castrated rats with the corresponding radioiodinated analog ([^{125}I]**8e**) showed low accumulation of radioactivity in AR-rich target tissues including prostate.[11] The lack of AR-mediated target uptake of [^{125}I]**8e** was ascribed to the metabolic conversion of its side-chain hydroxyl to a carboxylic acid based on the results of metabolic studies reported by Cousty-Berlin et al. for the structurally similar analogs RU 58841 and RU 59063.[12] Thus, it was of interest to explore the effect of alternative side-chains having improved metabolic stability on the AR binding affinity of iodinated RU 58841 and RU 59063 derivatives. Accordingly, the short series of such derivatives was chosen for development based on synthetic convenience and availability of side-chain precursors. We report herein, a limited SAR investigation of these derivatives to identify suitable high-affinity analogs for development as [^{123}I]-labeled AR ligands for SPECT imaging of PC.

The hydantoin derivatives **5a**–**g** were synthesized as outlined in Scheme 1. Treatment of 4-cyano-3-iodoaniline (**1**)[10] with phosgene provided the isocyanate **2**, which was condensed with 2-amino-2-cyanopropane[8] to give the imino derivative **3**. Acid hydrolysis of **3** provided **4** in 64% overall yield. Synthesis of compounds **5a**–**g** were achieved by the treatment of **4** with NaH in DMF and subsequent alkylation with the appropriate commercially available alkyl halide or tosylate. Preparation of the *N*-(4-hydroxybutyl) derivative **5g** was conducted via initial synthesis of the 4-(*tert*-butyldimethylsilyloxy) derivative **5f**, followed by deprotection of the silyl group with acid hydrolysis.

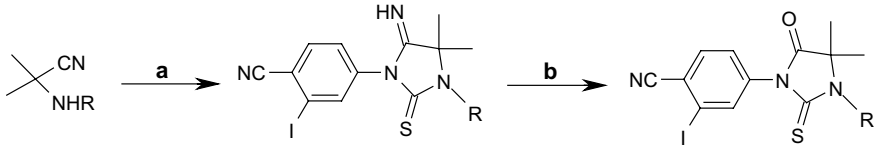
Synthesis of the thiohydantoin derivatives **8a**–**e** were accomplished as shown in Scheme 2. The 2-alkylamino-2-cyanopropane derivatives (**6a**–**e**) were readily prepared by the treatment of acetone cyanohydrin with the appropriate alkylamine using published procedures.[9] Condensation of 2-iodo-4-isothiocyanatobenzonitrile[10] with **6a**–**e** gave the imino derivatives **7a**–**e**, which were subsequently converted to the thiohydantoin derivatives **8a**–**e** by acid hydrolysis (2 N HCl). The final products were purified to homogeneity by flash chromatography and solids were recrystallized from EtOAc/hexane mixtures. The compounds gave ^{1}H NMR and elemental analysis or mass spectral data consistent with the assigned structures.

The binding affinities of new ligands and reference compounds to the rat prostate cytosolic AR were determined using a competitive binding assay in the presence of the high-affinity AR radioligand, [^{3}H]mibolerone.[13–15] These data (expressed as inhibition constants, K_i) are presented in Table 1. All ligands demonstrated monophasic radioligand displacement curves (Hill coefficient close to unity) indicating interaction with a single class of binding sites.

In the hydantoin series of ligands, the parent unsubstituted hydantoin (**4**) displayed weak binding affinity towards AR ($K_i = 400$ nM). However, introduction of either a methyl (**5a**) or hydroxybutyl (**5g**) group at the *N*(3) position resulted in a dramatic improvement in affinity (36- and 200-fold, respectively, over compound **4**). In contrast, introduction of a butyl group at this position resulted in only a slight enhancement (5-fold) in affinity suggesting the importance of the polar hydroxyl group in **5g** for high AR affinity. A dramatic loss



Scheme 1. Reagents: (a) $COCl_2$, toluene, reflux; (b) 2-amino-2-cyanopropane, Et_3N, 1,2-dichloroethane; (c) 2 N HCl, CH_3OH, reflux; (d) (1) NaH/DMF, (2) RX; (e) 2 N HCl, CH_3OH.



6a: R = CH_3 **7a - 7e** **8a**: R = CH_3

6b: R = $(CH_2)_3CH_3$ **8b**: R = $(CH_2)_3CH_3$

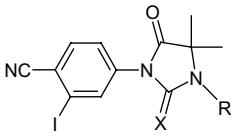
6c: R = $(CH_2)_2OCH_3$ **8c**: R = $(CH_2)_2OCH_3$

6d: R = CH_2Ph **8d**: R = CH_2Ph

6e: R = $(CH_2)_4OH$ **8e**: R = $(CH_2)_4OH$

Scheme 2. Reagents: (a) 2-iodo-4-isothiocyanatobenzonitrile, Et_3N, THF, reflux; (b) 2 N HCl, CH_3OH, reflux.

Table 1. Inhibition constants (K_i) for ligands at the rat androgen receptor



Compd	X	R	K_i[a] (nM)	Mp[b] (°C)	Yield[c] (%)
4	O	H	400 ± 41	198–200	64[d]
5a	O	CH_3	11 ± 5	193–194	61[e]
5b	O	$(CH_2)_3CH_3$	77 ± 17	100–102	68[e]
5c	O	$CH_2CH{=}CH_2$	59 ± 1	96–97	92[e]
5d	O	CH_2CN	20 ± 3	158–159	77[e]
5e	O	$(CH_2)_3Ph$	639 ± 141	Oil	86[e]
5g	O	$(CH_2)_4OH$	2 ± 1	Oil	37[e]
8a	S	CH_3	2.5 ± 0.7	183–184	73[f]
8b	S	$(CH_2)_3CH_3$	117 ± 19	Amorphous	56[f]
8c	S	$(CH_2)_2OCH_3$	37 ± 3	Oil	66[f]
8d	S	CH_2Ph	219 ± 8	Amorphous	49[f]
8e	S	$(CH_2)_4OH$	0.71 ± 0.22	Amorphous	76[f]
Testosterone			4.9 ± 1.8		
Mibolerone			0.75 ± 0.08		

[a] Data are presented as mean ± SEM of three independent determinations each conducted in duplicate.
[b] Solids were recrystallized from EtOAc/hexane mixtures.
[c] Isolated yields after chromatography.
[d] Yield based on **1**.
[e] Yield based on **4**.
[f] Yield based on 2-iodo-4-isothiocyanatobenzonitrile.

in AR affinity was observed for analog **5e**, which bears the sterically demanding 3-phenylpropyl side-chain, suggesting that there is limited tolerance for increased bulk at this position. Introduction of the propenyl (**5c**) or cyanomethyl (**5d**) side-chain afforded moderately high AR affinities of 59 and 20 nM, respectively. The latter compounds were chosen for investigation since a significant improvement in AR binding affinity was previously noted upon introduction of the iodopropenyl and cyanomethyl side-chains in the RU 58841 series.[16,17] The improved binding affinity of these derivatives over **4** could be due to a favorable π electronic interaction of the olefinic and cyano moieties with the AR binding site.

Thiohydantoin derivatives containing a methyl (**8a**) or hydroxybutyl (**8e**) side-chain displayed high AR binding affinity similar to that observed in the hydantoin series. Moreover, these derivatives showed a 3-fold improvement in affinity over their hydantoin counterparts supporting the previous observation of enhanced affinity with the thio function.[9,10] Bulky, hydrophobic side-chains such as butyl (**8b**) and benzyl (**8d**) were poorly tolerated as previously noted in the hydantoin series. In contrast, the more polar methoxyethyl derivative **8c** displayed moderately high affinity (K_i = 37 nM) similar to that seen with **5c** and **5d.** Taken together, this data suggests that electronic effects in the side-chains of these

ligands could play an important role in enhancing AR binding affinity.

In summary, a limited series of aryl(thio)hydantoin derivatives were synthesized and evaluated for their AR binding affinity to identify suitable alternative candidates to the metabolically labile analog **8e** for development as AR radioligands. Our SAR studies suggest that the high-affinity, *N*-methyl hydantoin and thiohydantoin derivatives (**5a**,**8a**) are promising candidates for radiolabeling and further biological evaluation. The binding affinity of these ligands towards the human AR will also need to be evaluated to address the possibility of cross-species differences. These investigations will be the subject of future reports.

Acknowledgements

We thank Dr. Craig Marhefka of Vertex Pharmaceuticals Inc., Cambridge, MA for helpful discussions. This research was supported by grants from the National Institutes of Health (CA 77287) and the SPORE in Prostate Cancer (P50 CA 69568).

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