J. Soc. Cosmet. Sci. Korea
Vol. 46, No. 1, March 2020, 43-47

pISSN 1226-2587
eISSN 2288-9507
http://dx.doi.org/10.15230/SCSK.2020.46.1.43

아다만틸을 기반한 *N*-아릴아미드 신규 안드로겐 수용체 길항제

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(2020년 3월 10일 접수, 2020년 3월 23일 수정, 2020년 3월 26일 채택)

Adamantyl-based *N*-arylamide as a Novel Series of Androgen Receptor Antagonists

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(Received March 10, 2020; Revised March 23, 2020; Accepted March 26, 2020)

요 약: 안드로겐 수용체 길항제로서 *N*-아릴아미드의 신규한 아다만틸 유도체들을 합성하고 항안드로겐 활성을 평가 하였다. 아다만틸 유도체를 함유하는 *N*-아릴아미드는 아다만틸 치환체가 없는 유도체보다 더 높은 활성을 가졌다. 아다만틸의 공간부피 및 방향족 고리에 전자밀도 상승효과를 주는 pendant 작용기들이 강력한 길항작용에 결정적인 영향을 미쳤다. 리간드와 수용체 사이의 상호 작용을 설명하기 위해 분자 모델링 연구를 수행 하였다.

Abstract: A novel series of adamantyl derivatives of *N*-aryl amides as androgen receptor antagonists were synthesized and their anti-androgenic activities were evaluated. The *N*-aryl amides containing adamantyl derivatives had more activity than those lacking adamantyl substitutions. The synergistic effect of bulkiness of the adamantyl group and modulation of electron density on the aromatic ring by pendant groups was crucial to the potent antagonism. Molecular modeling studies were performed to elucidate the interactions between ligands and receptors.

***Keywords*:** *androgen receptor, admantyl N-aryl amide, hair loss*

1. Introduction

The androgen receptor (AR) is an important member of the superfamily of nuclear hormone receptors that function as ligand-dependent regulators of transcription[1]. AR is responsible for mediating the physiological functions of testosterone (T) and dihydrotestosterone (DHT). The T-mediated functions are anabolic (muscle mass increase, vocal cord enlargement) and spermatogenesis (male sex drive and performance). The DHT-mediated effects include increases in facial and body hair, acne, scalp hair recession, and prostate enlargement[2]. Overproduction of androgens during puberty plays a critical role in excess sebum production and hair loss[3].

Anti-androgens can be applied to treat acne, hyperseborrhea, hirsutism, and male pattern alopecia[2]. Clinically, non-steroidal AR antagonists have advantages over steroidal antagonists

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because of their physicochemical and pharmacokinetic properties[4], and several structural classes of AR antagonists are being studied. To obtain an effective agent, we explored various non-steroidal AR antagonists and identified novel adamantyl substituted amide structures as antagonists. We initially analyzed AR antagonists, which have a similar structure as flutamide (Figure 1), and studied the aryl moiety because little is known regarding its SAR (structure-activity relationship)[5].

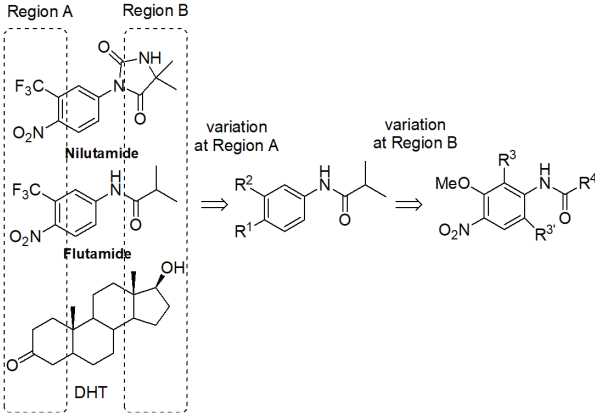


Figure 1. Design of aryl amide AR antagonists

2. Experimental

2.1. Reagent

4-Amino-2-chlorobenzonitrile, 4-amino-3-chlorobenzonitrile, 4-cyano-3-trifluoromethylaniline, 2-chloro-4-nitroaniline, 4-nitro -3-(trifluoromethyl)aniline, 4-amino-3-methylbenzoic acid was obtained from Sigma-Aldrich (USA).

4-Amino-2-methoxybenzonitrile, 4-amino-2-methylbenzonitrile, 3-chloro-4-nitroaniline, 3-methoxy-4-nitroaniline, 3-methyl-4-nitroaniline, 2-chloro-4-cyanobenzoic acid, 3-chloro-4-nitrobenzoic acid was obtained from Chemlin (China). Acyl chloride compounds was obtained from Amorepacific R&D (Korea).

2.2. General Synthetic Procedure

The amine compound 1 (1.1 eq) was dissolved in pyridine (1.0 M solution). Then acyl chloride 2 (1.0 eq)was added to dropwise with stirring at 0 ℃. The mixture was stirred for 3 - 5 hours at room temperature. The reaction mixture was diluted with EtOAc and washed two times with 1N HCl and brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography to give the title compound 3.

The spectroscopic data for representative compound 5c as follows:

Adamantane-1-carboxylic acid (3-methoxy-4-nitro-phenyl)-amide(5c)

^{1}H NMR (300MHz, $CDCl_3$) δ7.97 (d, 1H, J =2.1 Hz), 7.93 (d, 1H, J = 9.0 Hz), 7.51 (s, 1H), 6.76 (dd, 1H, J = 2.1 and J =9.0 Hz), 3.98 (s, 3H), 2.13(s, 3H), 2.04 - 1.90 (m, 6H), 1.83 - 1.72 (m, 6H) : ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.8, 153.7, 145.7, 133.1, 126.7, 110.8, 103.9, 56.3, 41.3, 37.9, 35.8, 27.5: HRMS calcd for $C_{18}H_{22}N_2O_4$ [M]+330.1579 found 330.1580.

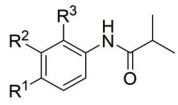
2.3. AR Antagonism Assay

The evaluation of AR antagonizing activity was performed by Cerep Co. (France). Human cytosolic AR proteins expressed in LNCaP cells were used in a modified Tris-HCl buffer, pH 7.4. The AR protein aliquot was incubated with a saturating concentration (1 nM) of [^{3}H]methyltrienolone, which is a potent anabolic steroid and a selective high affinity AR agonist, in the absence or presence of increasing concentrations of the compounds for 24 hours at 4 ℃. Non-specific binding of [^{3}H] methyltrienolone was estimated in separate incubates by adding excess unlabeled mibolerone. The seperation of bound and free radioactivity at the end of the incubation was achieved and the receptors were filtered and washed. And then the filters were countered to determine specially bound [^{3}H] methyltrienolone. The specific binding of [^{3}H] methyltrienolone at each concentration of the compounds was calculated by subtracting the non-specific binding of mibolerone and expressed as the percentage of inhibition. The concentration of compound that reduced binding by 50% (IC_{50}) was determined using the inhibition percentages.

3. Results and Discussion

As shown in Figure 1, the design rationale for novel antagonists was based on the hypothesis that the aryl moiety

on region A corresponds to the ketone moiety of DHT or satisfies the electron deficiency on the aryl part for antagonistic activity[6]. In addition, the role of additional region B was thought to be important for antagonistic activity. Therefore, we synthesized several *N*-arylalkyl-amides and evaluated their AR antagonistic activities (Table 1).



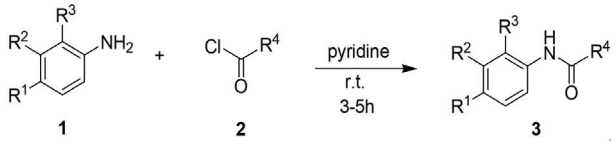
4

Table 1. *In Vitro* AR Antagonistic Activity of *N*-aryl-isobutyramides **4**

Entry	R_1	R_2	IC_{50} (μM)[a]
4a	NO_2	OMe	< 1 (55%)[b]
4b	NO_2	Me	2.0
4c	OMe	CF_3	> 30
flutamide	NO_2	CF_3	9.07

[a] Value represents the mean of at least two measurements.
[b] percent inhibition at 1 μM

The general synthesis of aryl amide **3** is illustrated in Scheme 1. The alkanoic acid was refluxed in thionyl chloride to afford acid chlorides. The acid chloride was reacted immediately with various anilines in pyridine to produce the corresponding alkanoic acid carboxamide derivatives. The general synthetic procedure was presented in **reference 7.**



1 2 3

Scheme 1. Synthesis of AR antagonists.

The compounds with electron-donating groups (**4a**, **4b**) on R^2 showed more potent antagonistic activity than compounds with the CF_3 group (flutamide). The flutamide with NO_2 at the R^1 position was more potent than compound **4c** with OMe. Therefore, electron density on the aromatic ring position is considered an important factor for antagonistic activity. Based on these results, the optimal electron density in region A and additional structural factors in region B were thought be useful for modulation of activity. To investigate the influence of steric bulkiness on R^4, several alkyl groups were selected. Among them, the adamantyl group has been studied in drug design[7]. Recently, improved depigmentation activities of kojic acid and resorcinol derivatives containing the adamantyl moiety were also reported[8]. AR antagonistic activities for various alkyl substituted derivatives **5** are shown in Table 2.

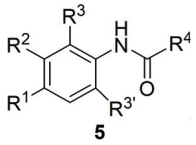


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Table 2. *In Vitro* AR Antagonistic Activity of *N*-aryl-alkyl-amides **5**

Entry	R^1	R^2	R^3	$R^{3'}$	R^4	IC50 (μM)[a]
5a	NO_2	OMe	H	H	Me	> 30
4a	NO_2	OMe	H	H	i-Pr	< 1 (55%)[b]
5b	NO_2	OMe	H	H	cyclohexyl	< 1 (62%)[b]
5c	NO_2	OMe	H	H	Ad[c]	0.24
5d	H	H	H	H	Ad	> 15
5e	H	OMe	H	H	Ad	> 15
5f	OMe	H	H	H	Ad	> 15
5g	OMe	OMe	H	H	Ad	> 15
5h	Br	OMe	H	H	Ad	5.3
5i	CO_2Me	OMe	H	H	Ad	> 15 (32%)[d]
5j	CN	OMe	H	H	Ad	2.2
5k	H	NO_2	H	H	Ad	13.5
5l	NO_2	H	H	H	Ad	> 15 (48%)[d]
5m	NO_2	Me	H	H	Ad	1.2
5n	NO_2	CF_3	H	H	Ad	3.6
5o	NO_2	H	OMe	H	Ad	1.2
5p	NO_2	OMe	H	OMe	Ad	> 15

[a] Value represents the mean of at least two measurements.
[b] percent inhibition at 1 μM
[c] Adamantyl
[d] percent inhibition at 15 μM

Compound **5a** containing the methyl (IC_{50} > 30 μM) did not show AR antagonistic activity. However, compound **5b** (R^4 = cyclohexyl) and compound **4a** (R^4 = isopropyl) showed more potent activity than compound **5a.** The most sterically congested compound, **5c** (R^4= adamantyl group), showed more potent activity than compound **5b**. From the substitution of the alkyl group on R^4, we speculated that as the size of the alkyl group increases, the potency in antagonism also increases. Therefore, we further examined the anti-androgenic effect of adamantyl bearing compounds **5d–5p** with various electron densities on region A. As shown in Table 2, the parent compound **5d** and compounds with an electron - donating MeO group on aromatic substituent R^1–R^2 (**5e–5g**) were inactive, but the compounds with electron - withdrawing groups at R^1–R^3(**5h–5o**) showed antagonistic activity, and their activities were relatively weak compared to compound **5c**. For compound **5p**, the addition of MeO on R^3 to compound **5c** deteriorated its activity. Therefore, the number and position of the MeO group influenced its activity. Compound **5c** showed the most potency for AR antagonistic activity in these compounds. As a result, introduction of an electron - withdrawing group R^1 such as NO_2 or CN on the aromatic ring, and balance of the electronic environment via additional electron - donating MeO groups together with bulky R^4 substituents are crucial for their antagonistic activity.

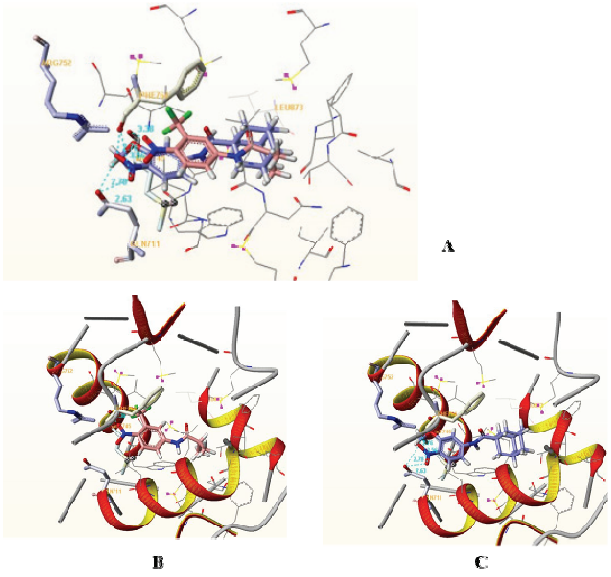

Figure 2. Binding mode between residues in the hAR binding pocket and result pose (A), flutamide (B), and **5c**(C).

To understand the interaction between **5c** and hAR, we performed molecular docking simulations between **5c** and the hAR active site (X-ray crystal structure with protein databank [PDB] code 1E3G)[9] with the Surflex-Dock v.2.7 SYBYL-X 2.1.1 software program (Tripos, L.P., USA). Using SYBYL, we extracted the ligand from the protein complex in the PDB file, and hydrogen atoms were added to the enzyme; staged minimization was performed using the Tripos Force Field. The partial atomic charges were calculated using the Gasteiger-Hückel method. The docked conformations with the highest docking scores were selected for binding mode analysis.

Based on analysis of the protein and ligands, flutamide and compound **5c**, we observed that the ligand orientation in the binding site favored the hydrophilic interaction. A hydrophilic interaction observed in the interaction between ligands with the active site residue Gln711, Met745, and Phe764 indicates a possible explanation for the strong inhibition. In the interaction between **5c** and hAR, Gln711, Met745, and Phe764 in hAR interacted directly with the nitro group of **5c**, with O-O distance of 2.63 to 2.78 Å. The highly hydrophobic adamantine is located in the hydrophobic pocket of the hAR. As a result of the polar interaction, **5c** showed stronger inhibitory activity (IC_{50} = 0.24 μM) than flutamide (IC_{50} = 9.07 μM). Therefore, the *in vitro* values were in good agreement with the docking simulations.

4. Conclusion

In summary, we prepared a series of potent anti-androgenic agents with an adamantyl moiety. Although introduction of the adamantyl group seemed to improve antagonistic activity, electronic effect on *N*-aryl moiety showed synergistic influence that has a beneficial effect on AR antagonism. Among these analogues, adamantane-1-carboxylic acid (3-methoxy-4-nitro-phenyl)-amide (**5c**) s elected for *in vivo* efficacy evaluation, and further investigations to develop these compounds as hair growth stimulators are currently being performed.

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