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Preliminary investigations into triazole derived androgen receptor antagonists

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ABSTRACT

A range of 1,4-substituted-1,2,3-*N*-phenyltriazoles were synthesized and evaluated as non-steroidal androgen receptor (AR) antagonists. The motivation for this study was to replace the *N*-phenyl amide portion of small molecule antiandrogens with a 1,2,3-triazole and determine effects, if any, on biological activity. The synthetic methodology presented herein is robust, high yielding and extremely rapid. Using this methodology a series of 17 *N*-aryl triazoles were synthesized from commercially available starting materials in less than 3 h. After preliminary biological screening at 20 and 40 μM, the most promising three compounds were found to display IC_{50} values of 40–50 μM against androgen dependent (LNCaP) cells and serve as a starting point for further structure–activity investigations. All compounds in this work were the focus of an in silico study to dock the compounds into the human androgen receptor ligand binding domain (hARLBD) and compare their predicted binding affinity with known antiandrogens. A comparison of receptor–ligand interactions for the wild type and T877A mutant AR revealed two novel polar interactions. One with Q738 of the wild type site and the second with the mutated A877 residue.

1. Introduction

Prostate cancer (PC) is the most commonly diagnosed cancer in men and is a leading cause of death in the male population, in 2013 prostate cancer was estimated to account for almost 14% of all diagnosed cancers.[1] Antagonistic binding of the steroid-docking site within the androgen receptor (AR) is the most common form of small-molecular therapy for PC. The antagonistic binding mitigates cancer cell proliferation and disease progression by preventing the binding of endogenous testosterone or dihydrotestosterone (5α-DHT) into the AR. The initial inspiration of PC chemotherapy was the chemical modification of naturally occurring steroidal ligands. Though viable, these compounds were plagued with poor bioavailability, hepatotoxicity and a lack of tissue specific action, leading to their discontinued clinical use.[2] As such, non-steroidal AR antagonists, such as Flutamide® **1** (its active metabolite **2**), Enzalutamide (**MDV3100**) and Bicalutamide **3** (Casodex®) are the current state of the art in androgen blockade therapy (Fig. 1).

A common structural feature of non-steroidal AR antagonists is the extremely electron-deficient *N*-phenyl amide moiety. The aryl portion commonly bears a trifluoromethyl group (CF_3) in addition to a strongly deactivating group at the *para*-position, relative to the amide.[3–8] Typical examples of *para*-substitution are the nitro group (NO_2) in the case of **1** and **2**, or the nitrile (CN) of **3** and **MDV3100**. Also, electron deficient *N*-phenyl amides have found widespread use in a variety of fields such as antimicrobials, anti-HIV therapy, Raf inhibitors and have shown potential as antitubercular agents.[9–15]

Recently, we reported the synthesis of several anti-androgenic (±)-α-lipoic acid derivatives bearing *meta*- and *para*- substitution patterns of CF_3–NO_2 and CF_3–CN, respectively.[8] Though successfully synthesised, we have found low-to-moderate yields (typically <50%) of the amide formation due to the electron deficient aryl ring despite extensive optimisation (Scheme 1).

In addition to low yields, another common problem noted by us and others,[8,16] is the tedious removal of residual deactivated aniline present in the crude product from the desired *N*-phenyl amide. This occurs usually from the inability of the deactivated aniline to be protonated and washed out during acidic work-up and/or their tendency to possess poorly defined R_f values (streaking) during

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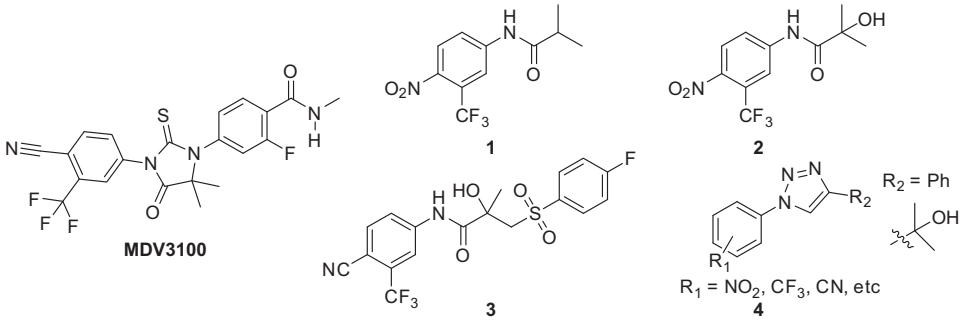
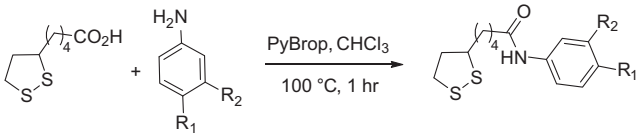


Figure 1. Enzalutamide **MDV3100**, flutamide **1**, hydroxyflutamide **2**, bicalutamide **3** and proposed triazoles **4**.



5 **6** $R_1 = NO_2$, $R_2 = CF_3$ **8** $R_1 = NO_2$, $R_2 = F_3$, 46%

7 $R_1 = CN$, $R_2 = CF_3$ **9** $R_1 = CN$, $R_2 = CF_3$, 34%

Scheme 1. Previously synthesised electron deficient *N*-arylamides.

column chromatography. To circumvent this problem, Sugai et al.[16] used an acetylation technique (derivatising both desired compound and residual starting aniline) to allow for chromatographic resolution. Though an effective strategy, the acyl group then had to be removed from their target compound, adding several synthetic steps which is not optimal. Despite these low yields and problematic purifications, there is no optimised methodology or alternative functional group easily accessible for this type of compound. As such we turned our attention to the use of 1,2,3-triazoles which have been used extensively as amide bond isosteres throughout medicinal chemistry for the past decade.[17–26] Recently, Antonella and co-workers have synthesised several triazole-derived compounds inspired by Bicalutamide **2**.[27]

As such, the impetus for this study was to replace the *N*-phenylamide moiety of simple AR antagonists with a 1,4-substituted-1,2,3-triazole and determine if there is any retention of biological activity against androgen dependent cells (LNCaP). Therefore, we designed a range of potential triazole derived AR antagonists inspired by anti-androgen Flutamide **1**. The synthetic procedures presented here are rapid, robust and require no purification techniques. Indeed, in contrast to the electron deficient anilines being a problematic for amide formation, these same deactivated anilines, using this approach, are the best performing substrates. The isosteric replacement of the amide with a triazole has an additional benfit in that these compounds will also probe the importance of the amide carbonyl present in many common AR antagonists. These compounds were then evaluated in vitro for their ability to inhibit androgen stimulated cell proliferation using an androgen dependent (LNCaP) cell line. Following this, compounds **23**–**39** were docked into the human ligand binding domain (hARLBD) of wild type AR and the mutated AR present in LNCaP cells and a comparison of interactions is presented. The latter of which possesses a key point mutation of threonine 877 for an alanine (T877A) allowing for correlation to in vitro studies.

2. Results and discussion

2.1. Synthesis of 1,4-substituted-1,2,3-triazoles

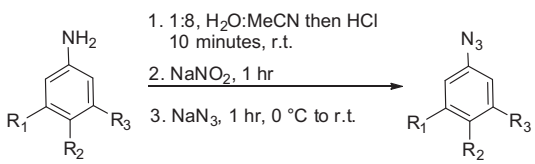
We began our investigation into the conversion of anilinic amines into phenyl azides, of general structure **13**, via diazo-phenyl formation in situ facilitated by sodium nitrite in HCl, using modified conditions reported by Lear et al. (Table 1).[28] Starting with aniline **12** (R_{1-3} = H), an electronically 'neutral' substrate, these conditions furnished phenyl azide **14** in moderate yield (62%) and in >95% crude purity (Table 1, entry 1). Encouraged by this result we moved on to 3-trifluoromethyl-4-cyanoaniline ($R_1 = CF_3$, $R_2 = CN$, $R_3 = H$), an aryl unit commonly used in prostate cancer therapy,[8,29–33] which proceeded much better than the previous example giving the desired azide **15** in 95% yield (Table 1, entry 2).

Applying these conditions to a series of substituted anilines gave the desired azides (**16**–**21**) in good to excellent yields (67–95%) and were isolated analytically pure as the crude material. Presumably the azide formation occurs much slower for anilines which were obtained in <70% yield (Table 1, entries 1, 3 and 6) than the other examples. Nevertheless these compounds were still isolated in synthetically usable yields.

With phenyl azides **14**–**21** in hand, our attention turned to the application of these compounds to the copper-alkyne-azide-cycloaddition (CuAAc) reaction. We chose the CuAAc reaction between **21** and phenylacetylene as our model reaction of choice as this provided a simple, yet novel, triazole **22**. Our initial reaction conditions used a combination of copper(II) sulfate and ascorbic acid in a water/ethanol mixture at room temperature (Table 2, entry 1) which only gave a trace (<5%) of the desired compound.

Repeating this reaction at a higher temperature (100 °C) using microwave irradiation, we found that the reaction proceeded in 30 min (Table 2, entry 2), giving the desired compound in excellent yield (89%). The drastic reduction of reaction duration using microwave irradiation has been shown several times within our group[34–38] and is a phenomena commonly observed in synthetic chemistry.[39–43] A copper source which is soluble in organic solvents

Table 1
Synthesis of aryl azides



12 **13**

Entry	Compound	R_1	R_2	R_3	Yield (%)
1	**14**	H	H	H	62
2	**15**	CF_3	CN	H	95
3	**16**	H	Cl	H	67
4	**17**	H	CN	H	95
5	**18**	CF_3	NO_2	H	76
6	**19**	CF_3	H	CF_3	68
7	**20**	H	NO_2	H	82
8	**21**	CF_3	Cl	H	95