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Discovery of AZD3514, a small-molecule androgen receptor downregulator for treatment of advanced prostate cancer

Robert H. Bradbury *, David G. Acton, Nicola L. Broadbent, A. Nigel Brooks, Gregory R. Carr, Glenn Hatter, Barry R. Hayter, Kathryn J. Hill, Nicholas J. Howe, Rhys D. O. Jones, David Jude, Scott G. Lamont, Sarah A. Loddick, Heather L. McFarland, Zaieda Parveen, Alfred A. Rabow, Gorkhn Sharma-Singh, Natalie C. Stratton, Andrew G. Thomason, Dawn Trueman, Graeme E. Walker, Stuart L. Wells, Joanne Wilson, J. Matthew Wood

Oncology iMed, AstraZeneca, Mereside, Alderley Park, Macclesfield SK10 4TG, UK

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ABSTRACT

Removal of the basic piperazine nitrogen atom, introduction of a solubilising end group and partial reduction of the triazolopyridazine moiety in the previously-described lead androgen receptor downregulator 6-[4-(4-cyanobenzyl)piperazin-1-yl]-3-(trifluoromethyl)[1,2,4]triazolo[4,3-*b*]pyridazine (**1**) addressed hERG and physical property issues, and led to clinical candidate 6-(4-{4-[2-(4-acetylpiperazin-1-yl)ethoxy]phenyl}piperidin-1-yl)-3-(trifluoromethyl)-7,8-dihydro[1,2,4]triazolo[4,3-*b*]pyridazine (**12**), designated AZD3514, that is being evaluated in a Phase I clinical trial in patients with castrate-resistant prostate cancer.

Prostate cancer is the second leading cause of death from cancer among men in developed countries, and was projected to account for 25% of newly-diagnosed cases and 9% of deaths due to cancer in the USA in 2010.[1] The androgen receptor (AR), a ligand binding transcription factor in the nuclear hormone receptor super family, is a key molecular target in the etiology and progression of prostate cancer.[2–6] Binding of the endogenous AR ligand dihydrotestosterone stabilizes and protects the AR from rapid proteolytic degradation. The early stages of prostate cancer tumor growth are androgen dependent and respond well to androgen ablation,[2–6] either via surgical castration or by chemical castration with a luteinizing hormone releasing hormone agonist in combination with an AR antagonist, such as bicalutamide.

Although introduction of androgen deprivation therapy represented a major advance in prostate cancer treatment, recurrence within 1–2 years typically marks transition to the so-called castrate-resistant state, in which the tumor continues to grow in the presence of low circulating endogenous ligand and is no longer responsive to classical AR antagonists.[2–6] Castrate-resistant prostate cancer (CRPC) is a largely unmet medical need with a 5-year survival rate of less than 15%. Antimitotic agents docetaxel and cabazitaxel, testosterone biosynthesis inhibitor abiraterone acetate and second generation AR antagonist enzalutamide (MDV3100) are the currently approved small-molecule drugs that have been shown to provide survival benefit.[7–10]

Recent evidence from both pre-clinical and clinical studies is consistent with the importance of re-activation of AR signaling in a majority of castrate-resistant prostate tumors.[2–6] It is also well established that the functional AR in castrate-resistant tumors is frequently mutated or amplified, and that over-expression can convert hormone-responsive cell lines to hormone refractory. Recent second-generation AR antagonists have been designed that retain antagonism in over-expressing cell lines, and among these agents enzalutamide[11] has recently successfully met efficacy criteria in a large Phase III clinical trial.[12]

By analogy with fulvestrant,[13] an estrogen receptor (ER) downregulator approved by the FDA in 2002 for treatment of advanced breast cancer and initially characterized as a pure ER antagonist, a

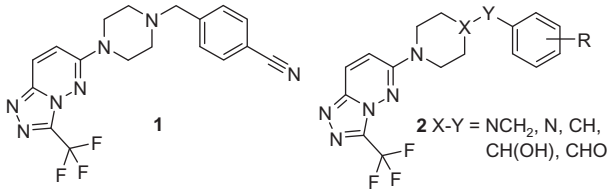

Figure 1. Structures of lead AR downregulator **1** and chemotype **2**.

* Corresponding author. Tel.: +44 1625 515708; fax: +44 1625 510097.
E-mail address: rob.bradbury@astrazeneca.com (R.H. Bradbury).

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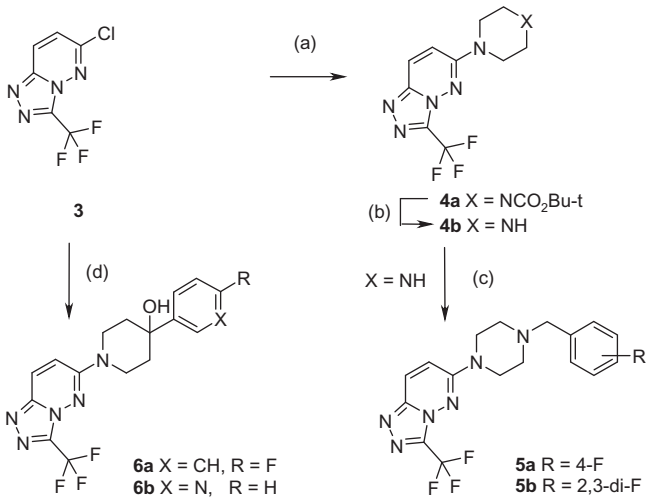
ligand which downregulates the AR represents one of a number of potential approaches[3–6] to treatment of CRPC via a sustained reduction in tumor AR content. We recently described derivation from a novel 3-(trifluoromethyl)-[1,2,4]triazolo[4,3-*b*]pyridazine ligand of AR inhibitor **1** (Fig. 1).[14] The compound also causes AR downregulation[15] and high plasma levels following oral administration in pre-clinical models compensate for moderate cellular potency.[14,15]

Although **1** showed excellent pharmacokinetic properties in rat and dog and low turnover in isolated cryopreserved human hepatocytes, two other properties of the compound precluded further progression. Firstly, **1** was moderately potent in an IonWorks™ hERG assay[16] (pIC_{50} 5.65), implying that there was unlikely to be sufficient margin between predicted efficacious human drug concentration and the concentration that could potentiate the hERG ion channel and increase the risk of adverse cardiovascular events in patients.[17] Secondly, due to low equilibrium $pH_{7.4}$ aqueous solubility (8 μM), the maximum absorbable dose (MAD) of **1** calculated using a gastrointestinal simulated model[18] was significantly lower than the predicted efficacious human dose derived from an in-house physiologically-based pharmacokinetic (PBPK) model.[19]

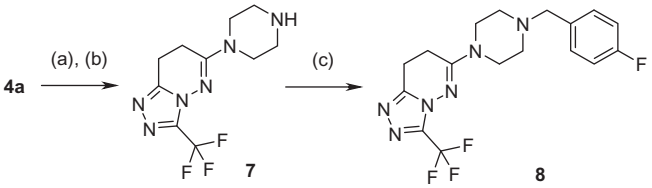
To address these issues, we carried out a comprehensive medicinal chemistry lead optimisation programme[20,21] centred around chemotype **2**, and in this publication we describe the key advances that led to clinical candidate AZD3514 (**12**) that is being evaluated in a Phase I clinical trial.[22]

Illustrative compounds selected from several hundred diverse examples prepared during the course of this work are listed in Table 1, and synthetic routes are outlined in Schemes 1–3.[20,21] As part of synthesis of wider compound libraries, benzyl piperazine derivatives **5a–b** and piperidinol derivatives **6a–b** (Scheme 1) were readily accessed from previously described precursors,[14] reductive amination of piperazine intermediate **4b** with the appropriate fluorinated aryl aldehyde giving **5a–b** and displacement of chlorotriazolopyridazine **3** with the appropriate aryl piperidinol[23,24] giving **6a–b**. Analogously to published work,[25] the dihydro triazolopyridazine intermediate **7** could be cleanly obtained by catalytic hydrogenation of the corresponding N-protected precursor **4a** under atmospheric pressure at 50 °C followed by de-protection (Scheme 2). Reductive amination with 4-fluorobenzaldehyde then gave **8**.

Also as part of synthesis of wider libraries, compounds **10–12** containing a neutral or moderately basic side chain were prepared by a route involving as the key step Mitsnobu reaction of the phenol precursors **9a–b**, readily obtained from **3** and the



Scheme 1. Synthesis of compounds **5a–b**, **6a–b**. Reagents and conditions: (a) *t*-Butyl piperazine-1-carboxylate, DIPEA, EtOH, 70 °C; (b) TFA, DCM, 20 °C; (c) 4-fluorobenzaldehyde or 2,3-difluorobenzaldehyde, (polystyrylmethyl)trimethylammonium cyanoborohydride, AcOH, DCM, 20 °C; (d) 4-(4-fluorophenyl)piperidin-4-ol[23] or 4-(pyridin-3-yl)piperidin-4-ol,[24] DIPEA, DMF, 80 °C.



Scheme 2. Synthesis of compound **8**. Reagents and conditions: (a) H_2, 5% Pd-C, MeOH, 50 °C; (b) TFA, DCM, 20 °C; (c) 4-fluorobenzaldehyde, (polystyrylmethyl)-trimethylammonium cyanoborohydride, AcOH, DCM, 20 °C.

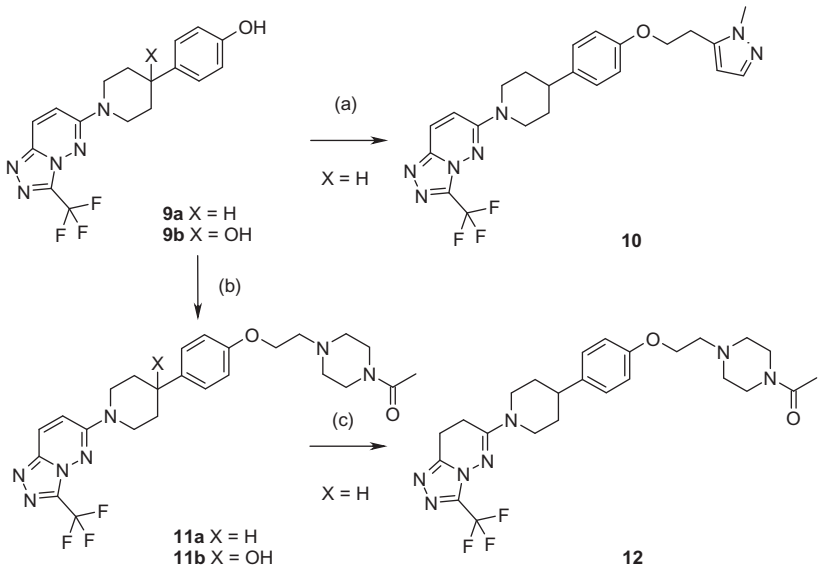
appropriate piperidine[26] (Scheme 3). Thus alkylation of **9a** with 2-(1-methyl-1*H*-pyrazol-5-yl)ethanol[27] gave **10** and alkylation of **9a–b** with 2-(4-acetylpiperazine-1-yl)ethanol[28] gave **11a–b**. Catalytic hydrogenation of **11a** then provided **12**.

Compounds listed in Table 1 were evaluated in a previously-described AR downregulation assay[14] that specifically quantifies nuclear AR levels in human LNCaP prostate cancer cells in the absence of androgen. Also included in Table 1 are data from a number

Table 1
AR-mediated cellular downregulation,[14] IonWorks™ activity,[15] log*D*,[29] aqueous solubility, rat and human protein binding, and human hepatocyte stability data for **1**, **5a–b**, **6a–b**, **8**, **10–12**

Entry	AR downregulation pIC_{50}[a]	IonWorks™ pIC_{50}[b]	log*D* $pH_{7.4}$	Solubility μM $pH_{7.4}$[c]	% Free rat/human[d]	Human hepatocyte Clint[e] (μl/min/10^6 cells)
1	5.82	5.65	2.9	8.4 (Cryst)	9.2/4.4	<3
5a	5.8	4.97	3.5	16 (Undefined)	3.4/2.0	52
5b	5.68	<4	3.6	NV[f]	1.8/1.7	22
6a	5.43	4.74	3.5	<0.9 (Cryst)	3.6/4.0	<3
6b	4.97	4.02	2.1	100 (Cryst)	34/26	<3
8	5.23	4.53	2.8	520 (Semicryst)	13/12	3.7
10	6.63	4.75	>4.3	<0.73 (Cryst)	0.21/0.16	12
11a	6.49	4.95	3.2	5.4 (Cryst)	1.2/2.7	12
11b	5.98	<4	2.2	190 (Semicryst)	6.4/17	<3
12	5.75	<4	2.5	577 (Cryst)	9.4/12	<3

[a] $n \geqslant 7$, SEM values are available in the Supplementary data.
[b] $n \geqslant 2$.
[c] 24 h thermodynamic solubility of solid sample determined in 0.1 M phosphate buffer (parentheses refer to visual examination of physical form of undissolved sample under a microscope at 24 h timepoint).
[d] Determined from DMSO stock solution by equilibrium dialysis in 10% plasma from Alderley Park Han Wistar rats or 10% human plasma supplied by Quintiles.
[e] Rate of metabolism determined from DMSO stock solution in isolated cryopreserved human hepatocytes diluted to 1×10^6 cells/ml.
[f] No value obtained.



Scheme 3. Synthesis of compounds **10**, **11a**–**b**, **12**. Reagents and conditions: (a) 2-(1-Methyl-1*H*-pyrazol-5-yl)ethanol,[27] Ph_3P, diisopropyl azodicarboxylate, THF, 20 °C; (b) 2-(4-acetylpiperazine-1-yl)ethanol,[28] Ph_3P, diisopropyl azodicarboxylate, THF, 20 °C; (c) H_2, 10% Pd-C, MeOH, 50 °C.

of routine in-house physical property, metabolic stability and safety assays.

To address the hERG activity of lead AR downregulator **1**, we considered a number of approaches reported in the medicinal chemistry literature,[16] including subtle structural effects, removal of the basic piperazine nitrogen and reducing lipophilicity. Of a wide range of aryl substituents investigated, replacement of the 4-cyano substituent by 4-fluoro (**5a**) maintained cellular potency and reduced activity in the IonWorks™ assay. More notably, 2,3-difluoro substitution (**5b**) obviated IonWorks™ activity (pIC_{50} <4) while maintaining cellular potency. These compounds were not progressed, however, as predicted efficacious human dose was significantly increased over **1** due to inferior human hepatocyte stability.

We were aware from earlier work[14] that removal of the basic piperazine nitrogen increased lipophilicity and compromised physical properties. By way of compensation, the corresponding aryl piperidinols were prepared (e.g., **6a**). For this sub-series of compounds, acceptable overall properties could only be achieved through replacement of the aryl ring with a heteroaryl moiety, for example **6b**, for which increased rat and human free fraction arguably compensate for reduced cellular potency. Partial reduction of the triazolopyridazine ring surprisingly gave a significant reduction in lipophilicity, with consequent improvement in overall compound profile (hERG, physical properties, human hepatocyte stability, e.g., compare **8** with **5a**).

Examination of the previously proposed[14] binding mode for the triazolopyridazine ligand to the AR suggested an alternative way to improve physical properties of compounds lacking the basic piperazine nitrogen, through incorporation of a solubilising end group attached via a linker to the 3- or 4-position of the aryl ring. Of a wide range of linkers and end groups investigated by parallel synthesis, polar and weak to moderately basic heterocycles attached via a 4-alkoxy linker emerged as of particular interest (e.g., **10** and **11a**), in that cellular potency and in vitro hERG margin were significantly improved over lead compound **1**.

As described earlier, the low aqueous solubility and free fraction of compounds such as **11a** was significantly improved by preparation of the corresponding piperidinol (**11b**) and dihydro triazolopyridazine (**12**). These compounds also showed low human hepatocyte turnover and no detectable activity in the hERG IonWorks™ assay. Binding of **12** to the AR was confirmed in ligand displacement assays, the estimated K_i of **12** being 5 μM in a fluorescence polarisation assay using rat AR ligand binding domain[30] and 2.2 μM in a radiolabelled assay using full length AR derived from LNCaP cell lysates.[31,32]

As representative of compounds with differing overall profile, in vivo efficacy of compounds **6b**, **10** and **12** was assessed in the Hershberger assay,[33] a longstanding model used in the discovery of the AR antagonist bicalutamide,[34] in which effects on accessory sex organ weight in immature castrated rats stimulated with testosterone propionate serve as a marker for intervention via the AR. Comparably to lead compound **1**,[14] compounds **6b**, **10** and **12** dosed orally at 50–100 mg/kg twice daily in the Hershberger model for 7 days caused a significant inhibition of testosterone-induced growth of rat seminal vesicles (data not shown), the magnitude of effect being comparable to that seen with bicalutamide dosed at 2 mg/kg. Analysis of plasma samples 18 h subsequent to administration of the final dose showed free concentrations comparable to the IC_{50} for nuclear AR downregulation.

For input into our in-house PBPK model, low dose rat and dog blood pharmacokinetic parameters were generated on compounds **6b**, **10** and **12** (Table 2), and MAD values were predicted using gastrointestinal simulated modelling.[18] In summary, developability risks for less potent but soluble compound **6b** and for more potent

Table 2
Rat[a] and dog[b] pharmacokinetic parameters for **6b**, **10**, **12**

Entry	Species	Vdss l/kg	Cl ml/min/kg	Bioavailability (%)
6b	Rat[c]	5.7	38	>75
	Dog[d]	1.3	18	>75
10	Rat[e]	2.3	22	43
	Dog[d]	2.7	9.2	18
12	Rat[f]	2.1	6.6	74
	Dog[d]	3.2	12	>75

[a] Mean blood PK parameters from 2 male Alderley Park Han Wistar rats.
[b] Mean blood PK parameters from 1 male zand 1 female Alderley Park beagle.
[c] Dosed at 20 μmol/kg iv and 50 μmol/kg po.
[d] Dosed at 2 μmol/kg iv and 10 μmol/kg po.
[e] Dosed at 3 μmol/kg iv and 10 μmol/kg po.
[f] Dosed at 4 μmol/kg iv and 5 μmol/kg po.

but less soluble compound **10** centred around high predicted dose and low MAD, respectively, whereas for compound **12** with the best overall property profile MAD significantly exceeded a predicted efficacious human dose in the low hundreds of milligrams twice daily.

Compound **12**, designated AZD3514, was chosen as clinical candidate and is being evaluated in a Phase I trial in patients with CRPC.[22] Detailed biological characterisation of compound **12**, including mode of action, cellular anti-proliferative and rodent CRPC tumour model data, has been published elsewhere.[35,36]

Supplementary data

Supplementary data (experimental procedures and characterisation data for compounds **5a–b**, **6a–b**, **8**, and **10–12**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.056.

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