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# Rational design and synthesis of novel anti-prostate cancer agents bearing a 3,5-*bis*-trifluoromethylphenyl moiety.

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**Abstract**

Prostate cancer is a major cause of male death worldwide and the identification of new and improved treatments is constantly required. Among the available options, different non-steroidal androgen receptor (AR) antagonists are approved also to treat castration-resistant forms. Most of these drugs show limited application due to the development of resistant mutants of their biological target.

Following docking-based studies on a homology model for the AR open antagonist conformation, a series of novel 3,5-*bis*-trifluoromethylphenyl compounds was designed with the aim to improve the antiproliferative activity of anti-androgen drugs bicalutamide and enzalutamide. The new structural modifications might impede the receptor to adopt its closed agonist conformation also in the presence of adaptive mutations.

Among the novel compounds synthesised, several displayed significantly improved *in vitro* activity in comparison with the parent structures, with $IC_{50}$ values in the low micromolar range against four different prostate cancer cell lines (LNCaP, VCaP, DU-145, 22Rv1). Selected hits demonstrated full AR antagonistic behaviour and promising candidates for further development were identified.

**Key words**

Homology modelling; rational drug design; prostate cancer; androgen-receptor.

Prostate cancer (PC) represents a major cause of male death worldwide and it is the second most common cancer for males, the fourth most common cancer overall.[1] Currently, among the several treatment options available, androgen deprivation is one of the main strategies to treat the disease at various stages of its development.[2] Flutamide (**1**) (in its active form as hydroxyflutamide (**2**)), bicalutamide (**3**) and enzalutamide (**4**) are non-steroidal androgen receptor antagonists approved for the treatment of PC (**Figure 1**). In many cases, these anti-androgens become ineffective after few years of treatment due to the emergence of different mutations on the AR, which cause the anti-androgen

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This work is dedicated to the memory of Prof. Chris McGuigan, a great colleague and scientist, invaluable source of inspiration and love for research.

compounds to function as agonists.[2]

In order to improve the antiproliferative properties of standard anti-androgens **3** and **4**, and to possibly impede the receptor to adopt its closed agonist conformation in the presence of adaptive mutations, diverse new analogues were designed to increase the molecule occupational volume in proximity to the receptor helix 12, responsible for the closure movement to the agonist conformation, with the aim to impede this conformational change. Specifically, different hydrophobic aromatic substituents were explored with docking studies, and in particular attention was focused on fluorinated groups, in order to take advantage of the potential of fluorine to enhance the pharmacological properties and drug-like characteristics of compounds.[3] A 3,5-*bis*-trifluoromethyl group was identified as the best substituent for aromatic ring B of the parent structures to achieve this result (**Figure 1**). The new analogues showed a marked improvement in *in vitro* antiproliferative activity on a range of human PC cell lines (VCap, LNCaP, DU-145 and 22Rv1), reaching sub-micromolar $IC_{50}$ values. In addition, full AR antagonistic effect was observed for selected compounds and preclinical candidates were identified.

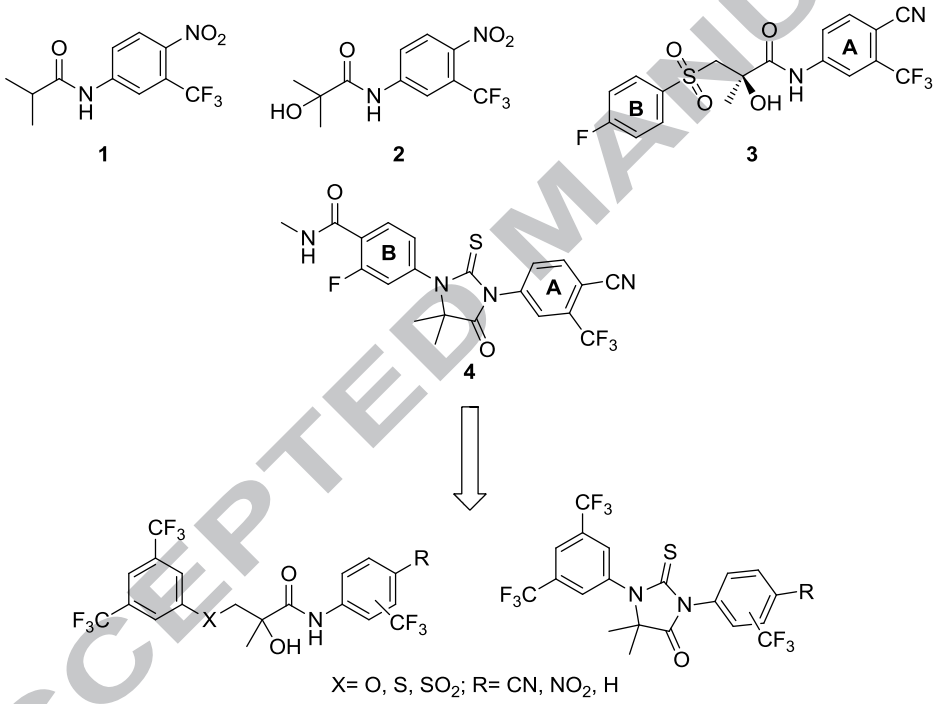



**Figure 1.** Structure of AR antagonists in the market for the treatment of PC and their proposed modifications.

A homology model of the human WT-AR open conformation was built using a single template approach in MOE2015.[4] The crystal structure of the progesterone receptor in its open antagonist form (PDB ID: 2OVM) was used as template (54% sequence identity with the human AR).[5] Docking of *(R)*-bicalutamide, enzalutamide and of the newly designed modifications was performed in the ligand binding domain (LBD) of the AR model using Plants docking software.[6] The predicted binding mode found for the parent compounds revealed the presence of free occupational space in the model structure in proximity to helix 12. As shown in **Figure 2a**, *in silico* studies suggest that the best substituent to fill this space is a 3,5-*bis*-trifluoromethyl group on aromatic ring B of the standard compounds. Due to the much bigger occupational volume in comparison with the 4-F substituent of bicalutamide, this

modification might impede the receptor closure even in the presence of adaptive mutations, such as the W741L mutation, the most common for bicalutamide.[7] As a further indication of this potential effect, docking of the newly designed compounds on the crystal structure of the complex bicalutamide-W741L AR (PDB ID 1Z95), which corresponds to the receptor closed conformation, reveals the presence of steric clashes between the new *bis*-trifluoromethyl portion of the molecule and helix 12 (**Figure 2b**).

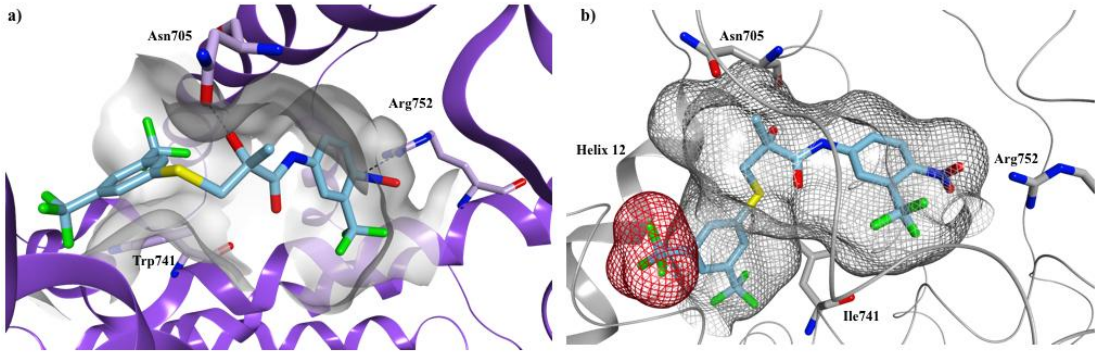
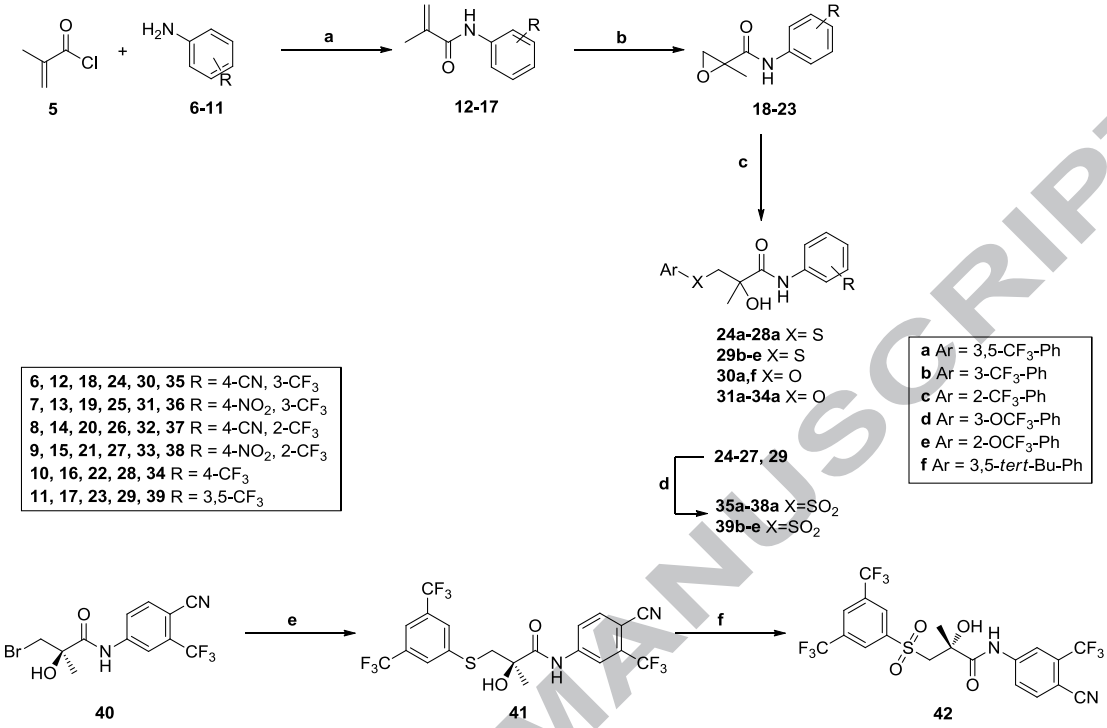



**Figure 2**. Predicted binding mode of **25a** in the AR homology model (a) and in the bicalutamide-W741A AR closed conformation crystal structure 1Z95 (b). The bis-$CF_3$ aromatic substituent in ring B shows major clashes (red grid) with the protein surface (grey grid) of the closed AR structure, indicating that it might impede the AR closure movement even in the presence of adaptive mutations.

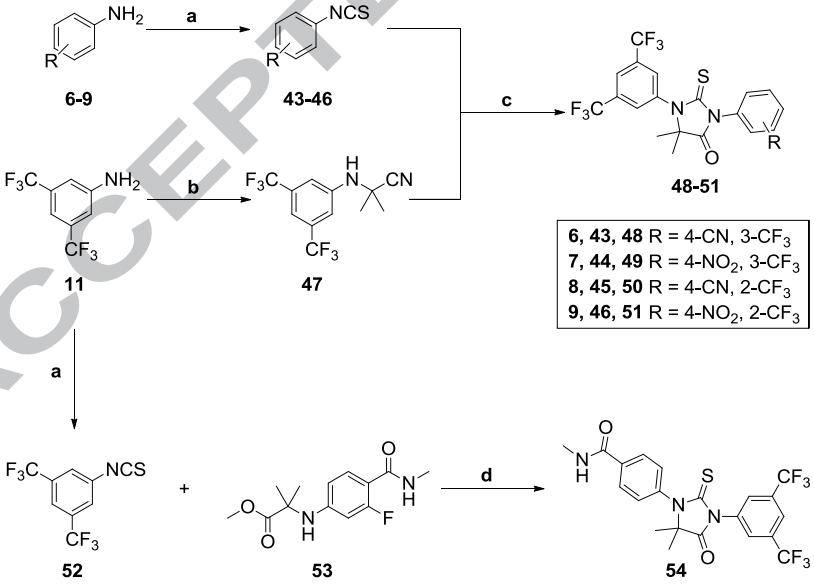
Along with the insertion of a 3,5-*bis*-trifluoromethyl group in aromatic ring B of the parent structures, its replacement with a bulkier 3,5-di-*tert*-butyl substituent was also envisaged (compound **30f**). Moreover, ring A was explored as well for the repositioning and replacement of its original substituents with a *bis*-$CF_3$ function. All planned modifications were carried out according to an optimised three or four-step synthetic pathway, adapted from literature procedures,[8-14] summarised in **Schemes 1** and **Scheme 2**.

Racemic bicalutamide derivatives, along with racemic bicalutamide,[10] were prepared by reacting methacryloyl chloride (**5**) with substituted anilines **6-11** in dimethylacetamide (DMA) to obtain phenylacrylamides **12-17**, which were converted into the corresponding epoxides **18-23** in the presence of a large excess of hydrogen peroxide and trifluoroacetic anhydride in dichloromethane. Opening of the epoxide rings of **18-23** with substituted phenols or thiophenols gave a series of ethers **30-34** and thioethers **24-29**. Thioethers **24-27** and **29** were oxidized to the corresponding sulfones **35-39** with mCPBA at 25 °C. For the synthesis of *R*-bicalutamide[12] and *R*-derivatives **41-42**, intermediate **40** was prepared according to literature,[11] and then reacted with the sodium salt of 3,5-*bis*-$CF_3$-thiophenol in tetrahydrofuran to give the desired (*R*)-thioether **41**.[12] Oxidation of **41** with mCPBA provided sulfone **42**. Enzalutamide analogues were obtained by the preparation of isothiocyanates **43-46**, treating the corresponding anilines **6-9** with thiophosgene.[13] Strecker reaction of substituted aniline **11** with acetone and trimethylsilyl cyanide generated the desired cyanoamine **47**, which was reacted with isothiocyanates **43-46** in DMF followed by HCl and MeOH addition to give the desired thiohydantoins **48-51**. Thiohydantoin **54** was prepared by heating intermediate **53**, obtained according to literature procedures,[14] with isothiocyanate **52** in a mixture of DMSO and isopropylacetate (iPAC) at 85 °C for 14 hours. Standard enzalutamide was also prepared according to this procedure.[14] All final compounds

were fully characterised by $^{1}H$ NMR, $^{13}C$ NMR, $^{19}F$ NMR and HRMS. Purity >95% was confirmed by HPLC (see Supplementary Material).




**Scheme 1** Synthetic pathways for the synthesis of racemic and *R*-bicalutamide analogues adapted from reported procedures.[8-12] Reagents and conditions: (a) **5** (8 equiv.), DMA, RT, 3 h (77-95%); (b) $H_2O_2$ (4 equiv.)/$(CF_3CO)_2O$ (5 equiv.), DCM, RT, 24 h (60-86%); (c) NaH (1.2 equiv.), phenol or thiophenol (1.2 equiv.), THF, RT, 24h (29-89%); (d) mCPBA (1.4 equiv.), DCM, RT, 4-6 h (40-89%); (e) 3,5-*bis*-$CF_3$-thiophenol (1.2 equiv.), 60% NaH (1.2 equiv.), THF, RT, 24 h (75%); (f) mCPBA (1.4 equiv.), DCM, RT, 6 h (68%).

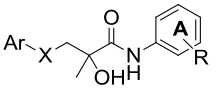
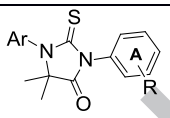



**Scheme 2.** Synthetic strategy toward enzalutamide derivatives. Adapted from literature procedures.[13, 14] Reagents and conditions: (a) $CSCl_2$ (1.5 equiv.), $NaHCO_3$, $H_2O$, DCM, RT, 24 h (98-100%); (b) TMSCN (5.1 equiv.), acetone, 80 °C, 12 h (80-97%); (c) DMF, RT 48 h, followed by HCl, MeOH, reflux, 6 h (10-51%). d) DMSO, iPAc, 85 °C, 14 h (67%).

All newly synthesised compounds were evaluated for their antiproliferative effect in an *in vitro* 2D monolayer assay using four human prostate cancer cell lines (**Table 1**, absolute $IC_{50}$ in µM). LNCaP,

VCaP and 22Rv1 cell lines exhibit some androgen sensitivity, whereas DU-145 is hormone-insensitive (Supporting Information, section S.3).

The activity of reference compounds **3** and **4** is consistent with previously reported data.[15-17] As recent research studies carried out in our group have shown the lack of significant difference in antiproliferative activity between racemic and *R*-bicalutamide analogues,[18] the major part of our new bicalutamide-derived structures have been prepared as racemic mixtures. Most of the new derivatives performed better than standard bicalutamide, significantly improving its antiproliferative activity up to 60-fold (overall antiproliferative activity in the four cell lines reported as geometric mean). Active inhibitors showed sigmoidal concentration-effect curves with total cell kill at high concentrations (Supplementary data).

| |  **Scaffold 1** | | |  **Scaffold 2** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **Scaffold** | **Ar (B ring)** | **X** | **R (A ring)** | **Absolute $IC_{50}$ (μM)** | | | |
| | | | | | 22Rv1 | DU-145 | LNCaP | VCaP | Geom.mean |
| **R,S-Bicalutamide** | 1 | 4-F-Ph | $SO_2$ | 4-CN, 3-$CF_3$ | 49.58 | 49.20 | 45.27 | 68.37 | 52.42 |
| **3 (R-Bicalutamide)** | 1 | 4-F-Ph | $SO_2$ | 4-CN, 3-$CF_3$ | 46.25 | 45.41 | 45.20 | 51.61 | 47.05 |
| **24a** | 1 | 3,5- $CF_3$-Ph | S | 4-CN, 3-$CF_3$ | 1.90 | 2.85 | 2.34 | 2.73 | 2.43 |
| **25a** | 1 | 3,5- $CF_3$-Ph | S | 4- $NO_2$, 3-$CF_3$ | 1.50 | 2.78 | 1.50 | 1.53 | 1.76 |
| **26a** | 1 | 3,5-$CF_3$-Ph | S | 4-CN, 2-$CF_3$ | 4.65 | 9.09 | 7.76 | 6.65 | 6.84 |
| **27a** | 1 | 3,5- $CF_3$-Ph | S | 4-$NO_2$, 2-$CF_3$ | 2.29 | 3.38 | 3.11 | 3.52 | 3.03 |
| **28a** | 1 | 3,5-$CF_3$-Ph | S | 4-$CF_3$ | 9.75 | 16.39 | 9.61 | 9.88 | 11.10 |
| **29b** | 1 | 3-$CF_3$-Ph | S | 3,5-$CF_3$ | 9.67 | 10.03 | 8.75 | 9.86 | 9.56 |
| **29c** | 1 | 2-$CF_3$-Ph | S | 3,5-$CF_3$ | 4.37 | 8.66 | 3.57 | 3.84 | 4.78 |
| **29d** | 1 | 3-$OCF_3$-Ph | S | 3,5-$CF_3$ | 6.42 | 9.61 | 7.76 | 9.29 | 8.17 |
| **29e** | 1 | 2-$OCF_3$-Ph | S | 3,5-$CF_3$ | 2.94 | 6.41 | 2.91 | 2.90 | 3.55 |
| **30a** | 1 | 3,5-$CF_3$-Ph | O | 4-CN, 3-$CF_3$ | 2.64 | 4.70 | 3.01 | 2.47 | 3.10 |
| **30f** | 1 | 3,5- *t*Bu-Ph | O | 4-CN, 3-$CF_3$ | 2.27 | 3.15 | 2.67 | 2.98 | 2.69 |
| **31a** | 1 | 3,5- $CF_3$-Ph | S | 4-$NO_2$, 3-$CF_3$ | 1.09 | 2.90 | 0.99 | 1.66 | 1.68 |
| **32a** | 1 | 3,5-$CF_3$-Ph | O | 4-CN, 2-$CF_3$ | 4.07 | 8.05 | 3.56 | 4.77 | 5.11 |
| **33a** | 1 | 3,5-$CF_3$-Ph | O | 4-$NO_2$, 2-$CF_3$ | 16.10 | 11.64 | 23.98 | 23.73 | 14.18 |
| **34a** | 1 | 3,5-$CF_3$-Ph | O | 4-$CF_3$ | 8.49 | 9.23 | 3.35 | 4.15 | 5.75 |
| **35a** | 1 | 3,5-$CF_3$-Ph | $SO_2$ | 4-CN, 3-$CF_3$ | 3.11 | 5.58 | 2.86 | 6.21 | 4.19 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **36a** | 1 | 3,5- $CF_3$-Ph | $SO_2$ | 4-$NO_2$, 3-$CF_3$ | 2.15 | 5.26 | 2.10 | 3.30 | 2.98 |
| **37a** | 1 | 3,5-$CF_3$-Ph | $SO_2$ | 4-CN, 2-$CF_3$ | n.d. | n.d. | n.d. | n.d. | n.d. |
| **38a** | 1 | 3,5-$CF_3$-Ph | $SO_2$ | 4-$NO_2$, 2-$CF_3$ | 2.41 | 3.05 | 3.09 | 7.11 | 3.57 |
| **39b** | 1 | 3-$CF_3$-Ph | $SO_2$ | 3,5-$CF_3$ | 10.27 | 11.48 | 9.50 | 9.98 | 10.28 |
| **39c** | 1 | 2-$CF_3$-Ph | $SO_2$ | 3,5-$CF_3$ | 9.45 | 11.02 | 8.51 | 8.55 | 9.33 |
| **39d** | 1 | 3-$OCF_3$-Ph | $SO_2$ | 3,5-$CF_3$ | 12.84 | 14.11 | 9.34 | 9.99 | 11.42 |
| **39e** | 1 | 2-$OCF_3$-Ph | $SO_2$ | 3,5-$CF_3$ | 8.99 | 12.91 | 8.02 | 9.95 | 9.81 |
| **41** | 1 | 3,5- $CF_3$-Ph | S | 4-CN, 3-$CF_3$ | 1.13 | 2.66 | 0.79 | 0.84 | 1.19 |
| **42** | 1 | 3,5- $CF_3$-Ph | $SO_2$ | 4-CN, 3-$CF_3$ | 2.50 | 6.36 | 1.99 | 4.11 | 3.38 |
| **4 (Enzalutamide)** | 2 | 3-F,4-CONHMe-Ph | - | 4-CN, 3-$CF_3$ | 31.76 | 32.27 | 11.47 | 53.04 | 28.10 |
| **48** | 2 | 3,5- $CF_3$-Ph | - | 4-CN, 3-$CF_3$ | n.d. | n.d. | n.d. | n.d. | n.d. |
| **49** | 2 | 3,5- $CF_3$-Ph | - | 4-$NO_2$, 3-$CF_3$ | 20.20 | 18.12 | 9.93 | 28.44 | 17.93 |
| **50** | 2 | 3,5- $CF_3$-Ph | - | 4-CN, 2-$CF_3$ | n.d. | n.d. | n.d. | n.d. | n.d. |
| **51** | 2 | 3,5- $CF_3$-Ph | - | 4-$NO_2$, 2-$CF_3$ | 6.97 | 10 | 4.67 | 18.59 | 8.82 |
| **54** | 2 | 3-F,4-CONHMe-Ph | - | 3,5-$CF_3$ | 26.37 | 47.86 | 11.29 | 19.77 | 29.78 |

**Table 1**. Antiproliferative activity for bicalutamide/enzalutamide derivatives. All data are mean values from triplicate experiments.

In the bicalutamide-derived series of analogues (compounds **24-42**), all the newly synthesised compounds performed better than the standards in the antiproliferative assay, with the only exception being **37a**, for which an antiproliferative effect could not be observed due to solubility issues. The presence of a 3,5-*bis*-trifluoromethyl substituent in aromatic ring B seems to strongly affect the antiproliferative activity in all four prostate cancer cell lines, with the best results obtained for **41**, which reaches sub-micromolar $IC_{50}$ values in two different cell lines (LNCaP and VCaP, both androgen-sensitive), **31a**, **25a**, **24a**, **36a**, **27a** and **30a**. Replacement of the *bis*-$CF_3$ substituent of **30a** with a bulkier 3,5-di-*tert*-butyl group (compound **30f**) leads to slightly lower $IC_{50}$ values, which indicate activity retention. Insertion of a 3,5-*bis*-$CF_3$ substituent in ring A of parent bicalutamide (compounds **29b-e**, **39b-e**), according to our modelling studies, would be associated with a better occupation of the inner portion of the AR ligand binding domain; this modification, combined with optimised substituents for aromatic ring B recently found in our group (unpublished data), is associated with a significant activity improvement in comparison with standard bicalutamide for all the new derivatives prepared.

Due to solubility issues, the effect of the modification of ring B of enzalutamide with a 3,5-*bis*-trifluoromethyl group could be assessed only for **49** and **51**: also in this case, this structural change is associated with activity improvement in all four prostate-cancer cell lines, with a particularly

significant effect found for **51**. Insertion of this substituent on aromatic ring A of enzalutamide (compound **54**) is associated with activity retention.

Considering the antiproliferative results obtained across the four different cell lines, the new compounds show significant activity also on androgen-insensitive DU-145 cells. Even if the $IC_{50}$ values obtained for most of the new compounds are higher in this cell line in comparison with the other three, the improved antiproliferative activity suggests the presence of a potential off-target effect, besides the canonical anti-androgen mechanism. Nevertheless, the presence of an off-target can be speculated also for parent bicalutamide, for which the results obtained in our assay further confirm the previously reported lack of selectivity between LNCaP and DU-145 cell lines.[15] Due to this last consideration, and given the marked improvement in antiproliferative activity associated with the new structures, the presence of a potential off-target was not considered a limitation at this stage, while further investigations to assess the molecular mechanism underlying this effect will be the object of future studies.

As representative of the new series of compounds, **25a** and **36a** were evaluated for their antagonist/agonist effect on the AR using the GeneBLAzer® Betalactamase reporter technology for Nuclear receptors (NRs), to assess whether the antiproliferative activity found is correlated with any interference with the AR function.[19] Both compounds were found to be full antagonists of the androgen receptor in a single concentration antagonism experiment (10μM concentration, antagonistic effect > 80%), reducing the receptor activation induced by the standard agonist R1881.[20,21] A 10-concentration antagonism assay showed that **25a** possess an antagonistic $IC_{50}$ in the same range of *(R)*-bicalutamide and enzalutamide (**Table 2**). The $IC_{50}$ value found for *(R)*-bicalutamide is comparable with previously reported values in similar assays.[22,23]

| Compound | Antagonistic effect (%)* | $IC_{50}$ (μM)** | Agonistic effect (%)*** |
|---|---|---|---|
| **3 (*R*-Bic.)** | 83 | 0.490 | 5 |
| **4 (Enzal.)** | 95 | 0.361 | N.E. |
| **25a** | 90 | 0.710 | 7 |
| **36a** | 93 | 3.1 | 1 |

**Table 2:** AR antagonist/agonist assay results. * Compounds were considered full antagonist if at a single concentration of 10μM the reduction of receptor activation by R1881 was greater than 80%; ** Compounds tested at 10 different concentrations; *** Compound at 10 μM concentration in absence of R1881 was used.

Despite the fact that **36a** shows a significantly improved antiproliferative effect, its action as AR antagonist seems to be reduced in terms of inhibitory $IC_{50}$ in comparison with standard bicalutamide, thus indicating the presence of other aspects that contribute to its anti-prostate cancer properties, as already observed from the antiproliferative data. As a further confirmation of this potential off-target effect, additional *in vitro* studies on **36a** and **41**, our most active new analogue, were performed in order to evaluate their general cytotoxicity in comparison with **3**, along with their metabolic stability (**Table 3**).

| Compound | Metabolic stability[a] | MTT assay[b] | | Antiproliferative results[c] |
|---|---|---|---|---|
| | $t_{1/2}$ (min) | MEC (μM)* | $AC_{50}$ (μM)** | $IC_{50}$ (μM) |

| | | | | |
|---|---|---|---|---|
| **3 (R-bical)** | 214 | 19.2 | 54.3 | 47.05 |
| **36a** | 567 | 3.6 | 9.01 | 2.98 |
| **41** | 6.49 | 1.22 | 2.35 | 1.19 |

**Table 3:** Metabolic stability and cytotoxicity assay results for selected compounds. [a] $t_{1/2}$ calculated in human liver microsomes; compounds metabolically stable with no loss of parent detected for the duration of the assay. [b] MTT assay; compounds have been tested at 8 different concentrations. [*] MEC: minimum effective concentration that significantly crosses vehicle control threshold; [**] AC50: concentration at which 50% of maximum effect is observed. [c] Antiproliferative assay results are expressed in terms of geometric mean of the four different cell lines.

The MTT cell viability assay was performed in a human hepatocarcinoma (HepG2) cell line, as a means to measure the effect of **36a** and **41** on cell proliferation and other events that eventually lead to cell death.[24] Data are reported as minimum effective concentration (MEC) related to a vehicle control (DMSO) and $AC_{50}$. The tested compounds showed a concentration-dependent decrease in formazan production across the range studied (data not shown), indicating a decrease in cell viability associated with some growth inhibitory and toxic effects. Compared with control *(R)*-bicalutamide **3**, **36a** and **41** are characterized by a higher cytotoxicity, which at least partially correlates with the results obtained in the antiproliferative assay. These results might further indicate that the newly prepared analogues, besides the canonical AR antagonistic activity, possess an extra effect, which positively contributes to their antiproliferative action. The presence of the new bis-$CF_3$ substituent in aromatic ring B seems to increase the metabolic stability of the bicalutamide scaffold, as suggested by the $t_{1/2}$ found for **36a**, which is 2.6 fold higher than the value found for **3**. The presence of the thioether group, which likely undergoes rapid oxidation to sulfoxide or sulfone, is the most probable cause of the short half-life of **41**, the most active new derivative found in the antiproliferative assay, thus making the sulfide scaffold an unsuitable candidate for further development.

Due to the promising results obtained in the antiproliferative assay, different *in vitro* properties were evaluated for selected new derivatives (sulfone, ether or thiohydantoin analogues), in order to aid the identification of pre-clinical candidates. In particular, metabolic stability ($t_{1/2}$), plasma protein binding and cardiotoxicity were considered (**Table 4**).

| **Compound** | **Metabolic stability[a]** | **PPB[b]** | **Cardiotoxicity[c]** |
|---|---|---|---|
| | $t_{1/2}$ (min) | % bound | % hERG inhib. At 25 µM |
| **3 (R-bical)** | 214 | 96[24] | n.d. |
| **30a** | 1930 | 94.7 | 7.64 |
| **39e** | 290 | 78.3 | 13.4 |
| **42** | Metabolically stable | 99.9 | 29.3 |
| **51** | 71.5 | 99.9 | 9.44 |

**Table 4:** Metabolic stability, plasma protein binding and cardiotoxicity assay results for selected compounds. [a] $t_{1/2}$ calculated in human liver microsomes; compounds metabolically stable with no loss of parent detected for the duration of the assay. [b] Plasma protein binding assay; results are expressed in terms of mean % bound from duplicate experiments. [c] Cardiotoxicity expressed in terms of % hERG inhibition at 25 µM compound concentration.

Except for **51**, the compounds analysed are characterised by an increased metabolic stability in comparison with standard bicalutamide. The tested analogues share a high affinity for human serum albumin, with values similar to the ones of R-bicalutamide found for all except **39e**. Finally, none of

the compounds analysed showed any significant cardiotoxic effect (no $IC_{50}$ could be evaluated). As a combination of its *in vitro* properties and antiproliferative profile in the four prostate-cancer cell lines, **42** has been chosen as pre-clinical candidate for the treatment of prostate cancer and will be evaluated in 22Rv1 prostate cancer mice models. In particular, **42** is one of the most active compounds identified in the antiproliferative assay, with an $IC_{50}$ of 1.99 μM in the LNCaP cell-lines, the most active among the new sulfone derivatives. Being a sulfone this compound is also associated with the highest metabolic stability observed, and its plasma protein binding is highly similar to parent *R*-bicalutamide.
In summary, a novel series of anti-prostate cancer compounds bearing a 3,5-*bis*-trifluoromethylphenyl group have been identified through a rational approach. Most of the new structures showed a significant improvement in antiproliferative activity in comparison with the parent compounds against four different prostate cancer cell lines. Full AR antagonistic behaviour has been demonstrated for selected new structures and a pre-clinical candidate has been identified and is now undergoing further exploration. The biological data obtained so far suggests that the new compounds, besides their AR antagonist activity, might possess an off-target effect, which positively contributes to their anti-prostate cancer properties. The evaluation of the molecular basis of this extra effect will be the focus of future investigations.

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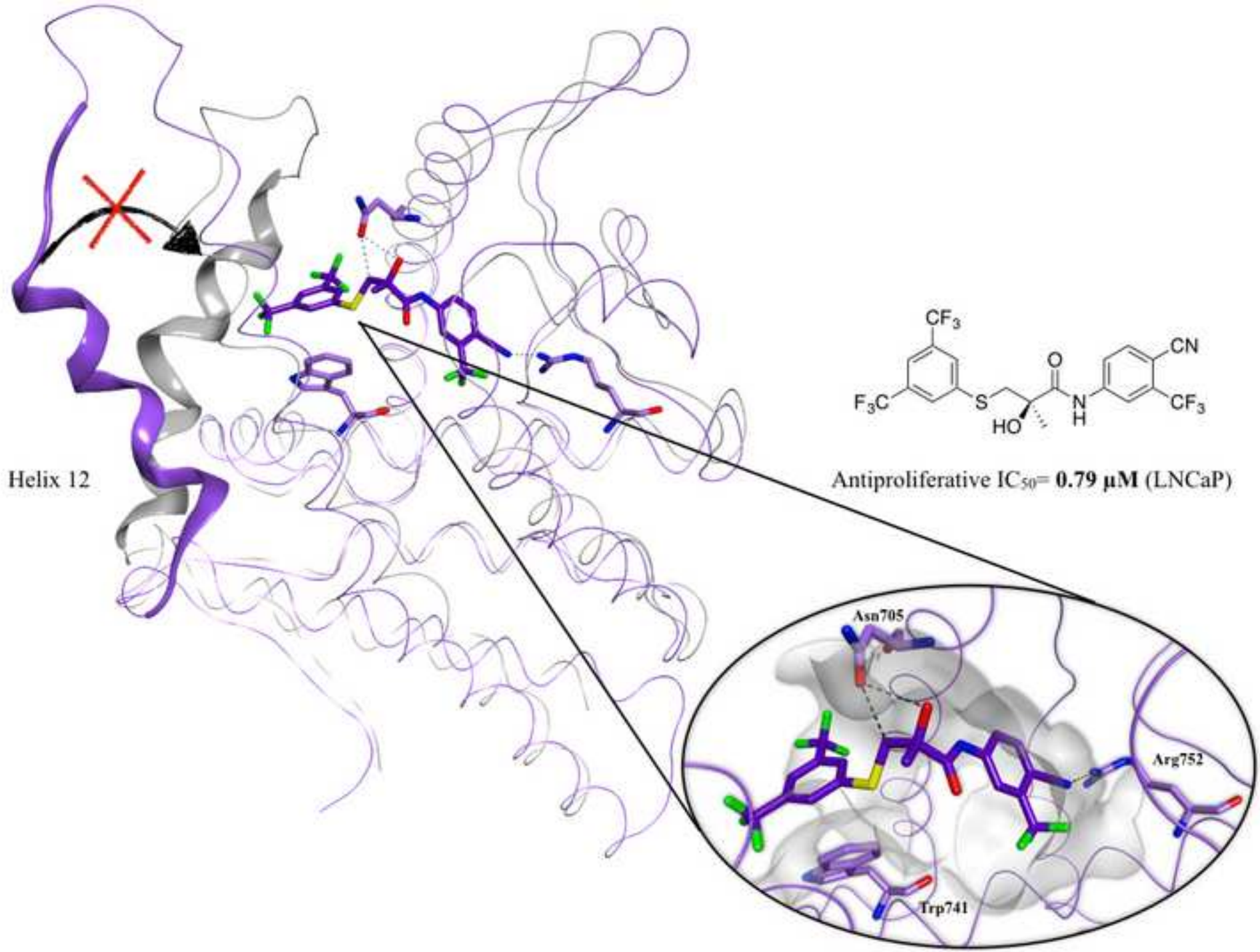
**References**

1. www.cancerresearchuk.gov (accessed April 8, 2016).
2. Ferlay, J.; Shin, H.-R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D. M. Int. J. Cancer. 2010, 127, 2893-2917.
3. Bassetto, M.; Ferla, S.; Pertusati, F. Future Med. Chem. 2015, 7, 527-546.
4. Gomaa, M. S.; Brancale, A.; Simons, C. J. Steroid Biochem. Mol. Biol. 2007, 104, 53−60.
5. Madauss, K.P.; Grygielko, E.T.; Deng, S.J.; Sulpizio, A.C.; Stanley, T.B.; Wu, C.; Short, S.A.; Thompson, S.K.; Stewart, E.L.; Laping, N.J.; Williams, S.P.; Bray, J.D. Mol.Endocrinol. 2007, 21, 1066-1081.
6. Korb, O.; Stützle, T.; Exner, T.E. Swarm Intell. 2007, 1, 115-134.
7. Bohl, C.E.; Gao, W.; Miller, D.D.; Bell, C.E.; Dalton, J.T. Proc. Natl. Acad. Sci. Usa. 2005, 102, 6201-6206.
8. James, K.D.; Ekwuribe, N.N. Synthesis. 2002, 7, 850-852.

9. Chen, B.-C.; Zhao, R.; Gove, S.; Wang, B.; Sundeen, J.E.; Salvati, M.E.; Barrish, J.C. J.Org.Chem. 2003, 26, 10181-10182.
10. Pizzatti, E.; Vigano, E.; Lussana, M.; Landonio, E. U.S. Patent 0,041,161, February 23, 2006.
11. Dalton, T.J.; Miller, D.D.; Yin, D.; He, Y. U.S. Patent 6,569,896 B2 May 27, 2003.
12. Tucker, H.; Chesterson, G.J. J. Med. Chem. 1988, 31, 885-887.
13. Jung, M.E.; Ouk, S.; Yoo, D.; Sawyers, C.L; Chen, C.; Tran, C.; Wongvipat, J. J. Med. Chem. 2010, 7, 2779-2796.
14. Jain, R.P.; Angelaud, R.; Thompson, A.; Lamberson, C.; Greenfield, S. WO Patent 0,657,0 A1 September 1, 2011.
15. Hwang, D.J.; Yang, J.; Xu, H.; Rakov, I.M.; Mohler, M.L.; Dalton, J.T.; Miller, D.D. Biorg. Med. Chem. 2006, 14, 6525-6538.
16. Colabufo, A.; Pagliarulo, V.; Berardi, F.; Contino, M.; Inglese, C.; Niso, M.; Ancona, P.; Albo, G.; Pagliarulo, A.; Perrone, R. Eur. J. Pharmacol. 2008, 601, 38-42.
17. Kuruma, H.; Matsumoto, H.; Shiota, M.; Bishpo, J.; Lamoureux, F.; Thomas, C.; Briere, D.; Los, G.; Gleave, M.; Fanjui, A.; Zoubeidi, A. Mol. Cancer. Ther. 2013, 12, 567-576.
18. Bassetto, M.; Ferla, S.; Pertusati, F.; Kandil, S.; Westwell, A.D.; Brancale, A.; McGuigan, C. Eur. J. Med. Chem. 2016, 118, 230-243.
19. Receptor Assays section. http://www.lifetechnologies.com/uk (accessed April 8, 2016).
20. Kelce, W. R.; Monosson, E.; Gamcsik, M. P.; Laws, S. C.; Gray Jr, L. E. Toxicol. Appl. Pharmacol. 1994,126, 276-285.
21. Waller, C.L.; Juma, B.W.; Gray Jr, L.E., Kelce, W. R. Toxicol. Appl. Pharmacol. 1996,137, 219-227.
22. Gao, W.; Kim, J.; Dalton, J.T. Pharm. Res. 2006, 8, 1641-1658.
23. Thompson, T.A.; Wilding, G. Mol. Cancer. Ther. 2003, 2, 797-803.
24. Senthilraja, P.; Kathiresan, K. J. App. Pharm. Sci. 2015, 03, 080-084.
25. Cockshott, I.D.; Plummer, G.F.; Cooper, K.J.; Warwick, M.J. Xenobiotica 1991, 21, 1347-1355.




Antiproliferative $IC_{50}$= **0.79 µM** (LNCaP)