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Research paper

Design, synthesis and biological evaluation of novel 5-oxo-2-thioxoimidazolidine derivatives as potent androgen receptor antagonists

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ABSTRACT

A series of novel highly active androgen receptor (AR) antagonists containing *spiro*-4-(5-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile core was designed based on the SAR studies available from the reported AR antagonists and *in silico* modeling. Within the series, compound (*R*)-**6** (ONC1−13B) and its related analogues, including its active *N*-dealkylated metabolite, were found to be the most potent molecules with the target activity (IC_{50}, androgen-sensitive human PCa LNCaP cells) in the range of 59−80 nM (inhibition of PSA production). The disclosed hits were at least two times more active than bicalutamide, nilutamide and enzalutamide within the performed assay. Several compounds were classified as partial agonists. Hit-compounds demonstrated benefit pharmacokinetic profiles in rats. Comparative SAR and 3D molecular docking studies were performed for the hit compounds elucidating the observed differences in the binding potency.

1. Introduction

Prostate cancer (PC), following after skin cancer, is the most common cancer occurred in American men, and the sixth cause of cancer-related death among the male population worldwide [1]. More than 230K new cases of prostate cancer have been estimated in the United States during 2014 by American Cancer Society and about 29,480 deaths will be, actually, registered [2]. The vast majority of PCs is initially androgen dependent, and androgen receptor (AR) is highly expressed throughout the various stages of disease playing the crucial regulatory functions in cells [3,4]. The activation of AR [5] strongly promotes prostate cancer growth and progression. Particularly, ligand binding triggers the heat-shock proteins (HSP70 and HSP90) release and subsequent receptor hyperphosphorylation leading to AR dimerization and binding to AR-associated promotor area in target genes. This signaling pathway can be efficiently blocked by competitive FDA-approved AR antagonists (antiandrogens) such as **1** (flutamide) [6], **2** (bicalutamide) [7], **3** (nilutamide) [8], **4** (enzalutamide) [9] or **5** (ARN-509) [10] (Fig. 1). Therefore, androgen deprivation/withdrawal therapy is currently regarded as the “first-line” therapeutic option for localized, early stage prostate cancer. In spite of initial high efficiency observed among a greater percentage of PCa patients, this therapeutic route leads only to a temporary reduction of PC resulting in a significant number of resistance outcomes. As a result, in clinics, classical (steroidal) AR antagonists are frequently ineffective for the treatment of advanced stages of the disease while nonsteroidal drugs are generally considered to be more potent in terms of disease prevention and selectivity thereby minimizing possible *off-target* as well as *on-target* side effects. Cells are able to

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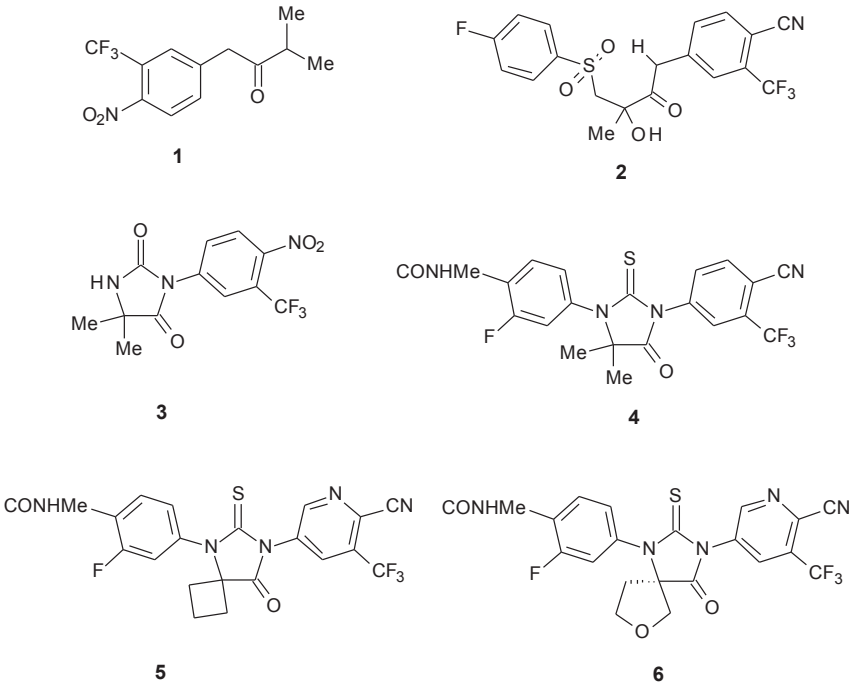


Fig. 1. Non-steroidal AR antagonists approved by FDA (**1**–**4**) and drug candidates currently evaluated in clinics (**5**,**6**).

regenerate and survive without the presence of high levels of testosterone. It is known as hormone-resistant (AIPC) forms and castration-resistant prostate cancer (CRPC) are commonly attributed to elevated AR gene expression or/and amplification [11,12], AR gene mutation [13] as well as ligand-independent AR activation, particularly through the related transcription factors and co-activators [14,15]. A great number of painstaking efforts have been made to develop novel AR antagonists with sufficient therapeutic potency against both types of PC cells with early and later stages of androgen dependence and independence, respectively [16]. In many cases the resistance is strongly associated with the pull of point mutations occurred predominantly in androgen binding site leading to the aberrant receptor up-regulation and higher sensitivity rather than down-regulation and relaxation upon antiandrogen therapy. It should be noted that AR agonists and AR antagonists share different modes of action towards the related transcriptional machinery inducing the “closed” and “open” conformations of AR-H12 helix [17–19]. Therefore, even minor modifications in the structure of a small molecule AR ligand can lead to dramatic alterations in the receptor–ligand interaction thereby providing opposite pharmacological responses. Accordingly, allosteric pockets in AR structure, including BF3 binding site [20], are currently described as promising pathway to overcome the resistance [for review see: [21–23]].

Recently, we have developed novel AR antagonist, (*R*)-**6** (Fig. 1), containing 2-thioxo-7-oxo-1,3-diaza-spiro[4.4]nonan-4-one core with *sub*-nanomolar activity [24] based on comprehensive SAR studies reported for the related analogue **4** launched in 2012 by Astellas Pharma and Medivation. Compound (*R*)-**6** showed similar to **4** and **5** mechanism of action and inhibited DHT (5α-dihydrotestosterone)-stimulated PSA expression and proliferation of prostate cancer cells, prevented binding of androgens to the AR ligand-binding domain, androgen-stimulated AR nuclear translocation and *co*-activator complex formation *in vivo* [25].

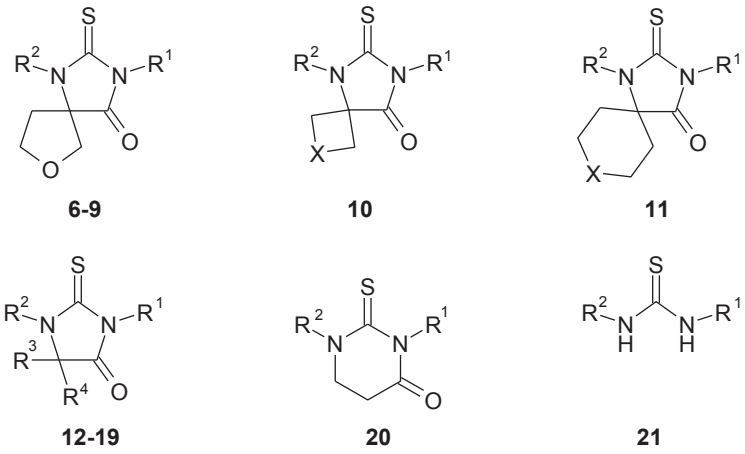
With the aim of obtaining more effective AR antagonists, we have synthesized and tested an extended series of novel (*R*)-**6** analogues (Fig. 2), including substituted 2-thioxo-7-oxa-1,3-diaza-spiro[4.4]nonan-4-ones **6**–**9**, heterocyclic analogues of 6-thioxo-5,7-diaza-spiro[3.4]octan-8-ones **10** and 2-thioxo-1,3-diaza-spiro [4.5]decan-4-ones **11**, 2-thioxo-1,3-diaza-spiro[4.5]decan-4-ones **12**–**19**, 2-thioxo-tetrahydro-pyrimidine-4-ones **20**, and thioureas **21**. We have also performed *in silico* modeling procedure for the disclosed compounds using 3D-molecular docking approach to estimate possible binding modes and diversity points.

2. Results and discussion

2.1. Chemistry

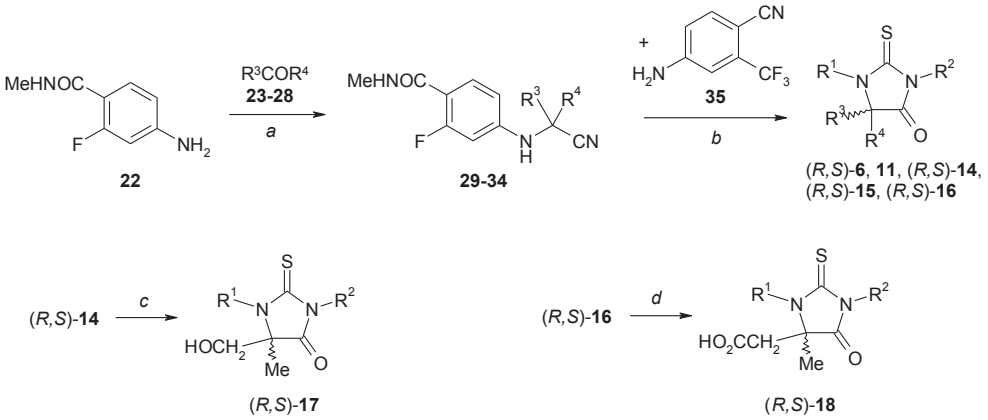
5-Methyl-2-thioxoimidazolidin-4-ones (*R*,*S*)-**6**, **11**, (*R*,*S*)-**14**, (*R*)-**14**, (*S*)-**14**, (*R*,*S*)-**15**, and (*R*,*S*)-**16** were readily obtained from 4-amino-2-fluoro-*N*-methylbenzamide (**22**) by reacting with the appropriate ketone (**23**–**28**) and trimethylsilylcyanide in the presence of ytterbium triflate (Scheme 1). The resulting product (**29**–**34**) was then treated with 4-amino-2-(trifluoromethyl)benzonitrile (**35**) and thiophosgene to generate the desired thiohydantoins mainly as **racemic mixtures**. Then, (*R*,*S*)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**6**) and (*R*,*S*)-5-methyl-5-(methoxymethyl)-2-thioxoimidazolidin-4-one (**14**) were successfully separated using chiral HPLC as optically pure compounds (*R*)-**6**, (*S*)-**6**, (*R*)-**14** and (*S*)-**14**. (*R*,*S*)-5-(Hydroxymethyl)-5-methyl-2-thioxoimidazolidin-4-one (**17**) was synthesized by BBr_3-mediated cleavage of (*R*,*S*)-5-methyl-5-(methoxymethyl)-2-thioxoimidazolidin-4-one (**14**), while (*R*,*S*)-(4-methyl-5-oxo-2-thioxoimidazolidin-4-yl)acetic acid (**18**) was obtained by the hydrolysis of ethyl ester **16** in mild alkaline conditions.

The desired (3*R*)-3-({3-fluoro-4-[(methylamino)carbonyl] phenyl}amino)tetrahydrofuran-3-carboxylic acid ((*R*)-**38**) was synthesized by the copper (I) catalysed reaction of 2-fluoro-4-iodo-*N*-methylbenzamide (**37**), which in turn was obtained from intermediate compound **22**, and (*R*)-3-aminotetrahydrofuran-3-carboxylic acid ((*R*)-**36a**) or its butyl ester (*R*)-**36b**. The reaction



6-21: R^1 = 6-cyano-5-trifluoromethylphenyl. **6**, **10-21**: R^2 = 3-fluoro-4-(methylcarbamoyl)phenyl. **(R)-7**: R^2 = 3-fluoro-4-(methoxycarbonyl)phenyl. **(R)-8**: R^2 = 4-carboxy-3-fluorophenyl. **(R)-9**: R^2 = 4-carbamoyl-3-fluorophenyl. **10a**: X = $NCHPh_2$. **10b** (X = NMe) and **10c** (X = O, synthesis was unsuccessful). **11a**: X = O. **11b**: X = NMe. **12**: R^3 = R^4 = H. **(R,S)-13**, **(R)-13**, **(S)-13**: R^3 = H, R^4 = Me. **(R,S)-14**, **(R)-14**, **(S)-14**,: R^3 = Me, R^4 = $MeOCH_2$. **(R,S)-15**: R^3 = Me, R^4 = $PhCH_2OCH_2$. **(R,S)-16**: R^3 = Me, R^4 = EtO_2CCH_2. **(R,S)-17**: R^3 = Me, R^4 = $HOCH_2$. **(R,S)-18**: R^3 = Me, R^4 = HO_2CCH_2. **19**: R^3 = R^4 = $MeOCH_2$.

Fig. 2. Structures of compounds described in the present work.



Reagents and conditions: (**a**) - AcOH, $MgSO_4$, 25 °C, 24 h; (**b**) - $CSCl_2$, DMF, 80 °C, 12 h; (**c**) - BBr_3, CH_2Cl_2, Ar, -78 °C, 3 h, then 25 °C, 3 h; (**d**) - EtOH, H_2O, NaOH, 25 °C, 12 h.

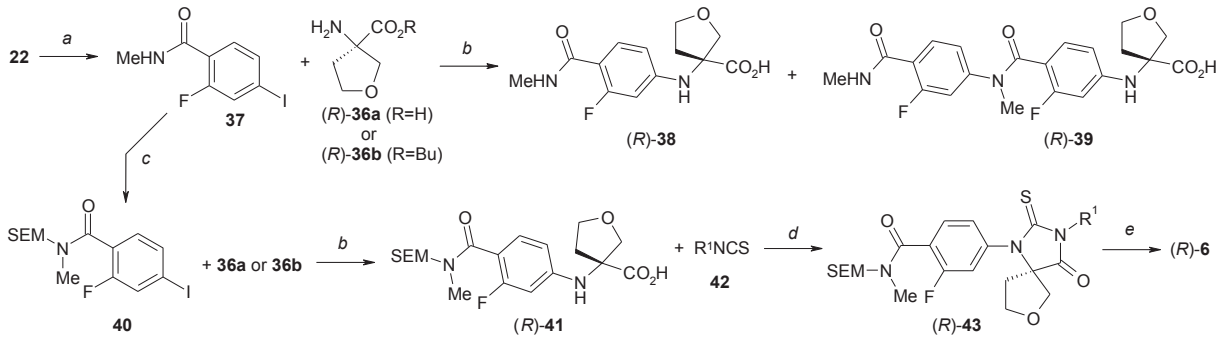
Scheme 1.

proceeded smoothly under relatively mild conditions to provide the desired molecule as well as a considerable amount of autoarylation side product **39** (up to 50% by LC-MS). To overcome this competitive by-reaction, we have protected the amide nitrogen in compound **37** as 2-(trimethylsilyl)ethoxymethyl (SEM) ether following the procedure depicted in Scheme 2. Then, the reaction of protected amide **40** with aminoacid (*R*)-**36a** or its ester (*R*)-**36b** led to individual arylated aminoacid (*R*)-**41** which was further cyclized upon the treatment with isothiocyanate **42** into the thiohydantoin (*R*)-**43**. The final product (*R*)-**6** was readily obtained after SEM-deprotection.

It should be noted that in contrast to iodide **37** 4-bromo-2-fluoro-*N*-methylbenzamide **44** that was found to be less reactive toward amines (*R*)-**36a** or (*R*)-**36b** produced aminoacid ((*R*)-**38**) as a single product (Scheme 3). The intermediate compound **38** was then readily converted into ester **45** using one of the classical methylation procedures followed by the reaction with isothiocyanate **42**. As a result the target compound (*R*)-**6** was obtained in better yield. This rational 3-step pathway is more adapted for the synthesis of antagonist (*R*)-**6** thereby providing a significantly higher overall yield.

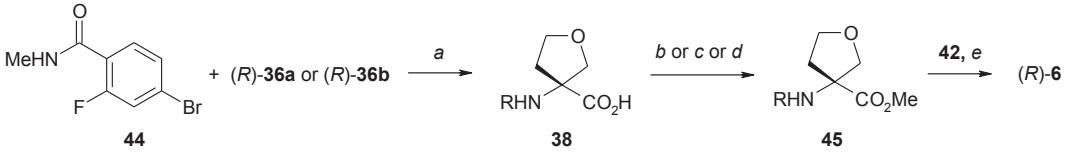
Methyl 4-{(5*R*)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluorobenzoate (**7**) was obtained starting from the aminolysis of 4-bromo-2-fluorobenzoic acid (**46**) by aminoacid (*R*)-**36a** (Scheme 4). The resulted diacid (*R*)-**47** was then converted into the corresponding diester (*R*)-**48** which was further treated by isothiocyanate **42** to afford compound (*R*)-**7**. Subsequent alkaline hydrolysis followed by coupling with ammonia led to the final amide (*R*)-**9**.

Synthesis of 6-thioxo-2,5,7-triaza-spiro[3.4]octan-8-one (**10a**) was performed following the route depicted in Scheme 5 below. Initially, 1-benzhydrylazetidin-3-one (**49**) was smoothly converted into 2-benzhydryl-2,5,7-triaza-spiro[3.4]octane-6,8-dione (**50**) by the *spiro*—junction with KCN and $(NH_4)_2CO_3$. The resulting product was readily hydrolysed by KOH/H_2O into 3-amino-1-benzhydrylazetidine-3-carboxylic acid (**51**) which was then



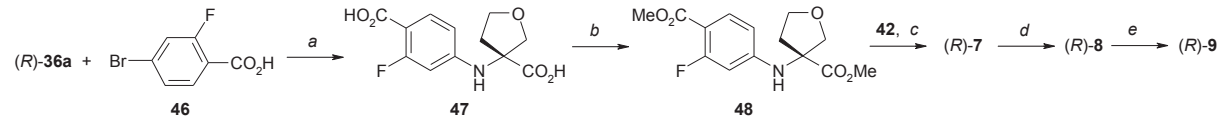
42, (***R***)-**43**: R^1 = 4-cyano-3-(trifluoromethyl)phenyl.
Reagents and conditions: (**a**) H_2O, H_2SO_4, $NaNO_2$, 30 min, 0-5 °C, then KI, 30 min, 80 °C; (**b**) - CuI, K_2CO_3, H_2O, DMF, Et_3N, 2-acetylcyclohexanone, 100 °C, 24 h; (**c**) - DMF, NaH, -10 °C, $Me_3Si(CH_2)_2OCH_2Cl$, 25 °C, 24 h; (**d**) - pyridine, 80 °C, 48 h; (**e**) - TFA, CH_2Cl_2, 25 °C, 3 h.

Scheme 2.



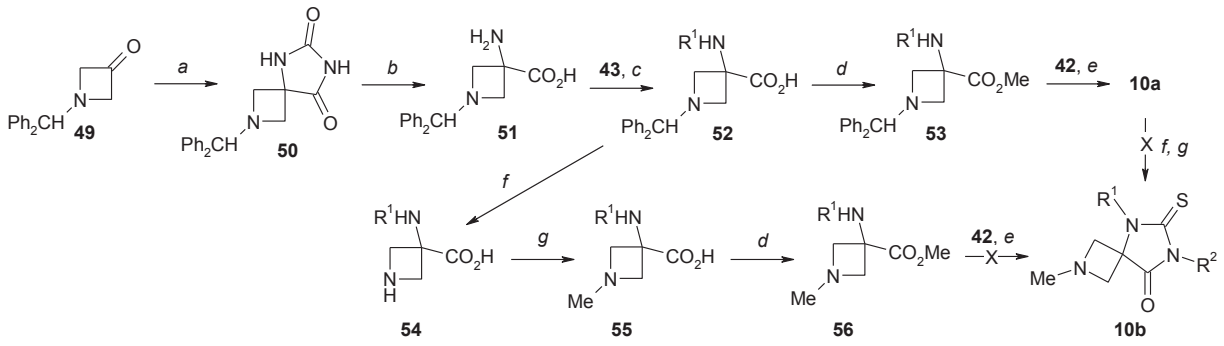
(***R***)-**38**, (***R***)-**45**: R = 3-fluoro-4-(methylcarbamoyl)phenyl.
Reagents and conditions: (**a**) - K_2CO_3, CuI, H_2O, DMF, Et_3N, 2-acetylcyclohexanone, 100 °C, 48 h; (**b**) - DMF, K_2CO_3, MeI, 40 °C, 1 h; (**c**) - MeCN, *N*,*N*-carbodiimidazole, MeOH, 60 °C, 2-3 h; (**d**) - MeOH, $SOCl_2$, reflux, 4 h; (**e**) - DMSO, EtOAc, Ar, 85 °C, 48 h.

Scheme 3.



Reagents and conditions: (**a**) - CuI, K_2CO_3, H_2O, DMF, 2-acetylcyclohexanone, 100 °C, 48 h; (**b**) - MeOH, $SOCl_2$, reflux, 15 h; (**c**) - DMSO, EtOAc, 85 °C, 48 h, then MeOH, 85 °C, 30 min; (**d**) - MeOH, H_2O, NaOH, 25 °C, 12 h; (**e**) - EDAC, HOBt, NH_4Cl, Et_3N, DMF, 25 °C, 24 h.

Scheme 4.



10b, **52-56**: R^1 = 3-fluoro-4-(methylcarbamoyl)phenyl; **10b**: R^2 = 4-cyano-3-(trifluoromethyl)phenyl.
Reagents and conditions: (**a**) - KCN, $(NH_4)_2CO_3$, H_2O, EtOH, 80 °C, 72 h; (**b**) - KOH, H_2O, reflux, 48 h; (**c**) - K_2CO_3, CuI, 2-acetylcyclohexanone, DMF, H_2O, 105 °C, 48 h; (**d**) - MeCN or DMF, CDI, 25 °C, 1h, then MeOH, 50 °C, 24 h; (**e**) - DMSO, EtOAc, 90 °C, 20 h; (**f**) - AcOH, 10% Pd/C, MeOH, H2, 25 °C, 12 h; (**g**) - H_2O, MeOH, HCHO, 25 °C, 30 min, then 10% Pd/C, H_2, 25 °C, 12 h

Scheme 5.

converted into 3-[3-fluoro-4-(methylcarbamoyl)phenylamino]-1-methylazetidine-3-carboxylic acid (**52**) upon the conditions described above. Esterification of **52** via CDI activation followed by the cyclization with isothiocyanate **42** led to the desired 2-benzhydryl-6-thioxo-2,5,7-triaza-spiro[3.4]octan-8-one **10a**. Our effort to convert **10a** into2-methyl-6-thioxo-2,5,7-triazaspiro[3.4]octan-8-one **10b** has failed due to the destruction of the *spiro*-frame upon the removal of benzhydryl protection group. We also did not succeed to obtain **10b** by sequential transformation of 1-benzhydrylazetidine-3-carboxylic acid **52** into 1*H*-azetidine-3-carboxylic acid **54**, 1-methylazetidine-3-carboxylic acid **55** and its methyl ester **56** finished with cyclisation to **10b** in the last step.

3-[3-Fluoro-4-(methylcarbamoyl)phenylamino]oxetane-3-carboxylic acid (**59**) and its methyl ester **61** were obtained starting from 3-aminooxetane-3-carboxylic acid (**57**) which was initially arylated with 4-iodo-*N*-methylbenzamide **40** providing compound **58** (Scheme 6) which was readily deprotected upon the treatment with acid or even during HPLC purification in the presence of TFA in mobile phase to give acid **59**. It should be noted that HPLC purification of acid **58** with neutral mobile phase retained SEM-protection group. Subsequent esterification of **58** followed by deprotection particularly during HPLC purification with TFA led to the formation of valuable intermediate **61**. Interestingly, the reaction of 3-aminooxetane-3-carboxylic acid **59** or its ester **61** with isothiocyanate **42** afforded *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]oxetane-3-carboxamide (**62**) as the main product instead of target 6-thioxo-2-oxa-5,7-diazaspiro[3.4]octan-8-one **10c**. Treatment of **62** with $CSCl_2$ did also not lead to any detectable amount of **10c**. Our efforts to construct thiohydantoin frame prior to 5-arylation in compound **66** by the reaction of aminoacid **57** with isothiocyanate **42** were also unsuccessful.

In acidic media the reaction provided one main product with molecular weight corresponding to compound **65** (LC-MS $[M+H]^+$ 346), NMR spectra (^{13}C NMR, DMSO-d_6: δ 64.65 (CH_2O) and 35.72 (CH_2N)) fitted to 1-[4-cyano-3-(trifluoromethyl)phenyl]-4-(hydroxymethyl)-2-thioxoimidazolidine-4-carboxylic acid (**63**). The formation of the later presumably occurs through oxetane ring opening in initially formed **65** followed by ring closure in the intermediate **64**.

2-Thioxoimidazolidin-4-one **12** was prepared from **37** by copper (I) catalysed reaction with glycine in microwave reactor at 140 °C followed by cyclization of the resulted [3-fluoro-4-(methylcarbamoyl)phenylamino]acetic acid (**67**) with isothiocyanate **42** (Scheme 7).

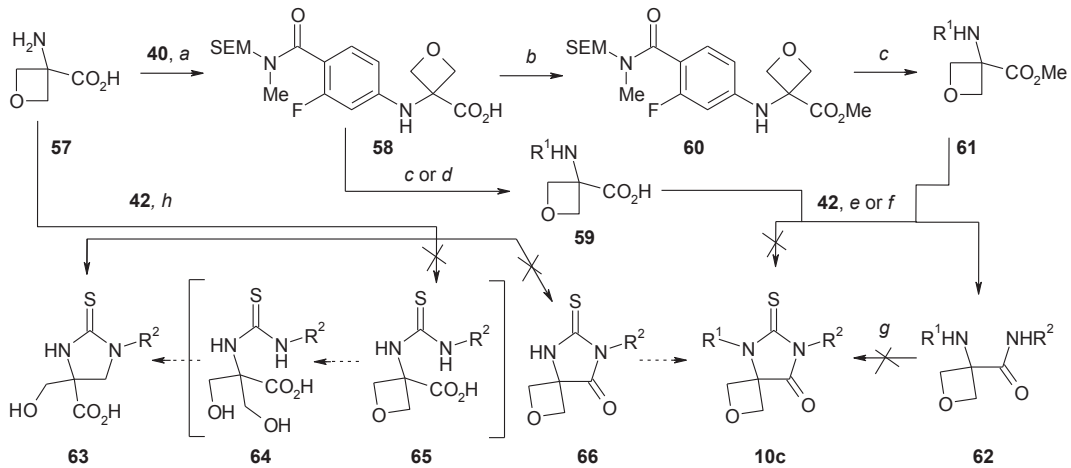
5-Methyl-2-thioxoimidazolidin-4-ones (*R,S*)-**13**, (*R*)-**13**, (*S*)-**13** were prepared generally following the same synthetic route described above for **12** using corresponding alanines D,L-**68**, D-**68** and L-**68** with minor variations in reaction conditions (Scheme 8).

Compound **19** was synthesized following the route depicted in Scheme 9. Thus, SEM-protected amide **40** was used instead of **37** to construct the desired thiohydantoin core since 2-amino-3-methoxy-2-(methoxymethyl)propionic acid was not sufficiently reactive due to steric clashes and conditions required for the arylation/autoarylation of **37** that also took place.

Addition of aniline **22** to ethyl acrylate resulted in aminopropionate **72** which then reacted with isothiocyanate **42** to form thioureidopropionic ester **73**. Subsequent mild hydrolysis of **73** followed by cyclization with TBTU led to the formation of the final 4-oxo-2-thioxotetrahydropyrimidine **20** (Scheme 10). Thiourea **21** was synthesized by the microwave-assisted reaction of aniline **22** and isothiocyanate **42** in DMF at 80 °C.

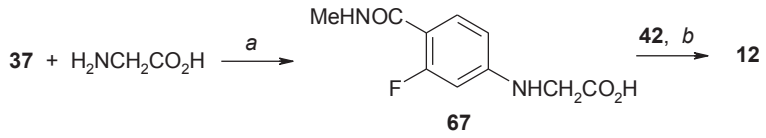
2.2. In vitro biological activity

All the synthesized molecules have been thoroughly evaluated *in vitro* using model LNCaP cells (see Experimental Section). As shown in Table 1, the activity of the tested compounds varies in a wide range. Among them, chiral methylamide (*R*)-**6**, ester (*R*)-**7** and amide (*R*)-**9** were found to be the most active AR binders with a K_i value in the range of 11.9–18.4 nM. It was certainly unexpected that the parent *spiro*-acid did not show any detectable activity against AR. 2-Thioxo-imidazolidin-4-one (*R*)-**14** which can be regarded as close structural analogue of the active triade was significantly less active under the same conditions. Thus, bearing the same peripheral substituents compounds (*R*)-**6** and (*R*)-**14** demonstrated dramatically different binding affinity and showed K_i values of 18.4 and 40.6 nM, respectively. It is especially important to note that (*S*)-enantiomers of compounds (*R*)-**6** and (*R*)-**14** were 11.7- and 19.6-times less active than the hit-compounds, respectively. Our painstaking efforts to synthesize and isolate 2-methyl-6-thioxo-2,5,7-triaza-spiro[3.4]octan-8-one **10b** and 6-thioxo-2-oxa-5,7-diaza-spiro[3.4]octan-8-one **10c** have failed ruling out any estimations of their binding potency. Either 2-benzhydryl-6-thioxo-2,5,7-triaza-



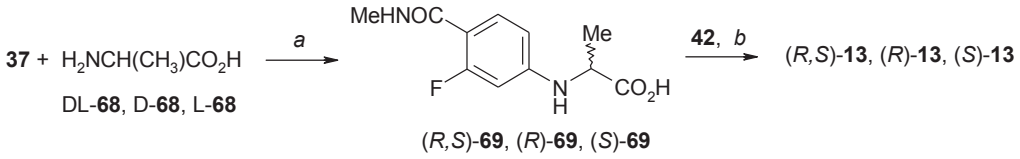
Reagents and conditions: (*a*) - DMF, H_2O, CuI, K_2CO_3, TEA, 2-acetylcyclohexanone; (*b*) - NaH, MeI, DMF, 0 °C - r.t.; (*c*) - TFA; (*d*) - HCl; (*e*) - DMF, 80 °C; (*f*) - DIPEA, MeCN; (*g*) - $CSCl_2$; (*h*) - AcOH, 80 °C.

Scheme 6.



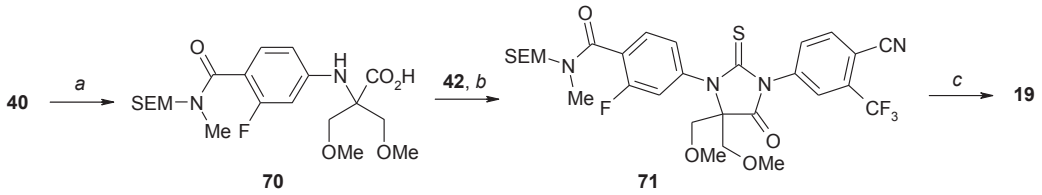
Reagents and conditions: (**a**) - DMF, K_2CO_3, CuI, MW, 140 °C, 18 min; (**b**) - DMF, 90 °C, 12 h.

Scheme 7.



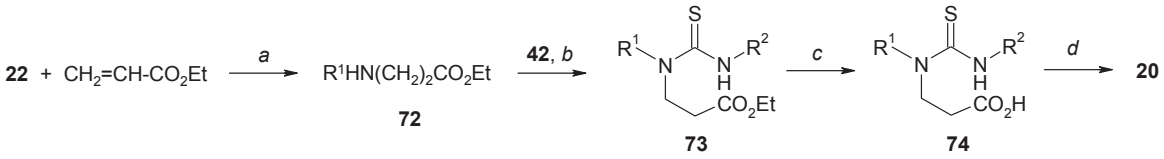
Reagents and conditions: (**a**) - DMSO, Cs_2CO_3, CuI, $Me_2NCH_2CO_2H$, 90 °C, 20 h; (**b**) - DMF, 80 °C, 18 h.

Scheme 8.



Reagents and conditions: (**a**) - 3-methoxy-2-(methoxymethyl)alanine, CuI, 2-acetylcyclohexanone, K_3PO_4, DMF, 100 °C, 48 h; (**b**) - DMF, pyridine, 90 °C, 36 h; (**c**) - DCM, TFA, 25 °C, 2 h.

Scheme 9.



72-74: R^1 = 3-fluoro-4-(methylcarbamoyl)phenyl; **73**, **74**: R^2 = 4-cyano-3-(trifluoromethyl)phenyl.
Reagents and conditions: (**a**) - DMSO, DBU, 70 °C, 24 h; (**b**) - DMF, MW, 60 °C, 8 h; (**c**) - EtOH, H_2O, NaOH, 80 °C, 2 h; (**d**) - DMF, TBTU, DIPEA, 45 °C, 15 h.

Scheme 10.

spiro[3.4]octan-8-one **10a** or 8-methyl-2-thioxo-1,3,8-triaza-spiro [4.5]decan-4-one **11b** did not show a considerable binding affinity toward AR. At the same time, oxygen-containing analogue, 8-methyl-2-thioxo-1,3,8-triaza-spiro[4.5]decan-4-one **11a**, demonstrated almost nanomolar binding potency, K_i = 126.1 nM. These results clearly indicate that compounds containing "*spiro*-oxocycloalkyl" moiety ((*R*)-**6**, **10c** and **11a**) are more promising AR ligands as compared to their "*spiro*-cycloalkyl" derivatives (**4, 5**, **10a**,**b** and **11b**).

Steric effect of the substituents in position 4 of 2-thioxo-imidazolidin-4-one fragment of AR modulators is ambiguous. Thus, an increase in their volume during the hopping from 4,4-dimethyl- **4** (K_i = 28.2 nM) to 4-methyl-4-methoxymethyl- (*R,S*)-**14**, 4-methyl-4-benzyloxymethyl-**15** and 4,4-dimethoxymethyl-2-thioxo-imidazolidin-4-one (*R,S*)-**19** is accompanied with a dramatic fold in the target activity, by 2.4-, 18.2- and 20.7-times, respectively. Simultaneously, the transition from 4,4-dimethyl- **4** (IC_{50} = 127.1 nM) to 4-methyl- (*R,S*)-**13** (IC_{50} = 944.4 nM) and unsubstituted (at position 4) 2-thioxo-imidazolidin-4-one **12** (IC_{50} > 10,000 nM) led not only to insignificant loss of activity but also shifted the response specificity — antagonistic action was changed for partially agonistic modulation (Fig. 3). Similar effect was observed in the case of hopping from 4-methyl-4-methoxymethyl- (*R,S*)-**14** to 4-hydroxymethyl-4-methyl-2-thioxo-imidazolidin-4-one (*R,S*)-**17** and accompanied by 1.57-fold decrease in the target activity. The replacement of 4-(ethoxycarbonylmethyl)-4-methyl- (*R,S*)-**16** (K_i = 235.9 nM) by 4-carboxymethyl-4-methyl-2-thioxo-imidazolidin-4-one (*R,S*)-**18** resulted in significant loss of activity. In contrast to a relatively weak binding potency of 2-thioxo-imidazolidin-4-one **12**, its related analogue, 2-thioxo-tetrahydro-pyrimidin-4-one **20**, showed a moderate affinity (K_i = 575.6 nM), therefore this core can be reasonably regarded as promising starting point for the development of novel highly potent AR ligands. Finally, compound **21** was also found to be completely inactive toward AR.

The second group of compounds includes partial (13.5%–29.2%)

Table 1
In vitro activity of the tested compounds in LNCaP cells.

№	Type	IC_{50}, nM	$K_i \pm$ SD, nM
2	antagonist	732.7	160.2 ± 95.7
4	antagonist	127.1	28.2 ± 12.5
5	antagonist	174.0	34.7 ± 12.6
(*R,S*)-**6**	antagonist	195.6	33.8 ± 6.3
(*R*)-**6**	antagonist	79.2	18.4 ± 10.4
(*S*)-**6**	antagonist	2000.0	215.4 ± 18.6
(*R*)-**7**	antagonist	59.0	11.9 ± 10.9
(*R*)-**8**	antagonist	NA[a]	NA
(*R*)-**9**	antagonist	71.4	14.1 ± 8.0
10a	antagonist	NA	NA
11a	antagonist	539.8	126.1 ± 23.6
11b	antagonist	>10,000	NC[b]
12	partial agonist	>10,000	NC
(*R,S*)-**13**	partial agonist	944.4	257 ± 334.1
(*R*)-**13**	partial agonist	1511.1	481.8 ± 222.3
(*S*)-**13**	partial agonist	398.1	95.3 ± 66.5
(*R,S*)-**14**	antagonist	344.0	68.0 ± 34.6
(*R*)-**14**	antagonist	184.8	40.6 ± 16.1
(*S*)-**14**	antagonist	3166.7	797.6 ± 270.6
(*R,S*)-**15**	antagonist	2300.4	514.5 ± 312.5
(*R,S*)-**16**	antagonist	1378	235.9 ± 57.0
(*R,S*)-**17**	partial agonist	212.2	43.3 ± 18.9
(*R,S*)-**18**	antagonist	NA[b]	NA
19	antagonist	3374.0	584.6 ± 386.2
20	antagonist	3005.3	575.6 ± 437.9
21	antagonist	>10,000	NC

[a] NA – not active.
[b] NC – not calculated.

agonists: **12** without any substituents in position 4 of 2-thioxo-imidazolidin-4-one fragment core as well as (*R,S*)-**13**, (*R*)-**13**, and (*S*)-**13** with one substituent in the same position (Fig. 1). It should be noted that in contrast to methoxymethyl- and benzyloxymethyl-containing derivatives (*R,S*)-**14**, (*R*)-**14**, (*S*)-**14** and (*R,S*)-**15** hydroxymethyl analogue (*R,S*)-**17** can be classified as partial AR agonist (37.7%) with a K_i value of 43.3 ± 18.9 nM that is 1.57-fold higher than that observed for compound (*R,S*)-**14** (K_i = 68.0 ± 34.6 nM). The hopping from poorly active partial (13.5%) agonist **12** (IC_{50} > 10 μM) without any substituents in position 4 of 2-thioxo-imidazolidin-4-one fragment to 4- and 5-unsubstituted 2-thioxo-tetrahydro-pyrimidin-4-one **20** led to a drastic increase in the target antagonistic activity (IC_{50} = 3 μM) with only a trace amount of agonistic action. It should also be mentioned that 3-(3-fluoro-4-methylcarbamoyl-phenylamino)-oxetane-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide (**62**) was formally attributed to partial (16.2%) agonist class with a relatively low activity (IC_{50} = 1425 nM, K_i = 310.3 nM).

2.3. Pharmacokinetics

Considering higher antagonistic activity of the most potent compounds from this series (*R*)-**6**, (*R*)-**7**, (*R*)-**9**, as compared with the reported AR ligands **4** and **5** (Table 1), main pharmacokinetic (PK) features have been investigated exclusively for this triade in rats (Table 2). During the study amide (*R*)-**9** and acid (*R*)-**8** were revealed as basic Phase I metabolites of methylamide (*R*)-**6** (*N*-dealkylation) and ester (*R*)-**7** (*O*-dealkylation), respectively. Bioavailability of the hit-compounds was also calculated and presented in Table 2. Upon oral (po) administration of ester (*R*)-**7** only its metabolite (*R*)-**8** was detected in plasma, therefore PK parameters were calculated based on the concentration of this metabolite. As shown in Table 2, the max bioavailability (F ~ 100%) was revealed for methylamide (*R*)-**6**, while the min value was observed for acid (*R*)-**8** (F = 19%) upon po administration of ester precursor (*R*)-**7**. The principal claim is in very favourable PK profile of methylamide (*R*)-**6** as compared to other compounds tested. Thus, the hit compound showed a relatively low clearance expressed in K_{el} and Cl values, extended half-life time ($T_{½}$) and, that seems to be the most important, — significantly higher exposition in plasma expressed in terms of C_{max} and AUC. Considering a prolonged stability and high activity of (*R*)-**6** and its active metabolite (*R*)-**9** in rats this compound (methylamide) was unambiguously selected for further evaluation as promising clinical candidate.

Table 2
PK parameters for the selected compounds after single po and iv doses in rats (n = 3).

Compound	(*R*)-6		(*R*)-7		(*R*)-8		(*R*)-9	
Administration	po	iv	po	iv	po	iv	po	iv
Dose, mg/kg	5	1	5	1	5	1	5	1
K_{el}, 1/h	0.13	0.167	0.18	0.051	0.19	0.25	0.39	0.46
$T_{½}$, h	5.2	4.1	4.3	17.1	4.0	3.3	1.8	1.5
T_{max}, h	2	0.083	1.67	0.083	0.42	0.083	2.0	0.083
C_{max}, ng/ml	1062	531	104	518	231	1177	261	185
$AUC_{(0-t)}$, h·ng/mL	13,001	2572	570	599	716	497	1280	295
$AUC_{(0-\infty)}$, h·ng/mL	13,105	2624	576	712	743	509	1389	303
V_d, L/kg	–	2.3	53.0	46	38.4	8.0	9.4	7.0
Cl, L/h/kg	–	0.4	8.8	1.7	7.0	2.0	3.6	3.3
MRT_{last}, h	6.9	4.8	4.6	4.3	3.5	0.5	3.1	1.6
MRT_{INF}, h	7.2	5.3	5.0	12.8	4.2	0.8	3.7	1.8
V_{ss}, L/kg	–	2.0	–	22.3	–	1.6	–	6.1
F, %	100	–	19	–	29	–	87	–

2.4. In silico modeling

Androgen receptors share similar to other nuclear receptors (e.g., estrogen receptor) three-dimensional structure containing 11 α-helices and two β-turns that forms three layers packed in the antiparallel "α-helical sandwich" cluster [26,27]. Upon agonist binding, the carboxylterminal helix 12 (H12) undergoes a conformational change thereby forming a cap that results in ligand stabilization and retaining. Simultaneously, the second β-turn is formed to lock the active conformation of H12. As a result, the activation function 2 (AF2) site is immediately formed. It plays a

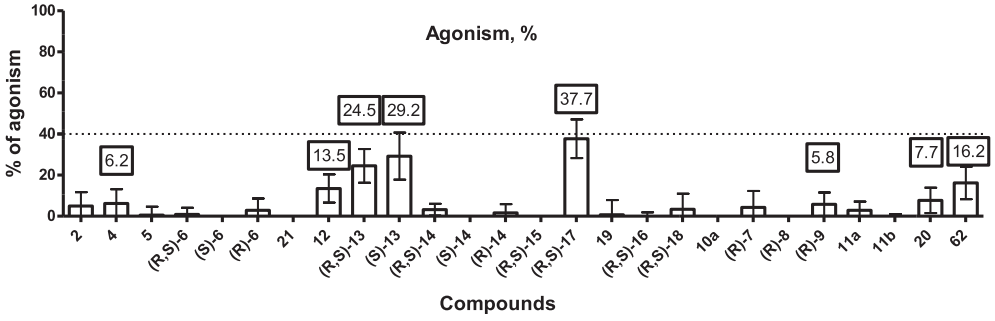


Fig. 3. Agonistic activity of the tested compounds.

crucial role in the recruitment of *co*-activators thereby controlling the activity of down-stream transcription factors related to AR [28]. Several small-molecule antagonists, e.g. **2**, induce a partial unfolding of H12 resulting in functionally disabled AF2 region similar to that previously observed for estrogen receptor complexed with the selective antagonist raloxifene [29].

To elucidate the possible mechanisms of action and the antagonistic potencies of the evaluated compounds, we initially performed a thorough computational analysis of the target structure based on available protein data (PDB codes: 2IHQ, 1Z95, 3V49 and 3V4A) and SAR evaluations. The results of several recently reported studies were taken into consideration [30,31]. At least one residue His874 was found to be conformationally flexible. In addition, three aminoacid residues positioned close to His874 were also suggested to be not strictly rigid in their native location. *In silico* modeling procedure was then performed for compounds (*R*)-**6**–**9** in ICM-Pro software [32] using the crystallographic structures published previously by Nique and colleagues [33] (PDB codes: 3V49 and 3V4A) as the major 3D template. Binding site was then completely reconstructed with flexible points assigned to His874, W741, M742 and I899. Reference compounds 4-[(4R)-4-(4-hydroxyphenyl)-3,4-dimethyl-2,5-dioxoimidazolidin-1-yl]-2-(trifluoromethyl)benzonitrile (from 3V49) and (5R)-3-(3,4-dichlorophenyl)-5-(4-hydroxyphenyl)-1,5-dimethyl-2-thioxoimidazolidin-4-one (from 3V4A) were docked into the active binding site as 2D structures with a predefined stereospecifity. Docking procedure with all the force-field components available classified the compounds as high-score AR ligands. The predicted active conformations correlated well with that observed in X-Ray structures (RMSD < 0.2). Then, compounds (*R*)-**6**–**9** were docked into the same binding site applying the semi-static mode using the same software settings. 3D representations of the supramolecular interfaces predicted for the active conformations, related scorings and correlation plot are presented in the Supporting Information (SI). Fig. 4 shows the resulting 2D supramolecular frame reconstructed based on the obtained docking results between crucial binding points (BPs) of AR antagonists **4**, **5**, (*R*)-**6**, (*R*)-**7**, (*R*)-**8**, (*R*)-**9** and key aminoacids of AR. As shown in Fig. 3, the binding mode predicted for the known AR antagonists **4** and **5** is absolutely identical to that observed for the reference compounds. The key conservative interactions were identified for all the compounds tested between nitrile moiety and R752 (H-bonding) as well as (trifluoromethyl)phenyl ring and W741/T877 (hydrophobic contacts). The aminoacid surrounding covering the *spiro*-moiety in **5**, (*R*)-**6**–**9** and *di*-methyl joint in **4** is also very similar and includes F876, L701 and M780. Considerable differences in binding of the reference molecules, known ligands and compounds (*R*)-**6**–**9** were observed within the ensemble of aminoacids surrounding the substituted (trifluoromethyl)phenyl ring in the opposite side of the molecules. Thus, the hydrogen bond is readily formed between the phenyl OH group in the structures of the reference compounds and H874, whereas for known amides **4** and **5** this contact was not predicted within the pull of generated conformations. However, these molecules provide the alternative H-bond observed between NH-group of methylamide fragment and the oxygen of peptide chain formed by M895. Contrariwise, two alternative binding modes were revealed for amide (*R*)-**6**. The first binding mode is similar to that predicted for compounds **4** and **5** (contact with M895 or Asn705), while the second one is stabilized by H-bonding with H847 as has been revealed for the template compounds. The analogous binding mode was predicted for the *N*-demethylated derivative (*R*)-**9** and acid (*R*)-**8**. However, the H-bond between acid OH group and M895 is less possible than with H874. Interestingly, acid (*R*)-**8** was completely inactive in the biological assay performed but showed virtual score comparative to active analogues. It can be reasonably elucidated by a drastically poor membrane permeability of polar (*R*)-**8** containing the negatively charged carboxylic acid moiety through the membrane of the model cells. It is not surprising, that only single binding mode (with H874) was predicted for ester (*R*)-**7**. Under the applied assay conditions compounds **4** and **5** were approximately two times less active than (*R*)-**6**. It may be due to the synergetic effect achieved by alternative binding mode and additional weak H-bond observed between oxygen atom in tetrahydrofuran *spiro*-moiety and T877 (not shown here).

To elucidate three-dimensional structure of the selected hit-compound (*R*)-**6** we have performed crystallographic analysis (Fig. 5a,b). As shown in Fig. 5, two conformations were observed in the crystal cell providing two different locations of the terminal amide bond (Fig. 5a). Two torsion angles (ta) of − 125° and − 49.3° (HN−C(O)-C_{ar}-C_{ar}(F)) are fixed by rotation of the amide group. The active conformation of (*R*)-**6** outputted from the docking study has the invert orientation realized by the rotation of benzamide fragment by 180° (ta = −66.6°). Conformational analysis (*in vacuo*) provided 15 possible conformations of compound (*R*)-**6** with the values of potential energy close to the calculated minimum (Fig. 5b). One of the most fitted conformations is presented in Fig. 5a (ta = 153°).

3. Conclusions

A small-sized library of novel substituted *spiro*-4-(5-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitriles were synthesized and evaluated *in vitro* against androgen-sensitive LNCaP cell line. Three compounds from the described series, (*R*)-**6**, (*R*)-**7** and (*R*)-**9**, were identified as the most promising hits with IC_{50} values of: 79.2, 59.0 and 71.4 nM (PSA expression/production inhibition), respectively. These molecules were 1.6-, 2.15- and 1.78-fold more active than **4**. On the basis of extensive PK study performed in rats compound (*R*)-**6** that showed an appropriate profile was selected for further clinical evaluation. This in-class small molecule antiandrogen can be reasonably regarded as the *pro*-drug of its *N*-dealkylated active metabolite (*R*)-**9**. SAR study performed for the evaluated compounds has revealed the key role of *spiro*-moiety and functionalities in *para*-position of fluorophenyl ring. Possible binding modes of the active compounds were predicted using 3D molecular docking approach and compared with the available X-Ray data for analogue compounds. Several distinctive aspects in binding were observed elucidating differences in the target activity.

4. Experimental section

4.1. Chemistry general procedures

Reference substance **2** was purchased from Tocris, catalogue no. 3389, **4** from Selleck Chemicals, catalogue no. S1250 and **5** from Selleck Chemicals, catalogue no. S2840. All chemicals, starting materials/reagents and solvents were used as received from the suppliers without further purification. The crude reaction mixtures were concentrated under reduced pressure by removing the organic solvents in a rotary evaporator. Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker DPX-400 spectrometer at room temperature (rt) with tetramethylsilane as an internal standard. The chemical shifts (δ) are reported in parts per million (ppm) and the signals are reported as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *m* (multiplet), or *br s* (broad singlet). The purities of the final compounds were determined by HPLC and were greater than 98%. The HPLC conditions for assessing purity were as follows: Shimadzu HPLC, XBridge™ C18, 4.6 mm × 250 mm (3.5 μm); gradient of 0.1% TFA in 5% acetonitrile/

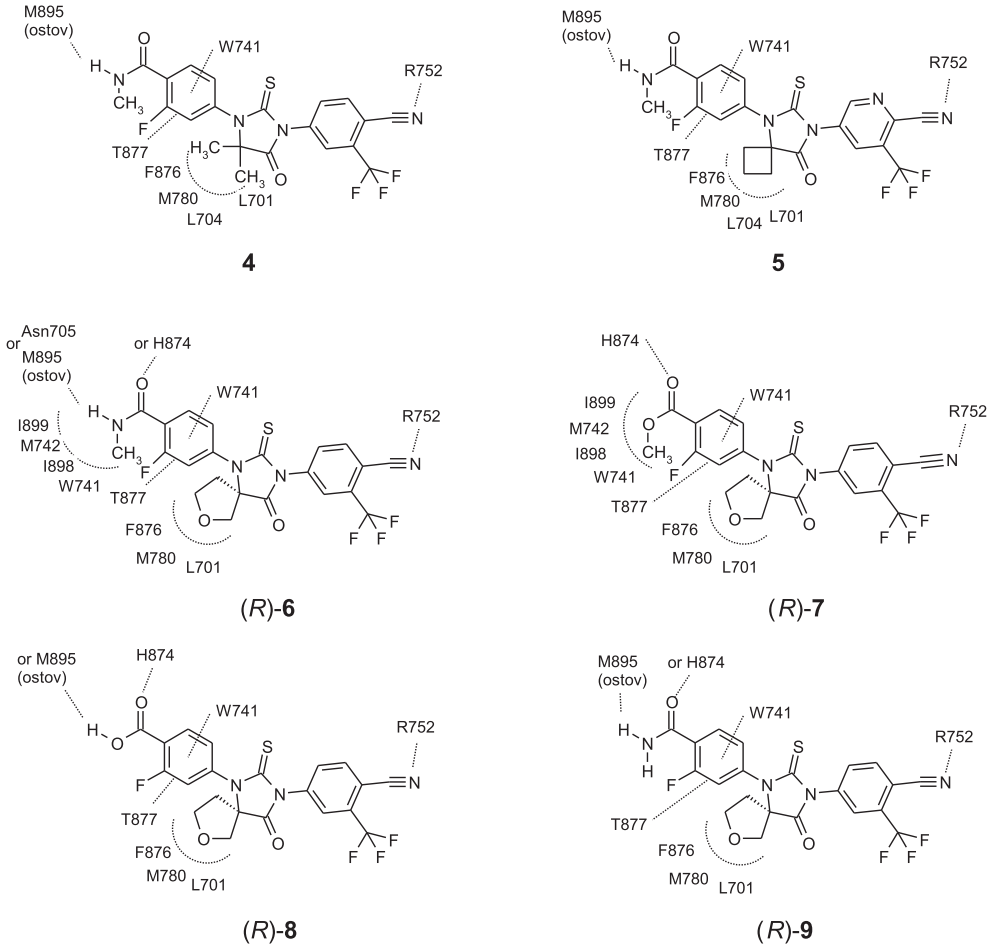


Fig. 4. 2D supramolecular interface predicted for AR antagonists **4**, **5**, (*R*)-**6**, (*R*)-**7**, (*R*)-**8** and (*R*)-**9**.

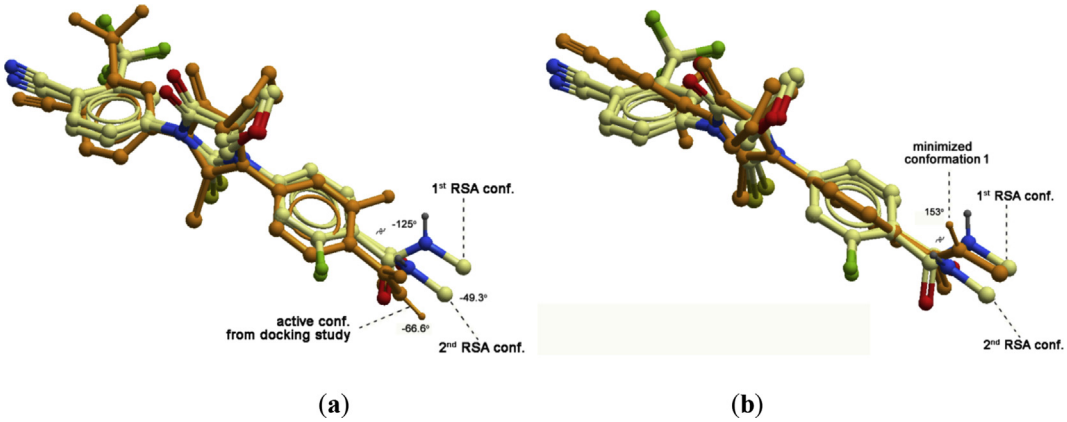


Fig. 5. 3D Representation of (*R*)-**6**: (**a**) rigid alignment of two X-Ray structures and active conformation resulted from the docking study; (**b**) rigid alignment of two X-Ray structures and conformation generated *in vacuo*.

water (A) and 0.1% TFA acetonitrile (B); flow rate, 0.5 mL/min; acquisition time, 20 min; wavelength, UV 214 and 254 nm. The preparative HPLC system included two sets of Shimadzu LC-8A pumps, a Shimadzu controller SCL 10Avp, and a Shimadzu detector SPD 10Avp. A Reprosil-Pur C-18-AQ 10 μm, 250 mm × 20 mm column was used. The mobile phase was a gradient of 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). LC/MS was conducted on a PE Sciex API 165 system using electrospray in positive ion mode, $[M+H]^+$, and a Shimadzu HPLC system equipped with a Waters XBridge C18 3.5 μm column (4.6 mm × 150 mm). High resolution mass spectra (HRMS) were acquired on an Orbitrap Elite mass spectrometer (Thermo, Bremen, Germany) equipped with an HESI ion source.

4.1.1. General procedure 1

4.1.1.1. 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5-substitute-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamides (R,S)-6, 11a, 11b, (R,S)-14, (R)-14, (S)-14, (R,S)-15, and (R,S)-16.

Thiophosgene (0.473 g, 4.15 mmol) was added dropwise to the solution of 4-[(cyanomethyl)amino]-2-fluoro-*N*-methylbenzamide **29**–**34** (3.77 mmol, procedure below) and 4-amino-2-(trifluoromethyl)benzonitrile (**35**) (0.772 g, 4.15 mmol, Apollo Scientific) in DMF (15 mL) keeping the temperature below 25 °C. The resulting mixture was stirred for 0.5 h at the ambient temperature and then for 12 h at 80 °C. After the stirring was completed, the mixture was mildly cooled down. The analogous procedure was applied for compound **35**. DMF was evaporated *in vacuo*, the formed residue was dissolved in 15 mL of MeOH and 1.5 mL of 3M HCl, then the resulting mixture was refluxed for 3 h. The solvent was removed under reduced pressure, the formed residue was treated with water, filtered off, washed with water and dried *in vacuo*. The desired product was then purified by HPLC.

4.1.1.2. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluoro-N-methylbenzamide ((R,S)-6). Yield 21%. MS (ESI) $[M+H]^+$ 493. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (t, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 1.6 Hz, 1H), 7.85 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 7.34 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 7.25 (dd, J_1 = 11.8 Hz, J_2 = 1.6 Hz, 1H), 6.78 (q, J = 4.4 Hz, 1H), 4.43 (d, J = 10.0 Hz, 1H), 4.16 (d, J = 10.0 Hz, 1H), 3.96 (m, 1H), 3.75 (m, 1H), 3.09 (d, J = 4.4 Hz, 3H), 2.74 (m, 1H), 2.48 (m, 1H). ESIHRMS *m/z* calcd for $C_{22}H_{17}F_4N_4O_3S$ $[M+H]^+$ 493.0952; found 493.0949. Separation of individual enantiomers (*R*)-**6** and (*S*)-**6** from racemic mixture was performed by HPLC with chiral column Phenomenex Lux Amylose-2 (φ 5 μm, 250 × 20 mm). Isocratic 15/85 system of MeOH/EtOH (A) and *n*-hexane (B) was used as a mobile phase, flow rate was 20 mL/min. Retention times for (*S*)-**6** and (*R*)-**6** were 19 and 22 min, respectively.

*4.1.1.3. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-8-oxa-1,3-diazaspiro[4.5]dec-1-yl}-2-fluoro-N-methylbenzamide (**11a**).* Yield 10%. MS (ESI) $[M+H]^+$ 507. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (t, J = 8.4 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.95 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.73 (m, 1H), 4.18 (m, 2H), 3.94 (m, 2H), 3.09 (d, J = 4.4 Hz, 3H), 2.07 (m, 4H). ESIHRMS *m/z* calcd for $C_{23}H_{19}F_4N_4O_3S$ $[M+H]^+$ 507.1109; found 507.1110.

*4.1.1.4. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-8-methyl-4-oxo-2-thioxo-1,3,8-triazaspiro[4.5]dec-1-yl}-2-fluoro-N-methylbenzamide (**11b**).* Yield 3%. MS (ESI) $[M+H]^+$ 520. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (brs, 1H), 8.48 (q, J = 4.4 Hz, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.84 (t, J = 8.0 Hz, 1H), 7.42 (d, J = 10.4 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 3.50 (m, 4H), 2.80 (d, J = 4.4 Hz, 3H), 2.78 (s, 3H), 2.72 (d, J = 14.0 Hz, 1H), 2.16 (m, 2H).

4.1.1.5. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-5-(methoxymethyl)-5-methyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide ((R,S)-14). Yield 16%. MS (ESI) $[M+H]^+$ 495. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (t, J = 8.4 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.92 (s, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.29 (dd, J_1 = 8.8 Hz, J_2 = 1.2 Hz, 1H), 7.21 (dd, J_1 = 11.6 Hz, J_2 = 1.2 Hz, 1H), 6.72 (q, J = 4.4 Hz, 1H), 3.71 (d, J = 10.0 Hz, 1H), 3.43 (s, 3H), 3.35 (d, J = 10.0 Hz, 1H), 3.09 (d, J = 4.4 Hz, 3H), 1.52 (s, 3H). ESIHRMS *m/z* calcd for $C_{22}H_{19}F_4N_4O_3S$ $[M+H]^+$ 495.1109; found 495.1107. Enantiomers were readily separated following the procedure described above. Isocratic 20/80 system of 0.02% Et_2NH in 20/80 MeOH/*i*-PrOH (A) and *n*-hexane (B) was used as a mobile phase, flow rate was the same. Retention times for (*S*)-**14** and (*R*)-**14** were 16 and 19 min, respectively.

4.1.1.6. 4-{5-(Benzyloxymethyl)-3-[4-cyano-3-(trifluoromethyl)phenyl]-5-methyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide ((R,S)-15). Yield 17%. MS (ESI) $[M+H]^+$ 571. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (t, J = 8.4 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.70 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 7.39 (m, 3H), 7.29 (m, 2H), 7.25 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 7.18 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 6.71 (q, J = 4.8 Hz, 1H), 4.59 (m, 2H), 3.79 (d, J = 10.2 Hz, 1H), 3.45 (d, J = 10.2 Hz, 1H), 3.08 (d, J = 4.8 Hz, 3H), 1.51 (s, 3H).

4.1.1.7. Ethyl {1-[4-cyano-3-(trifluoromethyl)phenyl]-3-[3-fluoro-4-(methylcarbamoyl)phenyl]-4-methyl-5-oxo-2-thioxoimidazolidin-4-yl]acetate ((R,S)-16). Yield 14%. MS (ESI) $[M+H]^+$ 536. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (t, J = 8.4 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 8.00 (s, 1H), 7.90 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.18 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.10 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 6.78 (q, J = 4.8 Hz, 1H), 4.26 (m, 1H), 3.13 (d, J = 18.0 Hz, 1H), 3.09 (d, J = 4.8 Hz, 3H), 2.64 (d, J = 18.0 Hz, 1H), 1.67 (s, 3H), 1.31 (t, J = 7.0 Hz, 3H).

4.1.1.8. (R)-3-Aminotetrahydrofuran-3-carboxylic acid ((R)-36a). NaOH (1N, 150 mL) was added to the solution of compound (*R*)-**36b** (16 g, 85 mmol) [36] in fresh methanol (150 mL). The resulting mixture was vigorously stirred for 15 h, then acidified with HCl to pH = 3–4 and rotovapped. The obtained product can be purified by the extraction with methanol. MS (ESI) $[M+H]^+$ 132. ^{1}H NMR (400 MHz, D_2O) δ 4.06 (m, 1H), 3.97 (m, 3H), 2.55 (m, 1H), 2.18 (m, 1H).

4.1.1.9. 4-{(R)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluoro-N-methylbenzamide ((R)-6)

4.1.1.9.1. Procedure A. The mixture of 4-amino-2-fluoro-*N*-methylbenzamide (**22**) (1.68 g, 10 mmol) [35], H_2SO_4 (0.68 mL) and water (13 mL) was gently heated until all the components were completely dissolved. The mixture was cooled to 0–5°C under stirring, then the solution of $NaNO_2$ (0.7 g, 10 mmol) in water (2 mL) was added dropwise. The resulting mixture was stirred at 0–5°C for 0.5 h and then slowly poured into the solution of KI (5 g) in cold water (20 mL). The solution was then heated up to 80°C and stirred for 0.5 h. After cooling it was treated with 30 mL of chloroform and filtered. The organic layer was washed with 5% Na_2SO_3 solution, dried over Na_2SO_4 and rotovapped. Column chromatography on silica gel (hexane/EtOAc = 6:1) afforded 2.33 g (83%) of ***2-fluoro-4-iodo-N-methylbenzamide*** **(37)**. MS (ESI) $[M+H]^+$ 280. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (m, 1H), 7.73 (d, J = 10.0 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 2.75 (d, J = 4.4 Hz, 3H). The solution of compound **37** (9 g, 32 mmol) in DMF (25 mL) was added to the ice cooled suspension of NaH (1.48 g, 36.8 mmol, 60% in oil, washed with hexane) in DMF (50 mL). The resulting mixture was vigorously stirred for 0.5 h in an ice bath, then SEM-chloride (6.46 g, 39 mmol) was added and the mixture was continuously stirred overnight at the ambient temperature. After the reaction was completed, the mixture was poured into water (400 mL) and the obtained product was extracted with benzene (2 × 200 mL), washed with water, dried over Na_2SO_4 and then filtered through 3 cm layer of silica gel washing with 5:1 hexane/EtOAc. The solvent was evaporated *in vacuo* providing 11 g (84%) of ***2-fluoro-4-iodo-N-methyl-N-[2-(trimethylsilyl)ethoxymethyl]benzamide*** **(40)**. MS (ESI) $[M+H]^+$ 520. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (m, 1H), 7.52 (m, 1H), 7.12 (m, 1H), 5.01 (s, 0.8H), 4.58 (s, 1.2H), 3.63 (t, J = 8.2 Hz, 0.8H), 3.31 (t, J = 8.2 Hz, 1.2H), 3.14 (s, 1.8H), 2.92 (d, J = 0.8 Hz, 1.2H), 0.99 (t, J = 8.2 Hz, 0.8H), 0.82 (t, J = 8.2 Hz, 1.2H), 0.04 (s, 3.6H), −0.01 (s, 5.4H). The mixture of 6.69 g (51 mmol) of aminoacid (*R*)-**36a** or 9.55 g of (*R*)-**36b**, 17.4 g (42.5 mmol) of **40**, 1.62 g (8.5 mmol) of CuI, 23.5 g (0.17 mol) of K_2CO_3, 36 mL of water, 145 mL of DMF and 3–5 drops of Et_3N was stirred for 10 min, then 6.56 g

(46.8 mmol) of 2-acetylcyclohexanone was added and stirring continued at 100°C for 24 h. After cooling the mixture was rotovapped, the residue was treated with 200 mL of water and acidified with hydrochloric acid to pH 2–3 (~30 mL). Then 200 mL of ether was added, the mixture was stirred for 0.5 h and the formed precipitate was filtered off, washed with 50 mL of ether and dried *in vacuo* to give 10.7 g (65%) of (R)-3-(3-fluoro-4-{methyl[2-(trimethylsilyl)ethoxymethyl]carbamoyl}phenylamino)-tetrahydrofuran-3-carboxylic acid **((*R*)-41)** (55% from ester (*R*)-**36b**). MS (ESI) $[M+H]^+$ 413. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (brs, 1H), 7.07 (m, 2H), 6.31 (brs, 1H), 6.17 (brs, 1H), 4.85 (brs, 0.67H), 4.60 (brs, 1.33H), 4.10 (brs, 1H), 3.87 (brs, 3H), 3.51 (brs, 0.67H), 3.24 (brs, 1.33H), 2.93 (s, 2H), 2.86 (brs, 1H), 2.56 (brs, 1H), 2.16 (brs, 1H), 0.89 (brs, 0.67H), 0.73 (brs, 1.33H), −0.03 (m, 9H). The mixture of 10.7 g (26 mmol) of (*R*)-**41** and 8.9 g (39 mmol) of 4-isothiocyanato-2-(trifluoromethyl)benzonitrile (**42**) [34] in pyridine (100 mL) was stirred at 80°C for 48 h. The residue formed after cooling and rotovapping was then treated with ethyl acetate and filtered through 2 cm layer of silica gel. The solvent was removed under reduced pressure and the desired product was crystallized from ethanol to obtain 4.85 g (30%) of ***4***-{(R)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluoro-N-methyl-N-[2-(trimethylsilyl)ethoxymethyl]benzamide **((*R*)-43)**. MS (ESI) $[M+H]^+$ 623. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.57 (m, 1H), 7.28 (m, 1H), 7.21 (m, 1H), 5.06 (s, 0.8H), 4.63 (s, 1.2H), 4.41 (d, J = 10.4 Hz, 1H), 4.16 (d, J = 10.4 Hz, 1H), 3.97 (q, J = 7.6 Hz, 1H), 3.78 (m, 1H), 3.66 (t, J = 8.2 Hz, 0.8H), 3.66 (t, J = 8.2 Hz, 1.2H), 3.20 (s, 1.8H), 2.99 (s, 0.8H), 2.72 (m, 1H), 2.47 (m, 1H), 1.01 (t, J = 8.2 Hz, 0.8H), 0.83 (t, J = 8.2 Hz, 1.2H), 0.06 (s, 3.6H), −0.01 (s, 5.4H). TFA (15 mL) was added to the solution of compound (*R*)-**43** (4.8 g, 7.7 mmol) in DCM (30 mL), then the resulting mixture was stirred for 3 h. The solvent was removed under reduced pressure and the formed residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH = 60:1) to give 2.96 g (78%) of desired product (*R*)-**6**.

4.1.1.9.2. Procedure B. The mixture of compound (*R*)-**36b** (3.29 g, 17.6 mmol), 4-bromo-2-fluoro-*N*-methylbenzamide **44** (3.40 g, 14.7 mmol) [41], CuI (0.53 g, 2.79 mmol), K_2CO_3 (7.97 g, 58.6 mmol) and Et_3N (0.2 mL) in water (6 mL) and DMF (27.5 mL) was stirred for 10 min, then 2-acetylcyclohexanone (0.41 g, 2.9 mmol) was added and stirring was continued at 100°C for 48 h. The mixture was cooled to rt, the solvent was removed under reduced pressure, then the formed residue was treated with water and acidified with hydrochloric acid to pH 2–3. The resulting solution was stirred for 0.5 h, the formed precipitate was filtered off, washed with cold water, dried in air, then washed with ether and dried again to provide 3.35 g (81%) of ***(R)-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]-tetrahydrofuran-3-carboxylic acid*** **((*R*)-38)**. MS (ESI) $[M+H]^+$ 283. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (brs, 1H), 7.65 (t, J = 4.4 Hz, 1H), 7.47 (t, J = 8.8 Hz, 1H), 7.11 (brs, 1H), 6.32 (dd, J_1 = 8.8 Hz, J_1 = 2.0 Hz, 1H), 6.13 (dd, J_1 = 14.4 Hz, J_1 = 2.0 Hz, 1H), 4.11 (d, J = 8.8 Hz, 1H), 3.88 (m, 3H), 2.73 (d, J = 4.4 Hz, 3H), 2.54 (m, 1H), 2.15 (m, 1H). Iodomethane (301 mg, 2.12 mmol) was added under vigorous stirring to the mixture of compound (*R*)-**38** (500 mg, 1.77 mmol) and K_2CO_3 (293 mg, 2.12 mmol) dissolved in DMF (4 mL) at 30°C. The resulting mixture was heated up to 40°C and stirred keeping the predefined temperature for 1 h, then diluted with 40 mL of water, heated up to 60°C and filtered. The filtrate was extracted with chloroform (2 ч 50 mL), organic phase was washed with water, dried over Na_2SO_4 and the solvent was evaporated using a vacuo pump. The formed residue was treated with ether, filtered off and dried to afford 372 mg (70%) of ***methyl (R)-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]-tetrahydrofuran-3-carboxylate*** **((*R*)-45)**. Following the alternative synthetic route, CDI (6.89 g, 42.5 mmol) was added to the solution of acid (*R*)-**38** (10 g, 35.4 mmol) in acetonitrile (100 mL) and the resulting mixture was stirred at 60°C for 1 h then methanol (20 mL) was added. Stirring was continued for 2 h, then the solvent was removed under reduced pressure and the formed residue was treated with water, filtered off, washed with cold water and dried *in vacuo* furnishing the desired product (*R*)-**45** in good yield (7.5 g, 71%). Following the third approach, thionyl chloride (4.81 mL, 65.9 mmol) was added dropwise (approximately for 10 min) to the ice cooled solution of acid (*R*)-**38** (15.5 g, 54.9 mmol) in methanol (150 mL). The resulting mixture was refluxed for 4 h. The solvent was removed under reduced pressure and the formed residue was dissolved in chloroform (200 mL), washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL), dried over Na_2SO_4, then the solvent was evaporated *in vacuo* and the residue was treated with ether. The precipitate was filtered off, washed with ether and dried in air to afford 12.5 g (77%) of (*R*)-**45**. MS (ESI) $[M+H]^+$ 297. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (t, J = 8.8 Hz, 1H), 6.59 (brs, 1H), 6.39 (dd, J_1 = 8.8 Hz, J_1 = 2.4 Hz, 1H), 6.20 (dd, J_1 = 14.4 Hz, J_1 = 2.4 Hz, 1H), 4.80 (brs, 1H), 4.19 (d, J = 9.6 Hz, 1H), 4.06 (m, 2H), 4.00 (d, J = 9.6 Hz, 1H), 3.77 (s, 3H), 3.01 (d, J = 4.4 Hz, 3H), 2.70 (m, 1H), 2.29 (m, 1H).

The mixture of acid (*R*)-**45** (4.44 g, 15 mmol), isothiocyanate **42** (6.85 g, 30 mmol), DMSO (1.1 mL) and ethyl acetate (5.9 mL) was stirred in a closed vessel at 85 °C for 48 h (till the absence of initial (*R*)-**45** by LC-MS control). After the reaction was completed, the solution was cooled to rt, the solvent was removed under reduced pressure and the product was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH = 60:1) providing 5.09 g (69%) of pure (*R*)-**6**. MS (ESI) $[M+H]^+$ 493. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (t, J = 8.4 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 1.6 Hz, 1H), 7.85 (dd, J_1 = 8.4 Hz, J_1 = 1.6 Hz, 1H), 7.33 (dd, J_1 = 8.4 Hz, J_1 = 1.6 Hz, 1H), 7.24 (dd, J_1 = 11.6 Hz, J_1 = 2.4 Hz, 1H), 6.73 (m, 1H), 4.42 (d, J = 10.4 Hz, 1H), 4.16 (d, J = 10.4 Hz, 1H), 3.96 (m, 1H), 3.75 (m, 1H), 3.09 (d, J = 4.8 Hz, 3H), 2.73 (m, 1H), 2.48 (m, 1H). ESIHRMS m/z calcd for $C_{22}H_{17}F_4N_4O_3S$ $[M+H]^+$ 493.0952; found 493.0947. A brief X-Ray description of (*R*)-**6**: monoclinic syngony, space group $P2_1$, a = 8.4706 (17) Å, b = 14.507 (3) Å, c = 17.925 (3) Å, β = 92.191 (5)°, V = 2201.0 (8) Å^3, Z = 4, T = 120 K, μ(MoKα) = 0.214 mm^{-1}, $Dcalc$ = 1.486 g/mm^3, 19,657 reflections measured (2Θ ≤ 54), 9431 independent (R_{int} = 0.0637). Final divergence factors: R_1 = 0.0603 (I > 2σ (I)), wR_2 = 0.1271 (all data). Detailed structural information, including tables of bond lengths and 3D-representations is presented in SI.

4.1.1.10. Methyl 4-{(5R)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluorobenzoate ((R)-7). The mixture of (*R*)-**36a** (85 mmol), 4-bromo-2-fluorobenzoic acid **46** (15.5 g, 71 mmol) [40], K_2CO_3 (39.3 g, 284 mmol), CuI (2.02 g, 10.6 mmol) and 2-acetylcyclohexanone (2 g, 14.3 mmol) dissolved in DMF (150 mL) and water (35 mL) was stirred for 48 h at 100°C. After the reaction was completed the mixture was rotovapped, the residue was treated with water, acidified to pH 2–3 with hydrochloric acid and the solvent was removed under reduced pressure. The obtained crude ***(3R)-3-[(4-carboxy-3-fluorophenyl)amino]-tetrahydrofuran-3-carboxylic acid*** **((*R*)-47)** (MS (ESI) 270 $[M+H]^+$) was further used without any purification. Thionyl chloride (13 mL, 177 mmol) was added dropwise to a cooled solution of (*R*)-**47** in methanol (150 mL) and the resulting mixture was refluxed for 15 h. After the reaction was completed, the solution was cooled to rt, solvent was evaporated *in vacuo*, the formed residue was treated with ethyl acetate (200 mL) and water (100 mL), then the solution was basified with $NaHCO_3$ (8.95 g, 107 mmol). The organic layer was separated, rotovapped and subjected to column chromatography on silica gel (CH_2Cl_2) to

afford 12.2 g (58%) of ***methyl (3R)-3-{[3-fluoro-4-(methoxycarbonyl)phenyl]amino}-tetrahydrofuran-3-carboxylate*** **((*R*)-48)**. MS (ESI) $[M+H]^+$ 298. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (t, J = 8.8 Hz, 1H), 6.32 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.21 (dd, J_1 = 13.6 Hz, J_2 = 2.4 Hz, 1H), 4.82 (brs, 1H), 4.18 (d, J = 9.4 Hz, 1H), 4.07 (m, 2H), 4.00 (d, J = 9.4 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 2.71 (m, 1H), 2.29 (m, 1H).

The mixture of compound (*R*)-**48** (12.2 g, 41 mmol), **42** (18.7 g, 82 mmol), DMSO (2.9 mL) and ethyl acetate (16 mL) was stirred for 48 h at 85 °C. After cooling the mixture was rotovapped and subjected to column chromatography on silica gel (CH_2Cl_2). After crystallization from methanol the final product (*R*)-**7** was obtained in good yield (6.9 g, 34%); MS (ESI) $[M+H]^+$ 494. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (t, J = 8.0 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.97 (d, J = 2.9 Hz, 1H), 7.85 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.27 (m, 2H), 4.42 (d, J = 10.2 Hz, 1H), 4.15 (d, J = 10.2 Hz, 1H), 3.99 (s, 3H), 3.96 (m, 1H), 3.76 (m, 1H), 2.73 (m, 1H), 2.47 (m, 1H). ESIHRMS *m*/*z* calcd for $C_{22}H_{16}F_4N_3O_4S$ $[M+H]^+$ 494.0792; found 494.0792.

4.1.1.11. 4-{(5R)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluorobenzoic acid ((R)-8). NaOH (16.5 mg, 0.41 mmol) in water (2 mL) was added to the solution of compound (*R*)-**7** (200 mg, 0.41 mmol) in methanol (2 mL). The resulting mixture was stirred for 12 h and then the solvent was evaporated under reduced pressure. The residue was treated with water (10 mL) and acidified to pH = 3 with hydrochloric acid. The formed precipitate was filtered off, washed with water and dried *in vacuo* to give 179 mg (92%) of (*R*)-**8**. MS (ESI) $[M+H]^+$ 490. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.53 (brs, 1H), 8.40 (d, J = 8.4 Hz, 1H), 8.26 (s, 1H), 8.08 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 8.03 (t, J = 8.4 Hz, 1H), 7.57 (dd, J_1 = 11.2 Hz, J_2 = 1.2 Hz, 1H), 7.48 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 4.43 (d, J = 10.8 Hz, 1H), 3.94 (d, J = 10.8 Hz, 1H), 3.75 (q, J = 8.0 Hz, 1H), 3.53 (q, J = 8.0 Hz, 1H), 2.58 (t, J = 7.2 Hz, 2H).

4.1.1.12. 4-{(5R)-3-[4-cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-7-oxa-1,3-diazaspiro[4.4]non-1-yl}-2-fluorobenzamide ((R)-9). The mixture of compound (*R*)-**8** (179 mg, 0.37 mmol), EDAC (107 mg, 0.55 mmol), HOBt (75 mg, 0.55 mmol), ammonium chloride (26 mg, 0.48 mmol) and TEA (52 μL, 0.37 mmol) dissolved in dry DMF (2 mL) was stirred for 24 h. The solvent was removed *in vacuo*, the residue was dissolved in DCM, washed with 10% sodium carbonate solution, dried over Na_2SO_4, the solvent was removed and the obtained product was subjected to HPLC to afford 86 mg (48%) of (*R*)-**9**. MS (ESI) $[M+H]^+$ 489. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 1.6 Hz, 1H), 8.08 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.92 (s, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.53 (dd, J_1 = 10.8 Hz, J_2 = 1.6 Hz, 1H), 7.43 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 4.42 (d, J = 10.6 Hz, 1H), 3.94 (d, J = 10.6 Hz, 1H), 3.75 (m, 1H), 3.53 (m, 1H), 2.58 (m, 2H). ESIHRMS *m*/*z* calcd for $C_{21}H_{15}F_4N_4O_3S$ $[M+H]^+$ 479.0796; found 479.0793.

*4.1.1.13. 4-{2-Benzhydryl-7-[4-cyano-3-(trifluoromethyl)phenyl]-8-oxo-6-thioxo-2,5,7-triazaspiro[3.4]oct-5-yl}-2-fluoro-N-methylbenzamide (**10a**).* The mixture of 1-benzhydrylazetidin-3-one (**49**) (21.4 g, 90 mmol) [37], KCN (6.5 g, 0.1 mol) and $(NH_4)_2CO_3$ (19.2 g, 0.2 mol) dissolved in water (80 mL) and ethanol (150 mL) was stirred for 72 h in a closed vessel at 80°C. After cooling the mixture was rotovapped, the residue was treated with water, filtered off, washed with cold water, then with methanol and dried in air to provide 23.5 g (85%) of ***2-benzhydryl-2,5,7-triaza-spiro[3.4]octane-6,8-dione*** **(50)**. MS (ESI) $[M+H]^+$ 308. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 8.57 (s, 1H), 7.43 (d, J = 7.6 Hz, 4H), 7.29 (t, J = 7.6 Hz, 4H), 7.18 (t, J = 7.2 Hz, 2H), 4.48 (s, 1H), 3.40 (d, J = 8.2 Hz, 2H), 3.20 (d, J = 8.2 Hz, 2H). The mixture of compound **50** (23 g, 75 mmol) and KOH (21 g, 0.375 mol) dissolved in water (115 mL) was refluxed for 48 h. The cooled solution was neutralized with citric acid, the formed precipitate was filtered off, washed with cold water, with ether and dried *in vacuo* to afford 18 g (85%) of ***3-amino-1-benzhydrylazetidine-3-carboxylic acid*** **(51)**. MS (ESI) $[M+H]^+$ 283. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (d, J = 7.6 Hz, 4H), 7.27 (t, J = 7.2 Hz, 4H), 7.17 (t, J = 7.2 Hz, 2H), 4.56 (s, 1H), 3.45 (d, J = 8.0 Hz, 2H), 3.13 (d, J = 8.0 Hz, 2H). The mixture of compound **51** (2.54 g, 9 mmol), compound **44** (2.32 g, 10 mmol), K_2CO_3 (3.45 g, 25 mmol), CuI (0.19 g, 1 mmol) and 2-acetylcyclohexanone (0.14 g, 1 mmol) dissolved in DMF (40 mL) and water (6 mL) was stirred for 48 h in a closed vessel at 105°C. The cooled mixture was rotovapped, diluted with water and extracted with ethyl acetate. The water layer was acidified with citric acid (10%), the formed precipitate was filtered off, washed with water and dried *in vacuo* to provide 2.73 g (70%) of ***1-benzhydryl-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]azetidine-3-carboxylic acid*** **(52)**. MS (ESI) $[M+H]^+$ 434. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (t, J = 6.4 Hz, 1H), 7.44 (m, 6H), 7.28 (t, J = 9.6 Hz, 4H), 7.19 (m, 2H), 6.21 (d, J = 12.0 Hz, 1H), 6.02 (d, J = 18.8 Hz, 1H), 4.58 (s, 1H), 3.60 (d, J = 10.4 Hz, 2H), 3.22 (d, J = 10.4 Hz, 2H), 2.72 (d, J = 5.6 Hz, 3H). The mixture of compound **52** (2.17 g, 5 mmol) and CDI (973 mg, 6 mmol) dissolved in acetonitrile (20 mL) was stirred for 1 h, then methanol (0.43 g, 15 mmol) was added and the solution was stirred for 24 h at 50°C. The solvent was evaporated under reduced pressure, the formed residue was treated with K_2CO_3 (5%), the precipitate was filtered off, washed with cold water and dried *in vacuo* to afford 1.79 g (80%) of ***methyl 1-benzhydryl-3-[3-fluoro-4-(methylcarbamoyl)phenylamino] azetidine-3-carboxylate*** **(53)**. MS (ESI) $[M+H]^+$ 448. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (m, 6H), 7.29 (m, 5H), 7.19 (m, 2H), 6.20 (d, J = 11.6 Hz, 1H), 6.04 (d, J = 18.8 Hz, 1H), 4.59 (s, 1H), 3.67 (s, 3H), 3.65 (d, J = 10.6 Hz, 2H), 3.25 (d, J = 10.6 Hz, 2H), 2.74 (d, J = 6.0 Hz, 3H). The mixture of compound **53** (224 mg, 0.5 mmol) and compound **42** (228 mg, 1 mmol) dissolved in DMSO (2 mL) and ethyl acetate (5 mL) was stirred for 20 h at 90°C. The cooled mixture was diluted with ethyl acetate (20 mL), washed with saturated NH_4Cl solution, dried over Na_2SO_4, rotovapped and subjected to HPLC to obtain 32 mg (10%) of **10a**. MS (ESI) $[M+H]^+$ 644. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (t, J = 8.0 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 12.0 Hz, 1H), 7.81 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.25 (m, 4H), 7.20 (m, 6H), 6.85 (q, J = 4.4 Hz, 1H), 4.40 (brs, 1H), 3.76 (m, 2H), 3.60 (m, 2H), 3.15 (d, J = 4.4 Hz, 3H). ESIHRMS *m*/*z* calcd for $C_{34}H_{26}F_4N_5O_2S$ $[M+H]^+$ 644.1738; found 644.1730.

4.1.1.14. 4-{7-[4-Cyano-3-(trifluoromethyl)phenyl]-2-methyl-8-oxo-6-thioxo-2,5,7-triazaspiro[3.4]oct-5-yl}-2-fluoro-N-methylbenzamide (10b, unsuccessful attempts to synthesize)

4.1.1.14.1. Procedure A. The solution of compound **53** (32 mg, 0.05 mmol) in methanol (15 mL) was stirred under hydrogen atmosphere with 100 mg of 10% Pd/C. No reaction was observed, as well as upon hydrogenation with hydrochloric acid or in acetic acid.

4.1.1.14.2. Procedure B. The mixture of compound **52** (1.734 g, 4 mmol), acetic acid (0.48 mL, 8 mmol) and Pd/C (0.1 g, 10%) dissolved in methanol (50 mL) was stirred for 12 h under hydrogen atmosphere. The solution was filtered through celite and rotovapped. The residue was treated with ether, filtered off and dried to afford 855 mg (80%) of ***3-[3-fluoro-4-(methylcarbamoyl)phenylamino]azetidine-3-carboxylic acid*** **(54)**. MS (ESI) $[M+H]^+$ 268. ^{1}H NMR (400 MHz, D_2O) δ 7.51 (t, J = 8.6 Hz, 1H), 6.26 (dd, J_1 = 8.6 Hz, J_2 = 2.2 Hz, 1H), 6.15 (dd, J_1 = 13.8 Hz, J_2 = 2.2 Hz, 1H), 4.56 (d, J = 11.4 Hz, 2H), 4.11 (d, J = 11.4 Hz, 2H), 2.84 (s, 3H). The mixture of compound **54** (802 mg, 3 mmol) and formalin (0.5 mL) dissolved in aqueous methanol (50 mL, 50% v/v) was stirred for 0.5 h, then Pd/C (0.3 g, 10%) was added and stirring was continued for 12 h. The

solution was filtered through celite and rotovapped. The residue was treated with ether, filtered off and dried to afford 717 mg (85%) of ***3-[3-fluoro-4-(methylcarbamoyl)phenylamino]-1-methylazetidine-3-carboxylic acid* (55)**. MS (ESI) $[M+H]^+$ 282. ^1H NMR (400 MHz, D_2O) δ 7.51 (t, $J = 8.6$ Hz, 1H), 6.27 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.0$ Hz, 1H), 6.20 (dd, $J_1 = 13.8$ Hz, $J_2 = 2.0$ Hz, 1H), 4.68 (d, $J = 11.4$ Hz, 2H), 4.23 (d, $J = 11.4$ Hz, 2H), 3.78 (s, 3H), 2.82 (s, 3H). The mixture of compound **55** (281 mg, 1 mmol) and CDI (195 mg, 1.2 mmol) dissolved in DMF (5 mL) was stirred for 1 h at rt. Then, methanol (0.405 mL, 10 mmol) was added and the mixture was stirred for 24 h at 50°C. The solvent was removed *in vacuo*, the precipitate was dissolved in ethyl acetate, washed with K_2CO_3 (5%), dried over Na_2SO_4 and the solvent was removed providing ***methyl 3-[3-fluoro-4-(methylcarbamoyl)phenylamino]-1-methylazetidine-3-carboxylate* (56)** with good yield (236 mg, 80%). MS (ESI) $[M+H]^+$ 296. ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (t, $J = 8.0$ Hz, 1H), 6.60 (m, 1H), 6.29 (dd, $J_1 = 11.2$ Hz, $J_2 = 2.4$ Hz, 1H), 6.09 (dd, $J_1 = 15.6$ Hz, $J_2 = 2.4$ Hz, 1H), 5.04 (s, 1H), 3.85 (d, $J = 10.6$ Hz, 2H), 3.77 (s, 3H), 3.42 (d, $J = 10.6$ Hz, 2H), 3.00 (d, $J = 6.4$ Hz, 3H), 2.46 (s, 3H). The same procedure was applied for the reaction of compound **56** with isothiocyanate **42** as for conversion of (*R*)-**45** to (*R*)-**6** described above, there was no product **10b** detected in the reaction mixture.

4.1.1.15. 4-{7-[4-Cyano-3-(trifluoromethyl)phenyl]-8-oxo-6-thioxo-2-oxa-5,7-diazaspiro[3.4]oct-5-yl]-2-fluoro-N-methylbenzamide (10c, synthesis was unsuccessful). The mixture of compound **40** (500 mg, 1.22 mmol), 3-aminooxetane-3-carboxylic acid (**57**) (215 mg, 1.84 mmol) [38], K_2CO_3 (590 mg, 4.27 mmol), CuI (47 mg, 0.24 mmol) and 1 drop of Et_3N dissolved in DMF (4 mL) and water (1 mL) was stirred for 15 min, then 2-acetylcyclohexanone (190 mg, 1.35 mmol) was added and the mixture was stirred for 15 h at 100°C. After cooling the mixture was rotovapped and subjected to HPLC with neutral mobile phase to afford 340 mg (70%) of ***3-(3-fluoro-4-{methyl-[2-(trimethylsilyl)ethoxymethyl]carbamoyl}phenylamino)oxetane-3-carboxylic acid* (58)**. MS (ESI) $[M+H]^+$ 399. ^1H NMR (400 MHz, DMSO-d_6) δ 7.61 (m, 1H), 7.53 (t, $J = 8.8$ Hz, 1H), 6.21 (d, $J = 8.4$ Hz, 1H), 5.96 (d, $J = 13.6$ Hz, 1H), 4.97 (d, $J = 6.0$ Hz, 2H), 4.55 (d, $J = 6.0$ Hz, 2H), 2.73 (d, $J = 4.4$ Hz, 3H), 2.21 (m, 2H), 1.12 (m, 2H), 0.14 (s, 9H). The mixture of compound **40** (500 mg, 1.22 mmol), 3-aminooxetane-3-carboxylic acid (**57**) (215 mg, 1.84 mmol),[38] K_2CO_3 (590 mg, 4.27 mmol), CuI (47 mg, 0.24 mmol) and 1 drop of Et_3N dissolved in DMF (4 mL) and water (1 mL) was stirred for 15 min, then 2-acetylcyclohexanone (190 mg, 1.35 mmol) was added and the mixture was stirred for 15 h at 100°C. After cooling the mixture was acidified with hydrochloric acid to pH = 3 and extracted with ethyl acetate. The organic layer was washed with cold water, with brine, dried over Na_2SO_4 and the solvent evaporated under reduced pressure to give 287 mg (88%) of ***3-[3-fluoro-4-(methylcarbamoyl)phenylamino]oxetane-3-carboxylic acid* (59)**. MS (ESI) $[M+H]^+$ 269. ^1H NMR (400 MHz, DMSO-d_6), δ 7.69 (m, 1H), 7.48 (t, $J = 8.8$ Hz, 1H), 6.15 (d, $J = 8.4$ Hz, 1H), 5.96 (d, $J = 13.6$ Hz, 1H), 4.97 (d, $J = 6.0$ Hz, 2H), 4.55 (d, $J = 6.0$ Hz, 2H), 2.73 (d, $J = 4.4$ Hz, 3H). Sodium hydride (66 mg, 1.65 mmol, 60% in oil) was added at 0°C to the solution of compound **58** (600 mg, 1.5 mmol) in DMF (6 mL). The resulting mixture was stirred for 0.5 h keeping same temperature. After the reaction was completed, iodomethane (0.14 mL, 2.25 mmol) was added to the mixture and stirring was continued for 6 h at 0°C. Finally, the solution was accurately poured into cold water, extracted with ethyl acetate, dried over Na_2SO_4 and rotovapped. The obtained crude ***methyl 3-(3-fluoro-4-{methyl-[2-(trimethylsilyl)ethoxymethyl]carbamoyl}phenylamino)oxetane-3-carboxylate* (60)** was subjected to HPLC with mobile phase containing 0.1% of TFA to afford 46 mg (11%) of ***methyl 3-[3-fluoro-4-(methylcarbamoyl)phenylamino]oxetane-3-carboxylate* (61)**. MS (ESI) $[M+H]^+$ 283. ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (t, $J = 9.2$ Hz, 1H), 6.60 (brs, 1H), 6.31 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.0$ Hz, 1H), 6.12 (dd, $J_1 = 14.4$ Hz, $J_2 = 2.0$ Hz, 1H), 5.13 (d, $J = 6.4$ Hz, 2H), 5.03 (brs, 1H), 4.76 (d, $J = 6.4$ Hz, 2H), 3.84 (s, 3H), 3.01 (d, $J = 4.4$ Hz, 3H). Compound **42** (63 mg, 0.276 mmol) was added to the solution of compound **59** or **61** (0.25 mmol) in DMF (1 mL). The resulting mixture was stirred for 12 h at 80°C under argon atmosphere. After the reaction was completed, the solution was poured into cold water, extracted with ethyl acetate, dried over Na_2SO_4 and rotovapped. Separation by HPLC afforded 17 mg (16%) of N-[4-cyano-3-(trifluoromethyl) phenyl]-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]oxetane-3-carboxamide **(62)**. MS (ESI) $[M+H]^+$ 437. ^1H NMR (400 MHz, DMSO-d_6) δ 10.59 (s, 1H), 8.30 (s, 1H), 8.16 (d, $J = 8.8$ Hz, 1H), 8.08 (d, $J = 8.4$ Hz, 1H), 7.73 (m, 1H), 7.55 (s, 1H), 7.49 (t, $J = 8.4$ Hz, 1H), 6.23 (d, $J = 10.0$ Hz, 1H), 6.10 (d, $J = 13.6$ Hz, 1H), 5.09 (d, $J = 6.8$ Hz, 2H), 4.59 (d, $J = 6.4$ Hz, 2H), 2.70 (d, $J = 4.0$ Hz, 3H).

*4.1.1.16. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide (**12**).* The mixture of compound **37** (279 mg, 1 mmol), glycine (80 mg, 1.07 mmol), K_2CO_3 (207 mg, 1.5 mmol) and CuI (19 mg, 0.1 mmol) dissolved in DMF (3 mL) was kept in microwave reactor for 20 min at 140 °C. After cooling the mixture was diluted with 10 mL of ethyl acetate and 10 mL of water, neutralized with hydrochloric acid to pH = 2–3 and the product was extracted with 5 × 20 mL of ethyl acetate. The extracts were washed with brine, dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. The product was then subjected to HPLC providing 113 mg (50%) of ***[3-fluoro-4-(methylcarbamoyl)phenylamino]acetic acid* (67)**. MS (ESI) $[M+H]^+$ 227. The mixture of compound **67** (113 mg, 0.5 mmol) and **42** (174 mg, 1.0 mmol) dissolved in DMF (2 mL) was stirred for 12 h at 90 °C. The cooled solution was then rotovapped and subjected to HPLC to give 86 mg (39%) of compound **12**. MS (ESI) $[M+H]^+$ 437. ^1H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, $J = 8.4$ Hz, 1H), 8.32 (m, 1H), 8.17 (s, 1H), 7.99 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.4$ Hz, 1H), 7.86 (dd, $J_1 = 12.0$ Hz, $J_2 = 1.4$ Hz, 1H), 7.75 (m, 2H), 4.95 (s, 2H), 2.79 (d, $J = 4.4$ Hz, 3H). ESIHRMS *m/z* calcd for $C_{19}H_{13}F_4N_4O_2S$ $[M+H]^+$ 437.0690; found 437.0686.

4.1.1.17. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-5-methyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamides ((R,S)-13, (R)-13, (S)-13). D,L-, D- or L-alanine (**69**) (638 mg, 7.16 mmol), Cs_2CO_3 (2.337 g, 7.17 mmol), CuI (68 mg, 0.358 mmol) and *N*,*N*-dimethylglycine (74 mg, 0.717 mmol) were added to the solution of compound **37** (1 g, 3.58 mmol) in DMSO (2.5 mL). The resulting mixture was stirred for 20 h at 90°C. After cooling the mixture was freeze-dried, the residue was extracted with ethanol and the extract was subjected to HPLC to obtain 413–450 mg (24–26%) of ***2-[3-fluoro-4-(methylcarbamoyl)phenylamino]propionic acid* ((*R,S*)-69, (*R*)-69, (*S*)-69). (*R,S*)-69**: MS (ESI) $[M+H]^+$ 241. ^1H NMR (400 MHz, DMSO-d_6) δ 12.66 (brs, 1H), 7.62 (m, 1H), 7.45 (t, $J = 8.8$ Hz, 1H), 6.67 (d, $J = 7.2$ Hz, 1H), 6.42 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.0$ Hz, 1H), 6.29 (dd, $J_1 = 14.8$ Hz, $J_2 = 2.0$ Hz, 1H), 4.03 (m, 1H), 2.73 (d, $J = 4.4$ Hz, 3H), 1.37 (d, $J = 7.2$ Hz, 3H). Compound **42** (104 mg, 0.456 mmol) was added to the solution of (*R,S*)-**69**, (*R*)-**69** or (*S*)-**69** (100 mg, 0.416 mmol) in DMF (1 mL). The resulting mixture was stirred for 18 h at 80°C, then the solvent was removed under reduced pressure and the product was subjected to HPLC to afford 28–37 mg (15–20%) of (*R,S*)-**13**, (*R*)-**13** or (*S*)-**13**. (*R,S*)-**13**: MS (ESI) $[M+H]^+$ 451. ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (t, $J = 8.6$ Hz, 1H), 8.01 (d, $J = 8.0$ Hz, 1H), 7.94 (d, $J = 1.2$ Hz, 1H), 7.81 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 7.48 (dd, $J_1 = 12.4$ Hz, $J_2 = 1.6$ Hz, 1H), 7.36 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H), 6.72 (m, 1H), 4.83 (q, $J = 7.2$ Hz, 1H), 3.08 (d, $J = 4.8$ Hz, 3H), 1.60 (d, $J = 7.2$ Hz, 3H). ESIHRMS *m/z*

calcd for $C_{20}H_{15}F_4N_4O_2S$ $[M+H]^+$ 451.0846; found 451.0844.

4.1.1.18. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-5-(hydroxymethyl)-5-methyl-4-oxo-2-thioxo-imidazolidin-1-yl}-2-fluoro-N-methylbenzamide ((R,S)-17). BBr_3 (53 μL, 0.55 mmol) was added to the solution of compound (*R,S*)-**14** (55 mg, 0.11 mmol) in CH_2Cl_2 (1.5 mL) under argon atmosphere at − 78°C. The resulting mixture was stirred for 3 h at − 78°C then for 3 h at rt, quenched with 10 mL of 5% sodium carbonate solution, extracted with ethyl acetate, dried over Na_2SO_4 and rotovapped. HPLC afforded 15 mg (28%) of (*R,S*)-**17** MS (ESI) $[M+H]^+$ 481. ^1H NMR (400 MHz, DMSO-d_6) δ 8.43 (m, 1H), 8.39 (d, $J = 8.4$ Hz, 1H), 8.13 (s, 1H), 7.98 (d, $J = 8.4$ Hz, 1H), 7.78 (t, $J = 8.0$ Hz, 1H), 7.42 (d, $J = 10.8$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 5.93 (t, $J = 4.4$ Hz, 1H), 3.81 (dd, $J_1 = 11.6$ Hz, $J_2 = 4.4$ Hz, 1H), 3.45 (dd, $J_1 = 11.6$ Hz, $J_2 = 5.0$ Hz, 1H), 2.79 (d, $J = 4.0$ Hz, 3H), 1.38 (s, 3H). ESIHRMS *m/z* calcd for $C_{21}H_{17}F_4N_4O_3S$ $[M+H]^+$ 481.0952; found 481.0951.

4.1.1.19. {1-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[3-fluoro-4-(methylcarbamoyl)phenyl]-4-methyl-5-oxo-2-thioxoimidazolidin-4-yl}acetic acid ((R,S)-18). NaOH (7 mg, 0.172 mmol) in water (0.5 mL) was added to the solution of compound (*R,S*)-**16** (46 mg, 0.086 mmol) in ethanol (2.5 mL). The resulting mixture was stirred for 12 h and rotovapped. Then isopropanol (2 mL) and hydrochloric acid (15 μL, 0.172 mmol) were added, the solution was filtered, rotovapped and subjected to HPLC to give 15 mg (38%) of (*R,S*)-**18**. MS (ESI) $[M+H]^+$ 469. ^1H NMR (400 MHz, DMSO-d_6) δ 13.31 (brs, 1H), 8.44 (m, 2H), 8.10 (s, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 7.81 (t, $J = 8.0$ Hz, 1H), 7.25 (d, $J = 10.8$ Hz, 1H), 7.19 (d, $J = 8.0$ Hz, 1H), 3.16 (d, $J = 17.6$ Hz, 1H), 2.79 (d, $J = 3.6$ Hz, 3H), 2.70 (d, $J = 17.6$ Hz, 1H), 1.59 (s, 3H). ESIHRMS *m/z* calcd for $C_{22}H_{17}F_4N_4O_4S$ $[M+H]^+$ 509.0901; found 509.0897.

*4.1.1.20. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-5,5-bis(methoxymethyl)-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide (**19**).* The mixture of 2-fluoro-4-iodo-*N*-methylbenzamide (**37**) (371 mg, 0.91 mmol), 3-methoxy-2-(methoxymethyl)alanine (222 mg, 1.36 mmol) [39], CuI (35 mg, 0.184 mmol), 2-acetylcyclohexanone (24 μL, 0.184 mmol) and dry K_3PO_4 (577 mg, 2.72 mmol) in DMF (3 mL) was stirred for 48 h at 100°C. After cooling the mixture was neutralized with acetic acid (163 μL, 2.72 mmol), diluted with water and extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The obtained ***2-(3-fluoro-4-{methyl[2-(trimethylsilyl)ethoxymethyl]carbamoyl}phenylamino)-3-methoxy-2-(methoxymethyl)propanoic acid*** **(40)** (MS (ESI) 445 $[M+H]^+$) was dissolved in DMF (3 mL) and pyridine (3 mL), then compound **42** (414 mg, 0.276 mmol) was added and the resulting mixture was stirred for 36 h at 90°C. After cooling the solution was rotovapped and ***4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-bis(methoxymethyl)-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methyl-N-[2-(trimethylsilyl)ethoxymethyl]benzamide*** **(71)** was subjected to HPLC. The isolated product was dissolved in DCM (3 mL) and TFA (3 mL), the solution was then stirred for 2 h and rotovapped. Column chromatography on silica gel ($CHCl_3$/MeCN = 15:1) afforded 46 mg of **19**. MS (ESI) $[M+H]^+$ 525. ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (t, $J = 8.4$ Hz, 1H), 7.98 (d, $J = 8.4$ Hz, 1H), 7.90 (d, $J = 1.2$ Hz, 1H), 7.79 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 7.33 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz, 1H), 7.25 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 6.71 (q, $J = 4.4$ Hz, 1H), 3.70 (d, $J = 9.8$ Hz, 2H), 3.43 (s, 6H), 3.35 (d, $J = 9.8$ Hz, 2H), 3.08 (d, $J = 4.4$ Hz, 3H). ESIHRMS *m/z* calcd for $C_{23}H_{21}F_4N_4O_4S$ $[M+H]^+$ 525.1214; found 525.1211.

*4.1.1.21. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-4-oxo-2-thioxo-tetrahydropyrimidin-1(2H)-yl}-2-fluoro-N-methylbenzamide (**20**).* DBU (0.81 g, 5.4 mmol) was added to the solution of compound **22** (9 g, 53.6 mmol) and ethyl acrylate (8 g, 80 mmol) in DMSO (90 mL). The resulting mixture was stirred for 24 h at 70°C, then freeze-dried and the residue was crystallized from 1:1 ethanol/water to obtain 5.46 g (38%) of ***ethyl 3-[3-fluoro-4-(methylcarbamoyl)phenylamino]propionate*** **(72)**. MS (ESI) $[M+H]^+$ 269. ^1H NMR (400 MHz, DMSO-d_6) δ 7.57 (brs, 1H), 7.48 (t, $J = 8.8$ Hz, 1H), 6.47 (brs, 1H), 6.42 (d, $J = 8.8$ Hz, 1H), 6.33 (d, $J = 14.8$ Hz, 1H), 4.07 (q, $J = 7.2$ Hz, 2H), 3.32 (m, 2H), 2.73 (d, $J = 4.4$ Hz, 3H), 2.55 (t, $J = 6.4$ Hz, 2H), 1.18 (t, $J = 7.2$ Hz, 3H). The mixture of compound **42** (320 mg, 1.51 mmol) and **72** (404 mg, 1.51 mmol) dissolved in DMF (8 mL) was kept in microwave reactor for 8 h at 60°C. The solvent was evaporated *in vacuo*, and the residue was subjected to column chromatography on silica gel (hexane/ethyl acetate = 1:2) to give 200 mg (27%) of ***ethyl 3-{3-[4-cyano-3-(trifluoromethyl)phenyl]-1-[3-fluoro-4-(methylcarbamoyl)phenyl]-thioureido}propionate*** **(73)**. MS (ESI) $[M+H]^+$ 497. The solution of NaOH (32 mg, 0.8 mmol) in water (0.25 mL) was added to the solution of compound **73** (200 mg, 0.4 mmol) in ethanol (1 mL). The resulting mixture was stirred for 2 h at 80°C. The cooled solution was neutralized with hydrochloric acid (69 μL, 0.8 mmol) and rotovapped. The residue was extracted with hot isopropanol and the solvent was removed under reduced pressure to afford 110 mg (59%) of ***3-{3-[4-cyano-3-(trifluoromethyl)phenyl]-1-[3-fluoro-4-(methylcarbamoyl)phenyl]-thioureido}-propionic acid*** **(74)**, MS (ESI) $[M+H]^+$ 469. TBTU (57 mg, 0.33 mmol) and DIPEA (96 mg, 0.74 mmol) were added to the solution of compound **74** (100 mg, 0.21 mmol) in DMF (1.5 mL). The resulting mixture was stirred for 15 h at 45°C. After the reaction was completed (LC-MS control), the mixture was poured into water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was removed by the standard procedure. Separation using HPLC afforded 17 mg (17%) of **20**. MS (ESI) $[M+H]^+$ 451. ^1H NMR (400 MHz, DMSO-d_6) δ 8.35 (q, $J = 4.4$ Hz, 1H), 8.27 (d, $J = 8.0$ Hz, 1H), 8.06 (d, $J = 1.6$ Hz, 1H), 7.83 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.6$ Hz, 1H), 7.71 (t, $J = 8.2$ Hz, 1H), 7.42 (dd, $J_1 = 11.0$ Hz, $J_2 = 1.8$ Hz, 1H), 7.33 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.13 (t, $J = 6.8$ Hz, 2H), 3.17 (t, $J = 6.8$ Hz, 2H), 2.78 (d, $J = 4.4$ Hz, 3H). ESIHRMS *m/z* calcd for $C_{20}H_{15}F_4N_4O_2S$ $[M+H]^+$ 451.0846; found 451.0843.

*4.1.1.22. 4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-thioureido}-2-fluoro-N-methylbenzamide (**21**).* The solution of compound **22** (0.34 g, 20 mmol) and **42** (0.46 g, 20 mmol) in DMF (1 mL) was stirred for 0.5 h in microwave reactor at 80 °C. The mixture was poured into water, the formed precipitate was filtered off, washed with water and dried *in vacuo*. Purification using column chromatography on silica gel (CH_2Cl_2/Et_3N = 20:1) afforded 0.3 g (44%) of **21**. MS (ESI) $[M+H]^+$ 397. ^1H NMR (400 MHz, $CDCl_3$) δ 10.67 (s, 1H), 10.65 (s, 1H), 8.29 (s, 1H), 8.15 (m, 1H), 8.09 (d, $J = 8.4$ Hz, 1H), 7.99 (d, $J = 8.0$ Hz, 1H), 7.62 (m, 2H), 7.32 (d, $J = 8.4$ Hz, 1H), 2.77 (d, $J = 4.0$ Hz, 3H). ESIHRMS *m/z* calcd for $C_{17}H_{13}F_4N_4OS$ $[M+H]^+$ 397.0741; found 397.0737.

4.1.2. General procedure 2. 4-(Cyanomethylamino)-2-fluoro-N-methylbenzamides 29–34

The mixture of compound **22** (336 mg, 2 mmol), corresponding ketone **23**–**28** (4 mmol), trimethylsilylcyanide (0.5 mL, 4 mmol) and ytterbium(III) (62 mg, 0.1 mmol) was stirred in a closed vessel for 12 h at 80 °C (36 h at 90 °C for 1-methylpiperidin-4-one). The cooled mixture was diluted with ethyl acetate, washed with cold water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification was performed using column chromatography on silica gel (hexane/EtOAc = 1:1).

*4.1.2.1. 4-(2-Cyano-1-methoxypropan-2-ylamino)-2-fluoro-N-methylbenzamide (**29**).* Yield: 413 mg (78%). MS (ESI) $[M+H]^+$ 266. ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, $J = 8.8$ Hz, 1H), 6.89 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz, 1H), 6.63 (m, 1H), 6.62 (dd, $J_1 = 14.4$ Hz, $J_2 = 2.4$ Hz, 1H), 4.77 (s, 1H), 3.69 (AB sys, $J = 9.4$ Hz, 1H), 3.62 (AB sys, $J = 9.4$ Hz, 1H), 3.51 (s, 3H), 3.01 (dd, $J_1 = 4.8$ Hz, $J_2 = 1.2$ Hz, 3H), 1.70 (s, 3H).

*4.1.2.2. 4-[1-(Benzyloxy)-2-cyanopropan-2-ylamino]-2-fluoro-N-methylbenzamide (**30**).* Yield: 506 mg (76%). MS (ESI) $[M+H]^+$ 342. ^1H NMR (400 MHz, DMSO-d_6) δ 7.78 (m, 1H), 7.54 (t, $J = 8.8$ Hz, 1H), 7.37 (m, 5H), 7.32 (m, 1H), 6.82 (s, 1H), 6.70 (d, $J = 8.4$ Hz, 1H), 6.60 (d, $J = 14.0$ Hz, 1H), 4.64 (m, 2H), 3.85 (d, $J = 9.2$ Hz, 1H), 3.60 (d, $J = 9.2$ Hz, 1H), 2.75 (d, $J = 4.4$ Hz, 1H), 1.67 (s, 3H).

*4.1.2.3. Ethyl 3-cyano-3-[3-fluoro-4-(methylcarbamoyl)phenylamino]butanoate (**31**).* Yield: 547 mg (89%). MS (ESI) $[M+H]^+$ 308. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (t, $J = 8.8$ Hz, 1H), 6.73 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H), 6.67 (dd, $J_1 = 14.4$ Hz, $J_2 = 2.0$ Hz, 1H), 6.63 (m, 1H), 5.14 (s, 1H), 4.23 (q, $J = 7.2$ Hz, 2H), 3.01 (d, $J = 4.4$ Hz, 3H), 3.00 (AB sys, $J = 15.6$ Hz, 1H), 2.95 (AB sys, $J = 15.6$ Hz, 1H), 1.84 (s, 3H), 1.29 (t, $J = 7.2$ Hz, 3H).

*4.1.2.4. 4-(3-Cyano-tetrahydrofuran-3-ylamino)-2-fluoro-N-methylbenzamide (**32**).* Yield: 305 mg (58%). MS (ESI) $[M+H]^+$ 264.

*4.1.2.5. 4-(4-Cyano-tetrahydro-2H-pyran-4-ylamino)-2-fluoro-N-methylbenzamide (**33**).* Yield: 338 mg (61%). MS (ESI) $[M+H]^+$ 278. ^1H NMR (400 MHz, DMSO-d_6) δ 7.79 (m, 1H), 7.55 (t, $J = 8.8$ Hz, 1H), 6.92 (s, 1H), 6.70 (d, $J = 8.8$ Hz, 1H), 6.60 (d, $J = 14.4$ Hz, 1H), 3.88 (m, 2H), 3.58 (m, 2H), 2.75 (d, $J = 4.0$ Hz, 3H), 2.35 (m, 2H), 1.86 (m, 2H).

*4.1.2.6. 4-(4-Cyano-1-methylpiperidin-4-ylamino)-2-fluoro-N-methylbenzamide (**34**).* Yield: 340 mg (59%). MS (ESI) $[M+H]^+$ 291. ^1H NMR (400 MHz, DMSO-d_6) δ 7.76 (m, 1H), 7.55 (t, $J = 8.8$ Hz, 1H), 6.83 (s, 1H), 6.69 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.0$ Hz, 1H), 6.58 (dd, $J_1 = 14.0$ Hz, $J_2 = 1.6$ Hz, 1H), 2.75 (d, $J = 4.4$ Hz, 3H), 2.34 (m, 2H), 2.22 (m, 2H), 2.20 (s, 3H), 1.94 (m, 1H), 1.83 (m, 2H), 1.73 (m, 1H). ESIHRMS *m/z* calcd for $C_{15}H_{19}FN_4O$ $[M+H]^+$ 291.1611; found 291.1616.

*4.1.2.7. 1-[4-Cyano-3-(trifluoromethyl)phenyl]-4-(hydroxymethyl)-2-thioxoimidazolidine-4-carboxylic acid (**63**).* Compound **42** (228 mg, 1 mmol) was added to the solution of compound **57** (117 mg, 1 mmol) in acetonitrile (3 mL). The resulting mixture was stirred for 18 h (LC-MS control). The solvent was removed following the standard procedure and the product was subjected to HPLC to give 159 mg (46%) of **63**. MS (ESI) $[M+H]^+$ 346. ^1H NMR (DMSO-d_6) δ 12.73 (brs, 1H), 10.35 (brs, 1H), 8.17 (s, 1H), 8.02 (d, $J = 8.0$ Hz, 1H), 5.17 (brs, 2H), 3.72 (t, $J = 11.2$ Hz, 2H), 3.62 (d, $J = 10.0$ Hz, 1H), 3.46 (d, $J = 11.6$ Hz, 1H). ^{13}C NMR (90 MHz, DMSO-d_6) δ 136.42, 132.40, 131.98, 131.56, 131.15, 124.37, 120.74, 116.13, 99.71, 64.65, 35.72.

4.2. In vitro biological activity

LNCaP cells (ATCC, catalogue no. CRL-1740) were propagated in RPMI-1640 (Invitrogen, catalogue no. 11,835-030) + 10% FCS (Fetal Calf Serum, Hyclone, catalogue no. SH300070.03) + 1%AAS (Sigma, catalogue no. 5955). Cultivated culture were plated into 96-well plates (BD, catalogue no. 354620) at 10,000 cells per well in 200 μL of RPMI 1640 + 10% CSS (Charcoal-stripped serum, Invitrogen, catalogue no. 12,676-011) + 1% AAS. Patterns were incubated for 3 days at 37 °C. After 3 days the culture medium was substituted with new one containing serial dilutions of the evaluated compounds in the absence (to estimate *agonism*) or presence (to estimate *antagonism*) of DHT. The final concentration of DHT was 1 nM and corresponded to EC_{80} value, while the final concentration of DMSO was 0.1%. Model cells were then incubated for 1 day at 37 °C. The related concentration of PSA (prostate-specific antigen) was then measured in the culture medium using ELISA (Vector-Best, catalogue no. T-8458) in full accordance with the procedure provided by manufacturer.

4.3. Pharmacokinetics

Male Sprague Dawley rats (250–350 g) obtained from the Animal Breeding Facility, Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow Region were used for pharmacokinetics studies. Compounds (*R*)-**6**, (*R*)-**7**, (*R*)-**8** and (*R*)-**9** were administered to animals at the dose of 1 mg/kg (iv) and at the dose of 5 mg/kg (po). For iv administration the compounds were dissolved in 20% HP-b-CD (hydroxypropylbetacyclodextran), while for po treatment — in 0.5% HPMC. Blood samples were taken in EDTA containing tubes 5, 15, 30 min, 1, 2, 4, 8 and 24 h after iv administration and 15, 30 min, 1, 2, 4, 8 and 24 h after po administration. Plasma samples (45 μL) were prepared by centrifugation of blood, then mixed with 5 μL of acetonitrile:H_2O (1:1 v/v) and precipitated with 150 μL of acetonitrile containing internal standard tolbutamide (50 ng/mL). Samples were incubated at − 20 °C for 15 min followed by centrifugation at 11,000 *g* for 10 min, then supernatants (130 μL) were transferred into a new plate for LC-MS/MS analysis. The analysis was performed using Agilent 1290 UPLC coupled with Qtrap5500 mass spectrometer (ABSciex, USA). The corresponding *m/z* values were: (*R*)-**6** — 493.1/380.0, (*R*)-**7** — 493.482/237.900, (*R*)-**8** — 480.2/224.0, (*R*)-**9** — 479/366. Pharmacokinetic parameters were calculated using non-compartmental analysis provided by the software WinNonlin Professional 5.2 (Pharsight Corporation).

4.4. In silico modeling

The 3D molecular docking study was performed in ICM Pro software (MolSoft).[30] All the *force-field* (FF) components available in the software, including H-bond capacity, dipole–dipole interactions and Van der Waals contacts, hydrophobic and–π-cationic interactions, were used for the model construction (grid size = 0.50 Å, map features: grid step = 0.50, dimensions: 55 × 51 × 43, size = 120,615) and further modeling. More than 10 different three-dimensional conformations were generated within the defined binding site with the potential energy close to the theoretical minimum. Binding energy (scoring) was calculated automatically for all the conformations predicted using the integral internal FF equation. Each "active" conformation within the pull was visually inspected and got the priority following the expert opinion. 2D supramolecular interface was constructed using MOE Software (Molecular Operating Environment, CCG) [42].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.039.

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