RSC Advances

PAPER

View Article Online
View Journal | View Issue



Cite this: *RSC Adv.*, 2017, **7**, 31866

# Novel thiohydantoin analogues bearing the 1-hydroxyl-2,2,2-trifluoro-1-ethyl moiety as androgen receptor inhibitors for the potential treatment of castration resistant prostate cancer†

Yingwei Wang, ‡[ab] Yufang Deng,‡[a] Xuehai Pang,[b] Jiang Yu,[a] Lei Fan,[c] Yuanwei Chen *[abc] and Lifeng Zhao*[d]

Received 21st February 2017
Accepted 31st May 2017

DOI: 10.1039/c7ra02142a

rsc.li/rsc-advances

Enzalutamide (ENT) is an approved drug for the treatment of castration resistant prostate cancer (CRPC). Despite its success, the duration of response in patients is still limited with drug resistance. More robust CRPC drugs with novel structural motifs are urgently needed. Here, we designed and synthesized a series of 1-hydroxyl or 1-amino-2,2,2-trifluoro-1-ethyl compounds as isosteres to replace the amide group of ENT. Among the compounds prepared and tested, compund **13b** is 2-fold more potent than ENT against LNCaP-AR cells. Western blot analysis showed that **13b** dose-dependently inhibits the expression of the prostate-specific antigen (PSA). Further in *vivo* efficacy studies established that **13b** has anti-tumor activity with oral administration at 15 mg kg$^{-1}$ once daily.



## 1 Introduction

Prostate cancer is the most commonly diagnosed cancer and the third-leading cause of cancer deaths in men.[1] Although approximately 75% of these patients will be cured by surgery or radiation when cancer remains within the prostate, the remainder will inevitably progress to a state of metastatic prostate cancer.[2] Androgen deprivation therapy or other combination therapy, through lowering serum testosterone or competitively blocking the binding of androgens to the androgen receptor (AR), are initially effective in 90% of patients at this stage.[3] Nevertheless, the vast majority of these patients will eventually develop metastatic castration resistant prostate cancer (CRPC) after long term treatments.[4–6] Recent research efforts have indicated that CRPC growth is driven by AR signaling as well.[7] Furthermore, many possible mechanisms influencing AR signaling have been suggested for the acquired resistance, among which AR mutations, androgen synthesis by prostate cancer cells, and over-expression of AR or AR co-activators have been found to directly or indirectly render AR more sensitive to low androgen concentrations or sometimes turn antagonist responses to agonistic.[8]

Enzalutamide (ENT) was approved by FDA in 2012 for the treatment of CRPC. Unlike the first-generation AR inhibitors, such as flutamide, nilutamide, and bicalutamide (Fig. 1), ENT not only binds to AR, but also reduces nuclear translocation of AR and impairs AR binding to DNA.[9] In addition, further studies suggested that ENT possesses a higher binding affinity of AR than the first-generation AR inhibitors. Clinical studies also demonstrated unequivocally its survival benefit in men with CRPC.[10,11] Despite its success, the duration of response in patients is still limited. Recently, AR with a specific mutation (F876L) was identified to confer resistance to ENT.[12] This disappointing outlook suggests that better compounds with novel structure motif need to be developed urgently.

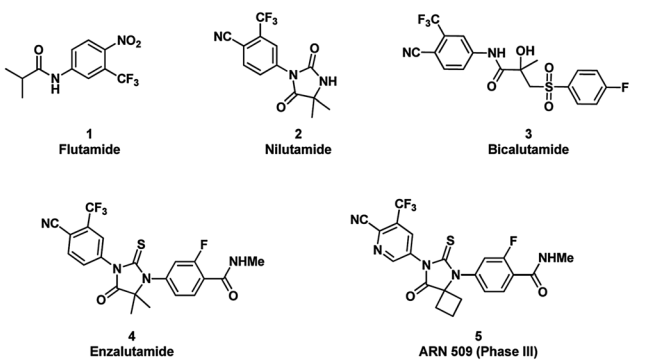



Fig. 1 The first and second generation AR inhibitors.

[a]*Lab of YW Chen, Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center, Chengdu, 610041, China. E-mail: ywchen@scu.edu.cn; Fax: +86 028 8598 0460; Tel: +86 028 8598 0460*

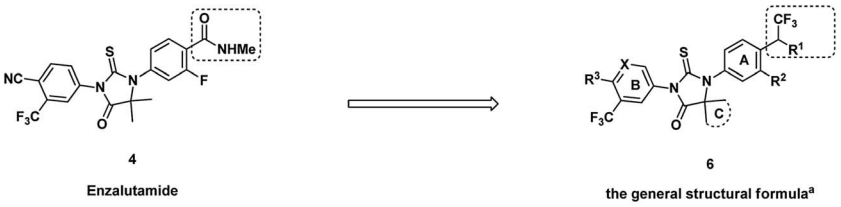
[b]*Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu, 610041, China*

[c]*Hinova Pharmaceuticals Inc., Suite 402, Building B, #5 South KeYuan Road, Chengdu, 610041, China*

[d]*Chengdu University, Sichuan Industrial Institute of Antibiotics, Chengdu, 610041, China. E-mail: lifengzhao@scu.edu.cn; Fax: +86 028 8505 8465; Tel: +86 158 8241 1095*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ra02142a

‡ These authors contributed equally.




[a] X = N, C; $R^1$ = hydrophilic group; $R^2$ = F, H; $R^3$ = CN, COOH, $CONH_2$, C = alicyclic ring.

Fig. 2 Proposed novel structure as potential AR inhibitors.



Replacement of amide group by other isosteres is a common practice in medicinal chemistry effort. Black and his colleagues from Merck reported to use trifluoroethylamine group as the isostere of amide in their searching of novel cathepsin K inhibitors.[13] After replacement of amide with trifluoroethylamine, the potency and selectivity were improved, also the new function group are stable for P1–P2 cleavage that was observed for other amide inhibitors. Considering the major metabolic path way of ENT is through *N*-demthylation and amide hydrolysis,[14,15] a series of isosteres analogs bearing trifluoroethylamine or trifluoroethanol were prepared as novel AR inhibitors. At the same time, C-ring was also optimized for potentially improvement of drug resistance (Fig. 2). Here we report our progress and also the *in vivo* and *in vitro* data of compound **13b** which is dose-dependently inhibiting expression of prostate-specific antigen and has anti-tumor activity at oral administration dose of 15 mg $kg^{-1}$ once daily.

# 2 Results and discussion

## 2.1 Chemistry

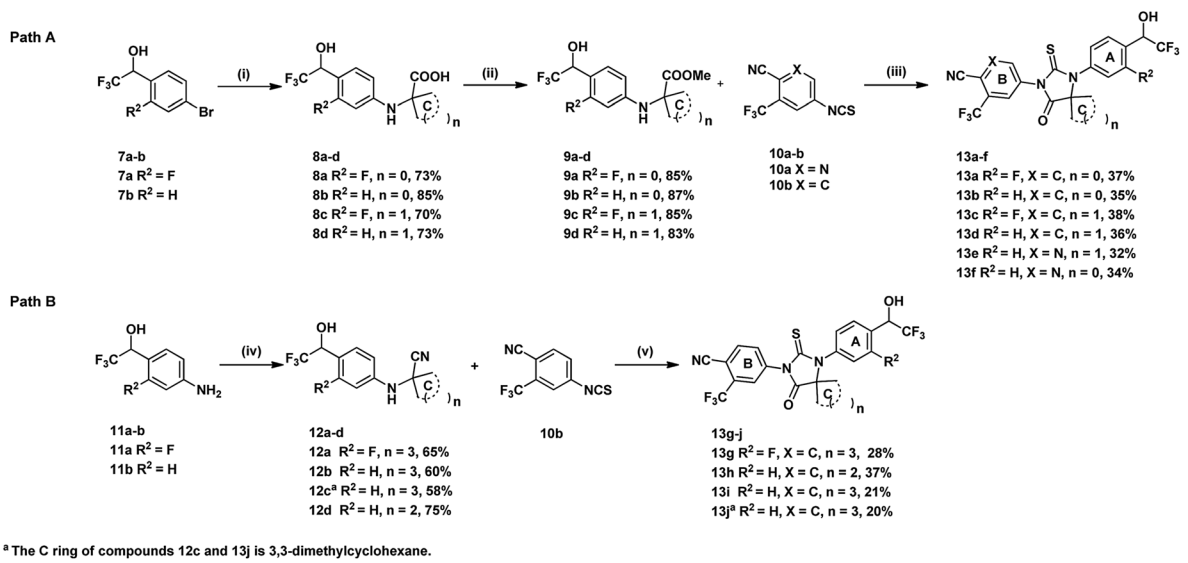
Synthetic routes of evaluated compounds **13a–f** were depicted in Scheme 1 as path A and path B, respectively. In path A, compounds **8a–d** was obtained by coupling of bromide **7a–b** with the appropriated amino-acid under Ullmann coupling reaction conditions. Then, compounds **8a–d** were directly subjected to esterification without further purification to afford compounds **9a–d** under conditions of $K_2CO_3$ and $CH_3I$. Finally, compounds **13a–f** were obtained through the reaction between **9a–d** and **10a–b**.

In path B, the starting material **11a–b** were provided from corresponding benzaldehyde according the reported procedure.[15] Compounds **12a–d**, then, were prepared under Strecker reaction conditions using compounds **11a–b**, TMSCN and corresponding ketone as substrates. Finally, compounds **13g–j** were obtained by reacting **12a–d** with **10b**. Other analogues, **14a–d** and **15a–i**, were synthesized form **13a–b** by substitution, alkylation, oxidation, or hydrolysis reaction as shown in Scheme 2.

## 2.2 Biology activity

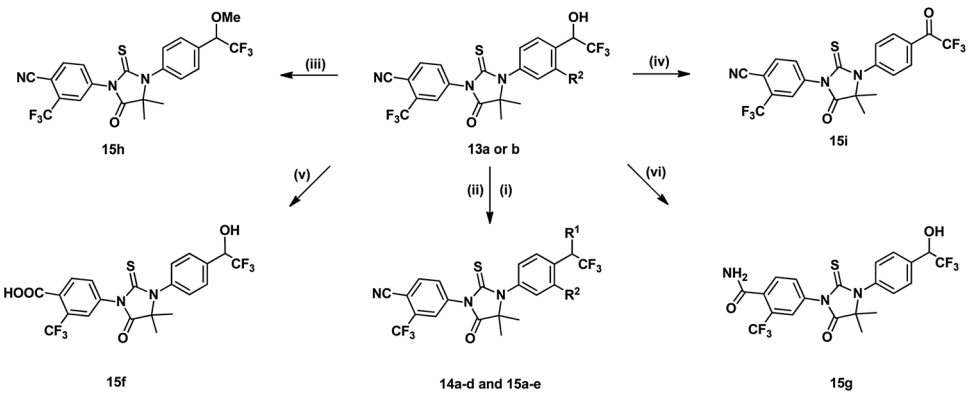
The *in vitro* anti-proliferation activities were evaluated by using LNCaP/AR cells (prostate cancer cells overexpressing AR). In order to mimic the CRPC state, the cells were cultured in charcoal stripped serum and treated with compounds for 6 days. The results were summarized in Tables 1, 2 and 3.

The hydroxyl trifluoroethyl analog compound **13a**, was first prepared because of its easily synthesis, showed 0.38 μM activity




[a] The C ring of compounds 12c and 13j is 3,3-dimethylcyclohexane.

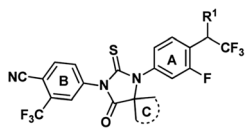
Scheme 1 Reagents and conditions: (i) 10% CuI, 2% Cu, 20% *N*,*N*-dimethylglycine as ligand, $K_2CO_3$, amino-acid, DMF, 80 °C; (ii) $K_2CO_3$, $CH_3I$, DMF, rt; (iii) **10a** or **10b**, DMAc, 80 °C, 24 h; (iv) TMSCN, AcOH, ketone, reflux; (v) **10b**, DMAc, 80 °C, 24 h, then MeOH and 1 N HCl, reflux.

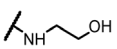
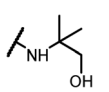
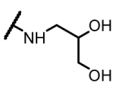



Scheme 2 Reagents and conditions: (i) $Et_3N$, MsCl, DCM, 0 °C, 2 h; (ii) amino or amino alcohol in THF, reflux, 12 h; (iii) NaH, MeI, DMF, rt; (iv) IBX, DMSO, rt, 12 h; (v) $H_2SO_4$, 100 °C, 24 h; (vi) 4 N NaOH, 30% $H_2O_2$, DMSO, 0 °C, 40 min.

against LNCaP-AR cells compared with ENT of 0.25 μM. Encourage by this result, C-ring analogs of cyclobutyl **13c** and cyclohexyl **13g** were also prepared and achieved sub-μM activity. However, to our surprise, the closest isostere of amide, *N*-methylamino trifluoroethyl analog **14a** lost the activity by 10-fold. Considering the poor solubility of ENT, more hydroxyl groups were introduced. To our delight, compounds **14b**, **14c** and **14d** were also expressed good activity with $IC_{50}$ from 0.35 to 0.92 μM.

Table 1 *In vitro* anti-proliferative activity ($IC_{50}$ μM) of compounds against LNCaP-AR cell[a]



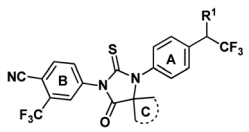
| ID | $R^1$ | C | $IC_{50}$ (μM) |
|---|---|---|---|
| ENT | — | — | 0.25 |
| **13a** |  |  | 0.38 |
| **14a** |  |  | 2.52 |
| **14b** |  |  | 0.35 |
| **14c** |  |  | 0.64 |
| **14d** |  |  | 0.92 |
| **13c** |  |  | 0.86 |
| **13g** |  |  | 0.98 |

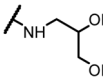
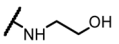
[a] $IC_{50}$ was average of three determinations and deviation from the average was <5% of average value.

Analogues with unsubstituted aromatic A-ring were also prepared in order to further study SAR (Table 2). Compound **13b** represented an approximate 2-fold improvement over ENT. The second most active compound is **15b**, with N–$CH_3$ trifluoroethyl substitutions. Compound **15e** with dihydroxyl propyl amino substitution provided 1.51 μM activity against LNCaP-AR. Other compounds **15a–d**, **15h–i** were found to have moderate to potent activities in the range of 0.15–0.83 μM against LNCaP-AR cell line.

ARN-509, which has substituted pyridine as B-ring and cyclobutyl as C-ring is under phase III trial for CRPC,[16] therefore modification of A-ring substitution and C-ring size were also conducted in this studies (Table 3). However, in comparison with compound **13b**, expanded the ring size of C-ring (compounds **13d** and **13h–j**) was detrimental to the activity. When C-ring was cyclobutyl group and B-ring is pyridine, compound **13e** regained activity with $IC_{50}$ of 0.26 μM. To our

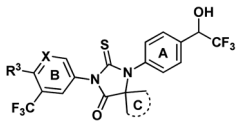
Table 2 *In vitro* anti-proliferative activity ($IC_{50}$ μM) of compounds against LNCaP-AR cell[a]



| ID | $R^1$ | $IC_{50}$ (μM) | ID | $R^1$ | $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **13b** |  | 0.10 | **15d** |  | 0.83 |
| **15a** |  | 0.15 | **15e** |  | 1.51 |
| **15b** |  | 0.13 | **15h** |  | 0.20 |
| **15c** |  | 0.44 | **15i** |  | 0.31 |

[a] Control compound ($IC_{50}$ of ENT is 0.25 ± 0.01 μM) used in all assays; $IC_{50}$ was average of three determinations and deviation from the average was <5% of average value.

Table 3 *In vitro* anti-proliferative activity ($IC_{50}$ μM) of compounds against LNCaP-AR cell[a]




| ID | C | X | $R^3$ | $IC_{50}$ (μM) | ID | C | X | $R^3$ | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|---|
| **13d** |  | C | CN | 9.72 | **13e** |  | N | CN | 0.26 |
| **13h** |  | C | CN | 8.10 | **13f** |  | N | CN | 5.60 |
| **13i** |  | C | CN | 7.63 | **15f** |  | C | COOH | >10 |
| **13j** |  | C | CN | 5.40 | **15g** |  | C | $CONH_2$ | 0.86 |

[a] Control compound ($IC_{50}$ of ENT is 0.25 ± 0.01 μM) used in all assays; $IC_{50}$ was average of three determinations and deviation from the average was <5% of average value.

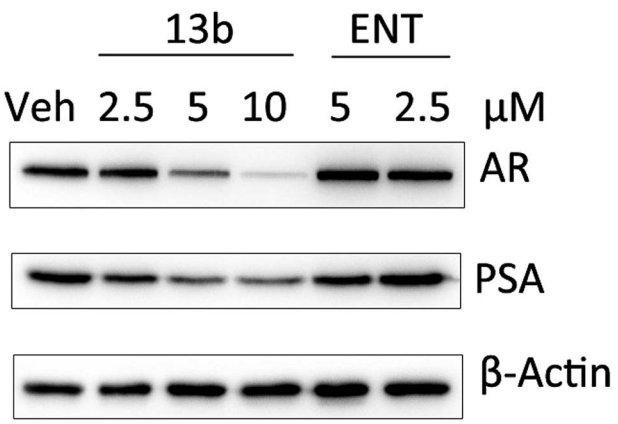



Fig. 3 Western blot analysis of PSA expression in LNCaP-AR cells.

surprise, the $IC_{50}$ value of compound **13f** is inferior compared with **13e**. In addition, replacement of CN group with carboxyl group (compound **15f**) totally lost activity and the carboxamide (compound **15g**) retained some activity.

To test whether **13b** has an effect on prostate-specific antigen (PSA) levels, compound **13b** was verified in LNCaP-AR cells by Western blot analysis. The result (Fig. 3) showed that compound **13b** inhibited the expression of PSA even at very low doses (2.5 μM) and the inhibition activity was dose-dependent. Interestingly, the result also showed that expression of AR was down-regulated, further investigation is needed to probe the mechanism.

The *in vivo* anti-tumor activity of **13b** was evaluated using the CPRC (LNCaP-AR) xenograft model. SCID mice were castrated and subcutaneously inoculated with LNCaP-AR cells. When the tumor volumes reached 100–200 $mm^3$, the mice were randomly grouped (8 animals each group) and oral administrated with compound

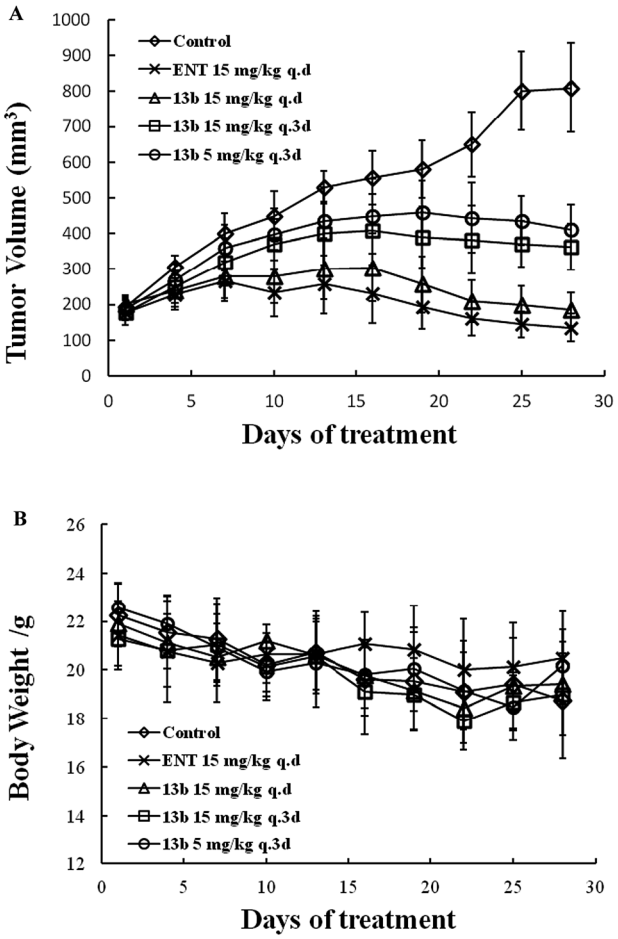



Fig. 4 Tumor volume and body weight changes of mice after oral administration of ENT and **13b**.

**13b** at 15 mg per kg q.d, 15 mg per kg q.3d, 5 mg per kg q.3d, and for ENT at 15 mg per kg q.d for 27 days. The tumor volumes and weight were measured every 3 days (Fig. 4). Compound **13b** at all dose levels inhibited the tumor proliferation compared with the control. 5 mg per kg q.3d of compound **13b** have a moderate activity. 15 mg per kg q.d has similar inhibition compared ENT. The tumor inhibition rate of **13b** at dose of 5 mg per kg q.3d, 15 mg per kg q.3d, 15 mg per kg q.d were 58.9%, 66.0%, 96.9%, respectively, whereas, the ENT was more than 100% (Fig. 4a). In addition, the weight change **13b**, ENT treated group and control group were slightly decreased (Fig. 4b).

## 3 Conclusions

In summary, we have synthesized a series of thiohydantoin analogues bearing substituted 2,2,2-trifluoro-1-phenylethanyl moiety. Compound **13b** showed good cell activity against CRPC LNCaP-AR cell. Western blot analysis also verified compound **13b** inhibited the expression of PSA in LNCaP-AR cells. Further evaluation in xenograft CRPC model showed compound **13b** has anti-tumor activity of 96.9% inhibition at 15 mg per kg q.d. Further optimization and relevant works are in progress.

# 4 Experimental

## 4.1 General information

The $^{1}H$ and $^{13}C$ $^{19}F$ NMR spectra were recorded on Avance DMX 400 MHz NMR spectrometers (Bruker, Germany) in $CDCl_3$ or deuterated DMSO using TMS as internal standard. Spectra are reported as follows: chemical shift $\delta$ (ppm), (integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constant $J$ (Hz), assignment). ESI-HRMS spectra were recorded on a commercial apparatus and methanol was used to dissolve the sample. Reagents were purchased from commercial sources and were used as received unless mentioned otherwise. Reactions were monitored by thin layer chromatography using silica gel GF 254 plates. Column chromatography was performed on silica gel (300–400 mesh).

## 4.2 Experimental section

Compounds **9**, **12**, **13** were prepared by using a slightly modified literature-known procedure.[17–20]

**General procedure for the synthesis of 9.** **7** (1.0 mmol), amino-acid (1.2 mmol), 10% CuI (0.1 mmol), 2% Cu (0.02 mol), 20% *N*,*N*-dimethylglycine (0.2 mmol) and $K_2CO_3$ (2.0 mmol) in DMF (15.0 mL) was stirred at 80 °C for 24 h, under the protection of nitrogen. After the reaction, 1 N HCl was added to adjust the PH value to 3–4. Then, the solution was partitioned between ethyl acetate and water. The organic layer was concentrated to give the crude product for the flowing reaction. A stirred solution of the crude product and $K_2CO_3$ (1.0 mmol) in DMF (5 mL) then, $CH_3I$ (1 mmol) was added. After the reaction, the solution was quenched with water (25 mL). Then, aqueous residue was extracted with EA (20 mL × 3), dried over $Na_2SO_4$, concentrated and purified by column chromatography (PE : EA/1 : 3) to given compounds **9** (yield from 59–76%).

**General procedure for the synthesis of 12.** The mixture of **11** (1.0 mmol), TMSCN (2.0 mmol), ketone (4.0 mmol) and 5 mL AcOH was stirred in a sealed tube at 80 °C for 24 h. The solution was neutralized with aqueous sodium hydrogen carbonate. Then, the mixture was extracted with ethyl acetate (20 mL × 3). The combined organics were dried over $Na_2SO_4$, filtered, concentrated and purified by column chromatography (PE : EA/1 : 2) to give **12** (yield from 58–75%).

**General procedure for the synthesis of 13a–f.** To the mixture of compound **9a** (53 mg, 0.17 mmol) and compound **10b** (79 mg 0.34 mmol) in DMAc (10 mL), the mixture was heated to 80 °C for 24 h. After the reaction, water (30 mL) was added. The resulting mixture was extracted with ethyl acetate, washed with brine, dried over sodium sulfate, concentrated and purified by column chromatography (PE : EA/1 : 1) to give a brown solid as crude product, which was purified by preparative chromatography to give compound **13a** as a white solid (32 mg, 37% yield).

Compounds **13b–f** were synthesized by a similar procedure as described for compound **13a**.

*4-(3-(3-Fluoro-4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**13a**).* $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.97 (dd, $J$ = 9.0, 4.9 Hz, 2H), 7.87–7.76 (m, 2H), 7.21 (dd, $J$ = 8.3, 1.7 Hz, 1H), 7.12 (td, $J$ = 9.7, 1.9 Hz, 1H), 5.43 (q, $J$ = 6.3 Hz, 1H), 3.88–3.45 (m, 1H), 1.61 (s, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$) $\delta$ 179.85, 174.78, 160.31 (d, $J$ = 248.6 Hz), 137.18 (d, $J$ = 9.2 Hz), 137.00, 135.41, 134.61 (q, $J$ = 33.1 Hz), 132.28, 130.24 (d, $J$ = 4.5 Hz), 127.15 (q, $J$ = 4.8 Hz), 125.94 (d, $J$ = 3.5 Hz), 123.93 (q, $J$ = 281.1 Hz), 123.81 (d, $J$ = 13.2 Hz), 121.87 (q, $J$ = 283.3 Hz), 117.43 (d, $J$ = 23.8 Hz), 114.83, 110.18, 66.75, 65.62 (q, $J$ = 33.5 Hz), 23.69. $^{19}F$ NMR (376 MHz, $CDCl_3$) $\delta$ −62.38, −78.42, −113.83. HRMS (EI) calcd for $C_{21}H_{14}F_7N_3O_2S$ $[M + H]^+$: 506.0768, found: 506.0769.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**13b**).* $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (d, $J$ = 9.2 Hz, 2H), 7.84 (dd, $J$ = 8.3, 1.6 Hz, 1H), 7.68 (d, $J$ = 8.2 Hz, 2H), 7.37 (d, $J$ = 8.4 Hz, 2H), 5.28–5.02 (m, 1H), 2.87 (s, 1H), 1.61 (s, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$) $\delta$ 179.88, 174.96, 165.29, 137.09, 136.00, 135.77, 135.32, 133.62 (q, $J$ = 34.1 Hz), 132.26, 129.76, 129.07, 127.16 (q, $J$ = 5.1 Hz), 124.04 (q, $J$ = 280.1 Hz), 121.89 (q, $J$ = 273.3 Hz), 114.83, 110.19, 71.97 (q, $J$ = 32.1 Hz), 66.60, 23.73. $^{19}F$ NMR (376 MHz, $CDCl_3$) $\delta$ −61.97, −78.16. HRMS (EI) calcd for $C_{21}H_{15}F_6N_3O_2S$ $[M + H]^+$: 488.0862, found: 488.0869.

*4-(5-(3-Fluoro-4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-7-yl)-2-(trifluoromethyl)benzonitrile (**13c**).* $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (dd, $J$ = 8.4, 5.0 Hz, 2H), 7.93–7.79 (m, 2H), 7.24 (dd, $J$ = 8.4, 1.9 Hz, 1H), 7.14 (dd, $J$ = 9.9, 1.9 Hz, 1H), 5.52 (q, $J$ = 6.3 Hz, 1H), 2.88 (ddd, $J$ = 5.3, 3.5, 2.5 Hz, 1H), 2.70 (ddd, $J$ = 8.9, 6.6, 3.4 Hz, 2H), 2.56 (ddd, $J$ = 20.0, 10.0, 2.4 Hz, 2H), 2.36–2.15 (m, 1H), 1.83–1.64 (m, 1H). $^{13}C$ NMR (101 MHz, CDCl3) $\delta$ 179.86, 174.51, 160.54 (d, $J$ = 252.3 Hz), 137.52 (d, $J$ = 9.6 Hz), 136.88, 135.28, 133.68 (d, $J$ = 33.5 Hz), 132.11, 130.39 (d, $J$ = 4.1 Hz), 127.04 (q, $J$ = 5.0 Hz), 126.43 (d, $J$ = 3.1 Hz), 124.38 (q, $J$ = 280.6 Hz), 123.57 (d, $J$ = 13.1 Hz), 121.73 (q, $J$ = 282.6 Hz), 117.89 (d, $J$ = 23.4 Hz), 114.78, 110.24, 67.51, 65.83 (q, $J$ = 32.1 Hz), 31.69, 13.67. $^{19}F$ NMR (376 MHz, CDCl3) $\delta$ −61.98, −78.38, −113.55. HRMS (EI) calcd for $C_{22}H_{14}F_7N_3O_2S$ $[M + H]^+$: 518.0768, found: 518.0772.

*4-(8-Oxo-6-thioxo-5-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-5,7-diazaspiro[3.4]octan-7-yl)-2-(trifluoromethyl)benzonitri-le (**13d**).* $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (dd, $J$ = 5.1, 3.2 Hz, 2H), 7.90–7.83 (m, 1H), 7.74 (t, $J$ = 9.1 Hz, 2H), 7.43–7.34 (m, 2H), 5.15 (q, $J$ = 6.6 Hz, 1H), 3.11–2.76 (m, 1H), 2.75–2.63 (m, 2H), 2.64–2.50 (m, 2H), 2.34–2.16 (m, 1H), 1.70 (dtt, $J$ = 10.5, 7.7, 5.1 Hz, 1H). $^{13}C$ NMR (101 MHz, $CDCl_3$) $\delta$ 179.91, 174.77, 137.04, 136.16, 135.72, 135.23, 133.61 (q, $J$ = 34.1 Hz), 132.16, 130.20, 129.25, 127.07 (q, $J$ = 5.1 Hz), 124.03 (q, $J$ = 280.1 Hz), 121.89 (q, $J$ = 273.3 Hz), 114.84, 110.12, 72.10 (q, $J$ = 32.1 Hz), 67.45, 31.59, 13.70. $^{19}F$ NMR (376 MHz, $CDCl_3$) $\delta$ −61.96, −78.09. HRMS (EI) calcd for $C_{22}H_{15}F_6N_3O_2S$ $[M + H]^+$: 500.0862, found: 500.0866.

*5-(8-Oxo-6-thioxo-5-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-5,7-diazaspiro[3.4]octan-7-yl)-3-(trifluoromethyl)picolino-nitrile (**13e**).* $^{1}H$ NMR (400 MHz, DMSO) $\delta$ 9.23 (s, 1H), 8.77 (d, $J$ = 1.9 Hz, 1H), 7.75 (d, $J$ = 8.3 Hz, 2H), 7.48 (d, $J$ = 8.4 Hz, 2H), 7.03 (s, 1H), 5.49–5.19 (m, 1H), 2.72–2.59 (m, 2H), 2.50–2.38 (m, 2H), 2.07–1.90 (m, 1H), 1.66–1.43 (m, 1H). $^{13}C$ NMR (101 MHz, DMSO) $\delta$ 180.05, 174.96, 154.07, 137.59, 136.19, 136.03 (q, $J$ = 4.2 Hz), 133.82, 130.31, 129.51, 129.24 (q, $J$ = 34.1 Hz), 129.20 (q, $J$ = 2.0 Hz), 125.43 (q, $J$ = 277.1 Hz), 122.04 (q, $J$ = 273.3 Hz),

114.73, 70.41 (q, $J$ = 31.5 Hz), 67.98, 31.49, 13.86. $^{19}$F NMR (376 MHz, DMSO) $\delta$ −60.83, −76.56. HRMS (EI) calcd for $C_{21}H_{14}F_6N_4O_2S$ $[M + H]^+$: 501.0814, found: 501.0815.

*5-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-hydro-xyeth-yl)-phenyl)imidazolidin-1-yl)-3-(trifluoromethyl)picolinonitrile (**13f**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.08 (dd, $J$ = 12.6, 2.2 Hz, 1H), 8.37 (t, $J$ = 3.9 Hz, 1H), 7.67 (dd, $J$ = 18.2, 8.3 Hz, 2H), 7.45–7.31 (m, 2H), 5.13 (q, $J$ = 6.6 Hz, 1H), 1.63 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.08, 174.67, 152.34, 135.96, 135.72, 134.16 (q, $J$ = 4.2 Hz), 132.41, 130.54 (q, $J$ = 34.3 Hz), 129.96, 129.67, 129.15, 124.04 (q, $J$ = 283.1 Hz), 121.26 (q, $J$ = 270.9 Hz), 113.75, 71.99 (q, $J$ = 26.3 Hz), 66.81, 23.76. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.87, −78.12. HRMS (EI) calcd for $C_{20}H_{14}F_6N_4O_2S^+$ $[M + H]^+$: 489.0814, found: 489.0815.

**General procedure for the synthesis of 13g–j.** To the mixture of compound **12a** (54 mg, 0.17 mmol) and compound **10b** (79 mg, 0.34 mmol) in DMAc (10 mL), the mixture was heated to 80 °C for 24 h, and then MeOH (2 mL) and 1 N HCl (1 mL) added in the mixture. The reaction mixture was stirred at 90 °C for 2 h. After completion of the reaction (TLC), water (30 mL) were added. The resulting mixture was extracted with ethyl acetate, washed with brine, dried over sodium sulfate, concentrated and purified by column chromatography (PE : EA/1 : 1) to give a brown solid as crude product, which was purified by preparative chromatography to give compound **13g** as a white solid (26 mg, 28% yield).

Compounds **13h–j** were synthesized by a similar procedure as described for compound **13g**.

*4-(1-(3-Fluoro-4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.5]decan-3-yl)-2-(trifluoromethyl)benzo-nitrile (**13g**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.99 (t, $J$ = 10.4 Hz, 1H), 7.94 (d, $J$ = 1.7 Hz, 1H), 7.89–7.78 (m, 2H), 7.17–7.10 (m, 1H), 7.05 (dd, $J$ = 9.9, 1.9 Hz, 1H), 5.61–5.38 (m, 1H), 3.03–2.80 (m, 1H), 2.26–1.98 (m, 4H), 1.87–1.64 (m, 5H), 1.17–1.00 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 180.12, 173.49, 160.36 (d, $J$ = 251.3 Hz), 137.10 (d, $J$ = 10.0 Hz), 136.95, 135.25, 133.32 (q, $J$ = 33.8. Hz), 132.31, 130.06 (d, $J$ = 4.6 Hz), 127.22 (q, $J$ = 3.2 Hz), 127.98 (d, $J$ = 3.1 Hz), 123.86 (q, $J$ = 280.1 Hz), 123.52 (d, $J$ = 14.0 Hz), 122.36 (q, $J$ = 283.3 Hz), 118.26 (d, $J$ = 23.3 Hz), 114.80, 110.19, 67.73, 65.97 (q, $J$ = 61.4 Hz), 32.82, 23.86, 20.90. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −62.04, −78.37, −113.90. HRMS (EI) calcd for $C_{24}H_{18}F_7N_3O_2S$ $[M + H]^+$: 546.1081, found: 546.1086.

*4-(4-Oxo-2-thioxo-1-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-1,3-diazaspiro[4.4]nonan-3-yl)-2-(trifluoromethyl)benzonitrile (**13h**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (dd, $J$ = 4.7, 3.2 Hz, 2H), 7.90–7.83 (m, 1H), 7.69 (d, $J$ = 8.2 Hz, 2H), 7.39 (d, $J$ = 8.3 Hz, 2H), 5.25–5.05 (m, 1H), 3.07–2.49 (m, 1H), 2.34 (dt, $J$ = 13.2, 6.2 Hz, 2H), 2.23–2.11 (m, 2H), 1.94–1.82 (m, 2H), 1.66–1.44 (m, 3H). $^{13}$C NMR (101 MHz, CDCl3) $\delta$ 180.19, 176.05, 137.19, 136.74, 135.58, 135.20, 133.61 (q, $J$ = 37.0 Hz), 132.19, 130.21, 129.09, 127.12 (q, $J$ = 4.8 Hz), 123.73 (q, $J$ = 279.6 Hz), 121.84 (q, $J$ = 270.7 Hz), 114.83, 110.17, 75.27, 72.10 (q, $J$ = 32.3 Hz), 36.16, 25.12. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.96, −78.19. HRMS (EI) calcd for $C_{23}H_{17}F_6N_3O_2S$ $[M + H]^+$: 514.1018, found: 514.1023.

*4-(4-Oxo-2-thioxo-1-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)-1,3-diazaspiro[4.5]decan-3-yl)-2-(trifluoromethyl)benzonitr-ile (**13i**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.05–7.90 (m, 2H), 7.83 (dd, $J$ = 8.2, 1.7 Hz, 1H), 7.67 (d, $J$ = 8.2 Hz, 2H), 7.31 (t, $J$ = 9.3 Hz, 2H), 5.12 (q, $J$ = 6.6 Hz, 1H), 3.28–2.83 (m, 1H), 2.19–2.00 (m, 4H), 1.70 (dd, $J$ = 17.4, 7.1 Hz, 5H), 1.17–1.00 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 180.07, 173.81, 137.12, 135.76, 135.21, 133.57 (q, $J$ = 34.0 Hz), 132.36, 130.69, 128.99, 127.32 (q, $J$ = 5.0 Hz), 124.05 (q, $J$ = 278.0 Hz), 121.90 (q, $J$ = 273.0 Hz), 114.86, 110.08, 72.05 (q, $J$ = 32.0 Hz), 67.62, 32.75, 23.88, 20.69. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.94, −78.07. HRMS (EI) calcd for $C_{24}H_{19}F_6N_3O_2S$ $[M + H]^+$: 528.1175, found: 528.1183.

*4-(7,7-Dimethyl-4-oxo-2-thioxo-1-(4-(2,2,2-trifluoro-1-hydro-xyeth-yl)phenyl)-1,3-diazaspiro[4.5]decan-3-yl)-2-(trifluoromethyl) benz-onitrile (**13j**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.04–7.89 (m, 2H), 7.90–7.77 (m, 1H), 7.75–7.64 (m, 2H), 7.28 (t, $J$ = 6.1 Hz, 2H), 5.27–5.01 (m, 1H), 3.17–2.69 (m, 1H), 2.36–2.20 (m, 1H), 2.21–2.10 (m, 1H), 1.98–1.86 (m, 1H), 1.73–1.60 (m, 3H), 1.57–1.46 (m, 2H), 1.22 (d, $J$ = 14.6 Hz, 3H), 0.98–0.90 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 180.04, 174.36, 137.23, 135.66, 135.17, 133.57 (q, $J$ = 34.0 Hz), 132.40, 131.28, 128.98, 127.32 (q, $J$ = 5.0 Hz), 124.05 (q, $J$ = 281.2 Hz), 121.89 (q, $J$ = 272.5 Hz), 114.84, 110.11, 72.09 (q, $J$ = 32.5 Hz), 68.81, 43.20, 37.41, 35.03, 32.48, 32.19, 26.36, 17.95. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.96, −78.04. HRMS (EI) calcd for $C_{26}H_{23}F_6N_3O_2S$ $[M + H]^+$: 556.1488, found: 556.1494.

**General procedure for the synthesis of 14a–d and 15a–e.** At 0 °C, DCM solution of **13a** (505 mg, 1 mmol), $Et_3N$ (151 mg, 1.5 mmol) and MsCl (137 mg, 1.2 mmol) were added in this order. The reaction was stirred at room temperature for 30 min, and concentrated under vacuum to give the crude product for the flowing reaction. A stirred solution of the crude product and aqueous ammonia in THF was refluxed overnight, and concentrated under vacuum to give the crude product. Purification by flash chromatography afforded the products **14a** (410 mg, 79% yield).

Compounds **14b–d** and **15b–e** were synthesized by a similar procedure as described for compound **14a**.

*4-(3-(3-Fluoro-4-(2,2,2-trifluoro-1-(methylamino)ethyl)phenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl) ben-zonitrile (**14a**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.04–7.91 (m, 2H), 7.89–7.78 (m, 1H), 7.68 (t, $J$ = 7.9 Hz, 1H), 7.22–7.16 (m, 1H), 7.16–7.05 (m, 1H), 4.75–4.38 (m, 1H), 2.49 (s, 3H), 1.70 (ddd, $J$ = 11.6, 10.6, 7.5 Hz, 1H), 1.62 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.78, 174.59, 162.34 (d, $J$ = 251.6 Hz), 136.89, 136.80 (d, $J$ = 9.8 Hz), 135.29, 133.66 (q, $J$ = 33.2 Hz), 132.13, 130.13 (d, $J$ = 4.0 Hz), 127.08 (q, $J$ = 4.1 Hz), 126.02 (d, $J$ = 3.8 Hz), 125.07 (q, $J$ = 277.2 Hz), 123.59 (q, $J$ = 14.0 Hz) 121.84 (q, $J$ = 273.1 Hz), 117.49 (q, $J$ = 23.9 Hz), 114.76, 110.36, 66.59, 58.65 (q, $J$ = 27.5 Hz), 34.84, 23.81. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.88, −78.43, −78.45, −113.50, −113.52. HRMS (EI) calcd for $C_{22}H_{17}F_7N_4OS$ $[M + H]^+$: 519.1084, found: 519.1093.

*4-(3-(3-Fluoro-4-(2,2,2-trifluoro-1-((2-hydroxyethyl)amino)ethyl) phenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoro-methyl)benzonitrile (**14b**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.04–7.91 (m, 2H), 7.85 (t, $J$ = 10.9 Hz, 1H), 7.68 (t, $J$ = 7.9 Hz, 1H), 7.18 (t, $J$ = 9.0 Hz, 1H), 7.12 (d, $J$ = 9.9 Hz, 1H), 4.67 (q, $J$ = 7.0 Hz, 1H), 3.91–3.51 (m, 2H), 3.13–2.51 (m, 2H), 2.28–1.90 (m, 2H), 1.62 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.81, 174.58, 161.20 (d, $J$ = 251.0 Hz), 136.92, 136.91 (d, $J$ = 9.9 Hz), 135.31, 133.69 (q, $J$ =

33.1 Hz), 132.18, 130.05 (d, $J$ = 4.0 Hz), 127.10 (q, $J$ = 4.8 Hz), 126.08 (d, $J$ = 3.0 Hz), 124.93 (q, $J$ = 281.1 Hz), 123.84 (d, $J$ = 13.8 Hz), 121.85 (q, $J$ = 273.1 Hz), 117.58 (d, $J$ = 24.3 Hz), 114.76, 110.36, 66.62, 61.26, 56.93 (q, $J$ = 24.3 Hz), 49.51, 23.81. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.94, −73.19, −113.75. HRMS (EI) calcd for $C_{23}H_{19}F_7N_4O_2S$ $[M + H]^+$: 549.1190, found: 549.1191.

*4-(3-(3-Fluoro-4-(2,2,2-trifluoro-1-((1-hydroxy-2-methylpro-pan-2-yl)amino)ethyl)phenyl)-4,4-dimethyl-5-oxo-2-thioxoi-midazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**14c**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.91 (d, $J$ = 8.6 Hz, 2H), 7.82–7.74 (m, 1H), 7.59–7.45 (m, 2H), 7.23 (dd, $J$ = 18.1, 4.6 Hz, 2H), 4.32–4.11 (m, 1H), 3.41–3.24 (m, 1H), 3.22–3.07 (m, 1H), 2.14–1.85 (m, 1H), 1.69–1.61 (m, 1H), 1.53 (s, 6H), 0.95 (d, $J$ = 11.9 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.80, 174.58, 161.35 (d, $J$ = 257.0 Hz), 136.92, 136.69 (d, $J$ = 10.0 Hz), 135.30, 133.65 (q, $J$ = 33.0 Hz), 132.15, 130.05 (d, $J$ = 4.0 Hz), 127.09 (q, $J$ = 5.0 Hz), 126.79 (d, $J$ = 14.1 Hz), 126.13 (d, $J$ = 4.0 Hz), 124.81 (q, $J$ = 280.3 Hz), 121.85 (q, $J$ = 273.1 Hz), 117.53 (d, $J$ = 24.0 Hz), 114.76, 110.33, 69.35, 66.54, 54.91, 51.21 (q, $J$ = 29.3 Hz), 24.81, 23.93, 23.82. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.95, −74.66, −114.24. HRMS (EI) calcd for $C_{25}H_{23}F_7N_4O_2S$ $[M + H]^+$: 577.1503, found: 577.1504.

*4-(3-(4-(1-((2,3-Dihydroxypropyl)amino)-2,2,2-trifluoroethyl)-3-fluorophenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**14d**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.10–7.91 (m, 2H), 7.90–7.74 (m, 1H), 7.74–7.58 (m, 1H), 7.18 (t, $J$ = 9.6 Hz, 1H), 7.13 (d, $J$ = 10.0 Hz, 1H), 4.73–4.51 (m, 1H), 3.92–3.69 (m, 2H), 3.67–3.52 (m, 1H), 2.92–2.83 (m, 1H), 2.82–2.70 (m, 1H), 2.49–2.05 (m, 2H), 1.62 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.82, 174.55, 161.20 (d, $J$ = 261.1 Hz), 137.03 (d, $J$ = 8.5 Hz), 136.99, 136.89, 135.31, 133.69 (q, $J$ = 33.4 Hz), 132.17, 129.94 (d, $J$ = 5.1 Hz), 127.11 (q, $J$ = 5.1 Hz), 126.14 (d, $J$ = 4.1 Hz), 124.84 (q, $J$ = 277.3 Hz), 119.15 (q, $J$ = 270.6 Hz), 117.68 (d, $J$ = 24.1 Hz), 114.74, 110.41, 70.15 (d, $J$ = 30.2 Hz), 66.62, 65.02 (d, $J$ = 12.3 Hz), 57.20 (q, $J$ = 30.3 Hz), 50.21, 23.83. $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ −61.99, −73.69, −73.71, −73.72, −113.60, −113.61, −113.66, −113.67. HRMS (EI) calcd for $C_{24}H_{21}F_7N_4O_3S$ $[M + H]^+$: 579.1295, found: 579.1299.

*4-(3-(4-(1-Amino-2,2,2-trifluoroethyl)phenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**15a**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.12–7.93 (m, 2H), 7.91–7.76 (m, 1H), 7.74–7.55 (m, 2H), 7.35 (d, $J$ = 8.4 Hz, 2H), 4.66–4.40 (m, 1H), 1.93–1.77 (m, 2H), 1.60 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.86, 174.90, 137.15, 137.09, 135.66, 135.27, 133.59 (q, $J$ = 33.1 Hz), 132.24, 129.84, 129.53, 127.14 (q, $J$ = 4.3 Hz), 125.37 (q, $J$ = 280.6 Hz), 121.89 (q, $J$ = 272.7 Hz), 114.83, 110.22, 66.53, 57.51 (q, $J$ = 31.2 Hz), 23.75. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.95, −76.41. HRMS (EI) calcd for $C_{21}H_{16}F_6N_4OS$ $[M + H]^+$: 487.1022, found: 487.1025.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-(methyl-amino)ethyl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl)ben-zonitrile (**15b**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.05–7.95 (m, 2H), 7.89–7.82 (m, 1H), 7.61 (d, $J$ = 8.3 Hz, 2H), 7.40–7.32 (m, 2H), 4.23–4.07 (m, 1H), 2.47 (s, 3H), 1.61 (d, $J$ = 2.0 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.82, 174.92, 137.11, 135.99, 135.71, 135.26, 133.57 (q, $J$ = 33.8 Hz), 132.24, 130.06, 129.85, 127.12 (q, $J$ = 5.1 Hz), 125.14 (q, $J$ = 280.2 Hz), 121.89 (q, $J$ = 272.5 Hz), 114.83, 110.21, 66.53, 66.02 (q, $J$ = 32.1 Hz), 34.85, 23.76. $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ −61.98, −73.81. HRMS (EI) calcd for $C_{22}H_{18}F_6N_4OS$ $[M + H]^+$: 501.1178, found: 501.1188.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-((2-hydroxy-ethyl)amino)ethyl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl) benzonitrile (**15c**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.99 (d, $J$ = 8.4 Hz, 2H), 7.92–7.77 (m, 1H), 7.62 (t, $J$ = 13.0 Hz, 2H), 7.42–7.27 (m, 2H), 4.28 (q, $J$ = 7.2 Hz, 1H), 3.85–3.51 (m, 2H), 2.97–2.65 (m, 2H), 2.01 (s, 2H), 1.61 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.83, 174.89, 137.07, 136.11, 135.77, 135.26, 133.60 (q, $J$ = 33.2 Hz), 132.23, 129.97, 129.93, 127.14 (q, $J$ = 4.8 Hz), 125.13 (q, $J$ = 281.2 Hz), 121.88 (q, $J$ = 273.2 Hz), 114.82, 110.23, 66.54, 64.06 (q, $J$ = 28.6 Hz), 61.37, 49.41, 23.77. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.96, −73.78. HRMS (EI) calcd for $C_{23}H_{20}F_6N_4O_2S$ $[M + H]^+$: 531.1284, found: 531.1288.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-((1-hydroxy-2-methylpropan-2-yl)amino)ethyl)phenyl)imida-zolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**15d**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.91 (d, $J$ = 8.6 Hz, 2H), 7.82–7.74 (m, 1H), 7.51 (d, $J$ = 8.1 Hz, 2H), 7.26 (d, $J$ = 8.2 Hz, 2H), 4.40–4.15 (m, 1H), 3.39–3.23 (m, 1H), 3.24–3.09 (m, 1H), 2.15–1.87 (m, 1H), 1.79–1.64 (m, 2H), 1.53 (s, 6H), 0.95 (d, $J$ = 11.9 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.80, 174.88, 139.13, 137.06, 135.48, 135.25, 133.60 (q, $J$ = 33.1 Hz), 132.18, 129.96, 129.51, 127.13 (q, $J$ = 4.9 Hz), 125.26 (q, $J$ = 279.3 Hz), 121.88 (q, $J$ = 273.4 Hz), 114.81, 110.24, 69.46, 66.47, 58.32 (q, $J$ = 29.6 Hz), 55.04, 25.05, 24.27, 23.80. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.97, −74.32. HRMS (EI) calcd for $C_{25}H_{24}F_6N_4O_2S$ $[M + H]^+$: 559.1597, found: 559.1600.

*4-(3-(4-(1-((2,3-Dihydroxypropyl)amino)-2,2,2-trifluoroethyl) phen-yl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-(trifluoro-meth-yl)benzonitrile (**15e**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.06–7.94 (m, 2H), 7.88–7.80 (m, 1H), 7.66–7.55 (m, 2H), 7.41–7.32 (m, 2H), 4.25 (qd, $J$ = 7.1, 2.3 Hz, 1H), 3.87–3.76 (m, 1H), 3.73 (dt, $J$ = 11.3, 3.3 Hz, 1H), 3.60 (ddd, $J$ = 11.5, 6.3, 5.4 Hz, 1H), 2.84 (dt, $J$ = 12.0, 3.6 Hz, 1H), 2.77–2.67 (m, 1H), 2.20 (s, 1H), 1.75–1.64 (m, 2H), 1.61 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.84, 174.93, 137.03, 135.90, 135.77, 135.26, 133.63 (q, $J$ = 33.3 Hz), 132.19, 130.02, 129.88, 127.13 (q, $J$ = 4.8 Hz), 124.83 (q, $J$ = 281.3 Hz), 118.27 (q, $J$ = 278.3 Hz), 114.79, 110.29, 70.21 (d, $J$ = 36.2 Hz), 66.54, 65.12 (d, $J$ = 8.1 Hz), 64.33 (q, $J$ = 28.2 Hz), 50.37 (d, $J$ = 45.3 Hz), 23.79. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.97, −73.75, −73.78. HRMS (EI) calcd for $C_{24}H_{22}F_6N_4O_3S$ $[M + H]^+$: 561.1390, found: 561.1390.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-hydroxyeth-yl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl)benzoic acid (**15f**).* In a 20 mL tube sealing, **13b** (49 mg, 0.1 mmol), concentrated $H_2SO_4$ (5 mL), were added. The mixture was sealed and stirred for 24 h at 100 °C. After the reaction, the mixture was poured into 100 mL ice water and saturated sodium bicarbonate was added to adjust pH to 4. The mixture was washed with EA (30 mL × 3) and the organic layer was separated and dried over $Na_2SO_4$. After filtration and evaporation, the resulting crude product was purified by column chromatography with petrol ether as eluent to afford **15f** (43 mg, 84% yield).

$^1$H NMR (400 MHz, DMSO) $\delta$ 8.06 (d, $J$ = 1.7 Hz, 1H), 8.02–7.92 (m, 1H), 7.88 (dd, $J$ = 8.2, 1.7 Hz, 1H), 7.68 (t, $J$ = 9.4 Hz, 2H), 7.48 (t, $J$ = 10.3 Hz, 2H), 5.52–5.13 (m, 1H), 1.52 (s, 6H). $^{13}$C NMR (101 MHz, CDCl3) $\delta$ 185.74, 180.49, 172.60, 142.00, 141.17, 140.70, 138.48, 135.54, 134.91, 133.94, 132.82 (q, $J$ = 2.9 Hz),

132.08 (q, $J$ = 32.1 Hz), 130.17 (q, $J$ = 282.7 Hz), 128.37, (q, $J$ = 273.0 Hz), 75.18 (q, $J$ = 29.8 Hz), 71.42, 28.23. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −53.41, −71.81. HRMS (EI) calcd for $C_{21}H_{16}F_6N_2O_4S$ $[M + H]^+$: 507.0808, found: 507.0817.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl)benza-mide (**15g**).* To a mixture of **13b** (49 mg, 0.1 mmol) and sodium hydroxide (4.0 M in water, 0.25 mL, 1.0 mmol) in DMSO (2.5 mL) was added hydrogen peroxide (30% aqueous solution, 0.05 mL, 0.49 mmol). The reaction mixture was heated to 35 °C. After 40 minutes, 20 mL $H_2O$ was added, the mixtures was extracted with ethyl acetate. The organic layer was separated and dried over $Na_2SO_4$. After filtration and evaporation, the resulting crude product was purified by column chromatography with petrol ether as eluent to afford **15g** (39 mg, 77% yield).

$^1$H NMR (400 MHz, DMSO) $\delta$ 8.19–8.10 (m, 1H), 8.04–7.91 (m, 1H), 7.87–7.78 (m, 1H), 7.70 (dd, $J$ = 16.7, 7.3 Hz, 4H), 7.46 (d, $J$ = 8.2 Hz, 2H), 7.09–6.91 (m, 1H), 5.37–5.24 (m, 1H), 1.51 (s, 6H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 181.07, 175.85, 168.88, 137.55, 137.21, 136.47, 134.84, 133.55, 130.18, 129.51, 129.17, 127.84 (q, $J$ = 5.1 Hz), 126.53, 125.43 (q, $J$ = 283.1 Hz), 123.81 (q, $J$ = 273.3 Hz), 70.47 (q, $J$ = 21.1 Hz), 66.62, 23.51. $^{19}$F NMR (376 MHz, DMSO) $\delta$ −57.99, −76.56. HRMS (EI) calcd for $C_{21}H_{17}F_6N_3O_3S$ $[M + H]^+$: 506.0968, found: 506.0975.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoro-1-methoxyethyl)phenyl)imidazolidin-1-yl)-2-(trifluoromethyl)benzo-nitrile (**15h**).* In a 25 mL round-bottomed flask, **13b** (49 mg, 0.1 mmol), DMF (5 mL), and NaH 60% (12 mg, 0.3 mmol) were added. The mixture was stirred and simultaneously $CH_3I$ (12.3 mg 0.3 mmol) was added then, the reaction was stirred for 2 h at room temperature. After the reaction, 20 mL saturated $NH_4Cl$ was added, the mixtures was extracted with ethyl acetate. The organic layer was separated and dried over $Na_2SO_4$. After filtration and evaporation, the resulting crude product was purified by column chromatography with petrol ether as eluent to afford **15h** (40 mg, 77% yield).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.22–7.93 (m, 2H), 7.86–7.84 (m, 1H), 7.63–7.57 (m, 2H), 7.39–7.34 (m, 2H), 4.61 (dd, $J$ = 11.0, 4.6 Hz, 1H), 3.52–3.48 (m, 3H), 1.61 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 179.83, 174.88, 137.11, 136.19, 135.26, 133.52 (q, $J$ = 33.0 Hz), 132.26, 129.82, 129.57, 127.15 (q, $J$ = 4.8 Hz), 123.64 (q, $J$ = 281.0 Hz), 121.85 (q, $J$ = 276.3 Hz), 114.84, 110.20, 80.79 (q, $J$ = 31.3 Hz), 66.56, 58.99, 23.75. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.96, −62.02, −76.36, −76.47. HRMS (EI) calcd for $C_{22}H_{17}F_6N_3O_2S$ $[M + H]^+$: 502.1018, found: 502.1020.

*4-(4,4-Dimethyl-5-oxo-2-thioxo-3-(4-(2,2,2-trifluoroacetyl)phenyl) imidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile (**15i**).* In a 25 mL round-bottomed flask, **13b** (49 mg, 0.1 mmol), DMSO (5 mL), and IBX (84 mg, 0.3 mmol) were added. The mixture was stirred for 12 h at room temperature. After the reaction, the mixture was poured over and filtered through a silica gel pad under vacuum the filtrate was added with EA (15 mL) and washed with water (20 mL × 3) and saturated brine (15 mL × 3). The organic layer was separated and dried over $Na_2SO_4$. After filtration and evaporation, the resulting crude product was purified by column chromatography with petrol ether as eluent to afford **15i** (45 mg, 90% yield).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.24 (dd, $J$ = 22.9, 8.3 Hz, 2H), 7.99 (dd, $J$ = 9.2, 4.7 Hz, 2H), 7.92–7.77 (m, 1H), 7.58 (dd, $J$ = 22.9, 8.6 Hz, 2H), 1.79–1.52 (m, 6H). $^{13}$C NMR (101 MHz, CDCl3) $\delta$ 179.73, 179.49 (q, $J$ = 35.1 Hz), 174.43, 141.68, 136.84, 135.35, 133.69 (q, $J$ = 34.9 Hz), 132.18, 131.66, 130.62, 130.55, 127.07 (q, $J$ = 5.1 Hz), 121.86 (q, $J$ = 273.4 Hz), 116.46 (q, $J$ = 290.1 Hz), 114.76, 110.42, 66.76, 23.95. $^{19}$F NMR (376 MHz, $CDCl_3$) $\delta$ −61.97, −71.55. HRMS (EI) calcd for $C_{21}H_{13}F_6N_3O_2S$ $[M + H]^+$: 486.0705, found: 486.0708.

### 4.3 Cell proliferation inhibition assay

Cell viability was determined using the CCK8 assay method. The cells were seeded in a 96-well plate at $3 \times 10^3$ cells per well for 24 hours (37 °C, 5% $CO_2$), and an equal volume of medium containing increasing concentration of inhibitors was added to each well. After a 5 days incubation, CCK8 reagent was added for 1.5 hours. The light absorption (OD) of the 96-well plate was measured at 490 nm using a SpectraMAX M5 microplate spectrophotometer (Molecular Devices). All experiments were performed in triplicate. The percentage of viable cells was calculated and compared with that of the control cells, the half maximal inhibitory concentration ($IC_{50}$) was calculated by GraphPad Prism5 software.

### 4.4 Western blot analysis

Cells were treated with a series of concentrations of **13b** and ENT for 5 days at 37 °C, then the cells were harvested, washed in ice-cold PBS, analyzed with RIPA buffer (10 mM Tris–HCl (pH 7.8), 1% NP40, 0.15 M NaCl, 1 mM EDTA, 10 μM aprotinin, 1 mM NaF and 1 mM $Na_3VO_4$), protease inhibitors, phosphatase cocktails A and B, and PMSF (1 mM). Protein concentration was determined by the BCA Protein Assay Kit (beyotime#p0012s), the sample proteins were separated by 10% SDS-PAGE gel and transferred onto 0.2 um polyvinylidene difluoride membranes (millpore#ISEQ00010), then incubated overnight at 4 °C with the AR or PSA antibody overnight at the indicated concentrations in 5% BSA/TBST buffer with gently shaking, then washing with 1 × TBS/T 3 times and followed by incubation for 1.5 hour with a 1/5000 dilution of secondary HRP antibody in 5% nonfat milk/TBST. The target blots were detected with a chemiluminescence system.

### 4.5 *In vivo* model

The animal studies were carried out under protocols approved by the animal protection law of the People's Republic of China Care and Use Committees. All rodent studies were also carried out in accordance with the guidelines of Institutional Animal Care and Treatment Committee of Sichuan University in China. The male CB17 SCID mice were purchased (Beijing HFK Bioscience Co. ltd., Beijing, China). LNCap-AR cells were harvested during the exponential-growth phase, washed twice with serum-free medium, and re-suspended at a concentration of $1 \times 10^7$ mL$^{-1}$. One hundred microliters of the cell suspension was injected subcutaneously into the hind flank of each male CB17 SCID mouse (6–7 weeks old) after castrated 2 days. The tumors were allowed to grow to 100–150 mm$^3$, at which point the mice

were randomized into 5 groups (8 mice for each group). The mice were dosed orally with **13b** (15 mg per kg q.d, 15 mg per kg q.3d, or 5 mg per kg q.3d), vehicle, and the reference compound ENT (15 mg per kg q.d). Tumor growth and body weight were measured every 3 days using vernier calipers for the duration of the treatment. The volume was calculated as follows: tumor volume $= a \times b^2/2$ ($a$, long diameter; $b$, short diameter).



## Acknowledgements

This work is financially supported by Hinova Pharmaceuticals Inc. and also partially supported by National Natural Science Foundation of China (No. 81472418, 81672951). We also thank the State Key Laboratory of Biotherapy and Cancer Center of Sichuan University for providing analytical service.

## References and notes

1 Y. N. Wong, R. Ferraldeschi, G. Attard and J. de Bono, *Nat. Rev. Clin. Oncol.*, 2014, **11**, 365.
2 J. K. Rane, D. Pellacani and N. J. Maitland, *Nat. Rev. Urol.*, 2012, **9**, 595.
3 J. K. Myung, C. A. Banuelos, J. G. Fernandez, N. R. Mawji, J. Wang, A. H. Tien, Y. C. Yang, I. Tavakoli, S. Haile, K. Watt, I. J. McEwan, S. Plymate, R. J. Andersen and M. D. Sadar, *J. Clin. Invest.*, 2013, **123**, 2948.
4 L. Yin, Q. Hu and R. Hartmann, *Int. J. Mol. Sci.*, 2013, **14**, 13958.
5 T. M. Beer, A. J. Armstrong, D. E. Rathkopf, Y. Loriot, C. N. Sternberg, C. S. Higano, P. Iversen, S. Bhattacharya, J. Carles, S. Chowdhury, I. D. Davis, J. S. de Bono, C. P. Evans, K. Fizazi, A. M. Joshua, C. S. Kim, G. Kimura, P. Mainwaring, H. Mansbach, K. Miller, S. B. Noonberg, F. Perabo, D. Phung, F. Saad, H. I. Scher, M. E. Taplin, P. M. Venner, B. Tombal and P. Investigators, *N. Engl. J. Med.*, 2014, **371**, 424.
6 A. Parray, H. R. Siddique, S. Nanda, B. R. Konety and M. Saleem, *Biol.: Targets Ther.*, 2012, **6**, 267.
7 C. Helsen, T. Van den Broeck, A. Voet, S. Prekovic, H. Van Poppel, S. Joniau and F. Claessens, *Endocr.-Relat. Cancer*, 2014, **21**, T105.
8 A. M. Moilanen, R. Riikonen, R. Oksala, L. Ravanti, E. Aho, G. Wohlfahrt, P. S. Nykanen, O. P. Tormakangas, J. J. Palvimo and P. J. Kallio, *Sci. Rep.*, 2015, **5**, 12007.
9 C. Tran, S. Ouk, N. J. Clegg, Y. Chen, P. A. Watson, V. Arora, J. Wongvipat, P. M. Smith-Jones, D. Yoo, A. Kwon, T. Wasielewska, D. Welsbie, C. D. Chen, C. S. Higano, T. M. Beer, D. T. Hung, H. I. Scher, M. E. Jung and C. L. Sawyers, *Science*, 2009, **324**, 787.
10 Y. M. Ning, W. Pierce, V. E. Maher, S. Karuri, S. H. Tang, H. J. Chiu, T. Palmby, J. F. Zirkelbach, D. Marathe, N. Mehrotra, Q. Liu, D. Ghosh, C. L. Cottrell, J. Leighton, R. Sridhara, A. Ibrahim, R. Justice and R. Pazdur, *Clin. Cancer Res.*, 2013, **19**, 6067.
11 H. I. Scher, K. Fizazi, F. Saad, M. E. Taplin, C. N. Sternberg, K. Miller, R. de Wit, P. Mulders, K. N. Chi, N. D. Shore, A. J. Armstrong, T. W. Flaig, A. Flechon, P. Mainwaring, M. Fleming, J. D. Hainsworth, M. Hirmand, B. Selby, L. Seely, J. S. de Bono and AInvestigators, *N. Engl. J. Med.*, 2012, **367**, 1187.
12 M. Korpal, J. M. Korn, X. Gao, D. P. Rakiec, D. A. Ruddy, S. Doshi, J. Yuan, S. G. Kovats, S. Kim, V. G. Cooke, J. E. Monahan, F. Stegmeier, T. M. Roberts, W. R. Sellers, W. Zhou and P. Zhu, *Cancer Discovery*, 2013, **3**, 1030.
13 W. C. Black, C. I. Bayly, D. E. Davis, S. Desmarais, J. P. Falgueyret, S. Leger, C. S. Li, F. Masse, D. J. McKay, J. T. Palmer, M. D. Percival, J. Robichaud, N. Tsou and R. Zamboni, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4741.
14 J. A. Gibbons, T. Ouatas, W. Krauwinkel, Y. Ohtsu, J. S. van der Walt, V. Beddo, M. de Vries and J. Mordenti, *Clin. Pharmacokinet.*, 2015, **54**, 1043.
15 J. Jiang, X. Pang, L. Li, X. Dai, X. Diao, X. Chen, D. Zhong, Y. Wang and Y. Chen, *Drug Des., Dev. Ther.*, 2016, **10**, 2181.
16 N. J. Clegg, J. Wongvipat, J. D. Joseph, C. Tran, S. Ouk, A. Dilhas, Y. Chen, K. Grillot, E. D. Bischoff, L. Cai, A. Aparicio, S. Dorow, V. Arora, G. Shao, J. Qian, H. Zhao, G. Yang, C. Cao, J. Sensintaffar, T. Wasielewska, M. R. Herbert, C. Bonnefous, B. Darimont, H. I. Scher, P. Smith-Jones, M. Klang, N. D. Smith, E. De Stanchina, N. Wu, O. Ouerfelli, P. J. Rix, R. A. Heyman, M. E. Jung, C. L. Sawyers and J. H. Hager, *Cancer Res.*, 2012, **72**, 1494.
17 H. Cheng, Y. Pei, F. Leng, J. Li, A. Liang, D. Zou, Y. Wu and Y. Wu, *Tetrahedron Lett.*, 2013, **54**, 4483.
18 N. D. Smith, C. Bonnefous and J. D. Julien, WO 2011103202A2, 2011.
19 M. D. Balabas, M. J. Evans, C. L. Sawyers, S. Yang, D. Hosfield and G. L. Greene, WO 2014066799A2, 2014.
20 Y. Chen and Y. Gong, WO 2014190895A1, 2014.