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Research paper

# Discovery and biological evaluation of novel androgen receptor antagonist for castration-resistant prostate cancer

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ABSTRACT

Prostate cancer (PC) is the second most common malignancy in men worldwide. Among current therapies, two antiandrogens, Abiraterone Acetate and Enzalutamide (Enza) have become the standard of care for patients with metastatic castration-resistant prostate cancer (mCRPC). Here, we designed and synthesized a new series of nonsteroidal compounds deriving from the hybridization of Abiraterone (Abi) and Enzalutamide, among which compound **4a** featuring the diphenylamine scaffold was identified as a potent and cell selective androgen receptor (AR) antagonist. In cell proliferation assays, compound **4a** exhibited better antiproliferative activities than Enzalutamide against AR-overexpressing VCaP cells and 22Rv1 cells bearing H874Y-mutated AR. In addition, **4a** suppressed the activity of AR-F876L mutant that confers resistance to Enzalutamide and efficiently blocked R1881-induced PSA and FKBP5 gene expression. In competitive binding assay, compound **4a** displayed higher binding affinity to AR than Enzalutamide. These results suggest compound **4a** as a potential candidate to treat Enza-resistant CRPC.


## 1. Introduction

Prostate cancer (PC) is the second most commonly diagnosed male malignancy and the fifth leading cause of cancer mortality in men worldwide [1]. The androgen receptor (AR), a ligand-dependent transcription factor, is highly expressed throughout various stages of prostate cancer [2,3]. The dysregulation of AR signaling is crucial for the occurrence and progression of prostate cancer. Multiple mechanisms have been identified to confirm maintained AR signaling in CRPC, including AR amplifications, increased AR expression and the presence of point mutations in ligand binding domain (LBD) [4–6].

Until 2010, the docetaxel-based chemotherapy was the only strategy known to extend survival in patients with mCRPC [7–10]. Since 2011, three AR-axis inhibitors (Fig. 1A) have been approved by FDA including Abiraterone Acetate, Enzalutamide for mCRPC and most recently Apalutamide (formerly known as ARN509) for non-metastatic CRPC [11–13]. Abiraterone, a steroidal antiandrogen, blocked dihydrotestosterone (DHT) biosynthesis through suppressing the 17-hydroxylase/17,20-lyase (CYP17A1), which represents a breakthrough for the treatment of CRPC [14–16]. Enzalutamide and Apalutamide are second-generation AR antagonists with superior binding affinity to the ligand binding domain of AR (AR-LBD) than the first-generation inhibitor, Bicalutamide [17,18]. Other second-generation AR antagonists (Fig. 1A) like Darolutamide (formerly known as ODM-201), Galeterone (Gal) and HC-1119, have entered clinical trials [19–21]. As a multi-target oral small molecule inhibitor, Galeterone blocked AR signaling through AR-LBD antagonism, AR degradation and inhibition of CYP17A1 [22]. Unfortunately, Galeterone failed in the phase III clinical trial (ARMOR3-SV) and the company announced the discontinuation of their phase II expansion (ARMOR2) as well on August 22, 2016 [23].

Despite the success achieved in CRPC with second generation androgen deprivation therapies (ADT), such as Abiraterone [24,25] and Enzalutamide [12,26], resistance to both therapies have been observed. The proposed mechanisms of acquired and primary resistance include up-regulation of systemic and intratumoral

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0223-5234/

**Abbreviations**

| | |
|---|---|
| AR | androgen receptor |
| AR-LBD | ligand binding domain of AR |
| PC | prostate cancer |
| CRPC | castration-resistant prostate cancer |
| mCRPC | metastatic castration-resistant prostate cancer |
| ADT | androgen deprivation therapies |
| Enza | Enzalutamide |
| Abi | Abiraterone |
| Gal | Galeterone |
| CYP17A1 | Cytochrome P450 17A1 |
| DHT | dihydrotestosterone |
| IC50 | 50% inhibitory concentration; |
| CCK-8cell counting kit-8PSA | cell counting kit-8PSAprostate specific antigen |
| FKBP5 | FK506 Binding Protein 5 |
| wt-AR | wild-type AR |
| EGFP | enhanced green fluorescence protein |
| TGI | tumor growth inhibition |
| DCM | dichloromethane |
| DMF | N,N-Dimethyl formamide |
| DMA | N,N-dimethylacetamide |
| PEG | poly(ethylene glycol) |
| 7-OHP | 17α-hydroxy progesterone. |

androgen biosynthesis, AR gene amplification or overexpression and AR mutations in the ligand-binding domain [27]. Recently, a F876L missense mutation in AR-LBD has been identified, which confers an antagonist-to-agonist switch driving the resistance to Enzalutamide and ARN-509 [28,29]. Other recurrent point mutations in AR-LBD including L701H, W741C (Bicalutamide resistance), H874Y and T877A (Flutamide resistance) are present in approximately 15–20% of CRPC cases, a frequency that grows to over 60% when AR gene amplification is included [30,31].

Therefore, there is an unmet and urgent need to develop novel and potent antiandrogens to block the AR signaling in CRPC. In this study, based on the structures of FDA-approved Enzalutamide and Abiraterone, a new class of compounds were designed, synthesized and evaluated.

## 2. Chemistry

Recently, the utilization of molecular hybridization strategy has led to the discovery of many active molecules [32–34]. Although Enzalutamide and Abiraterone feature different scaffolds, the structural similarity between these two antiandrogens attracted our attention. As described in Fig. 1B, both of them bear similar

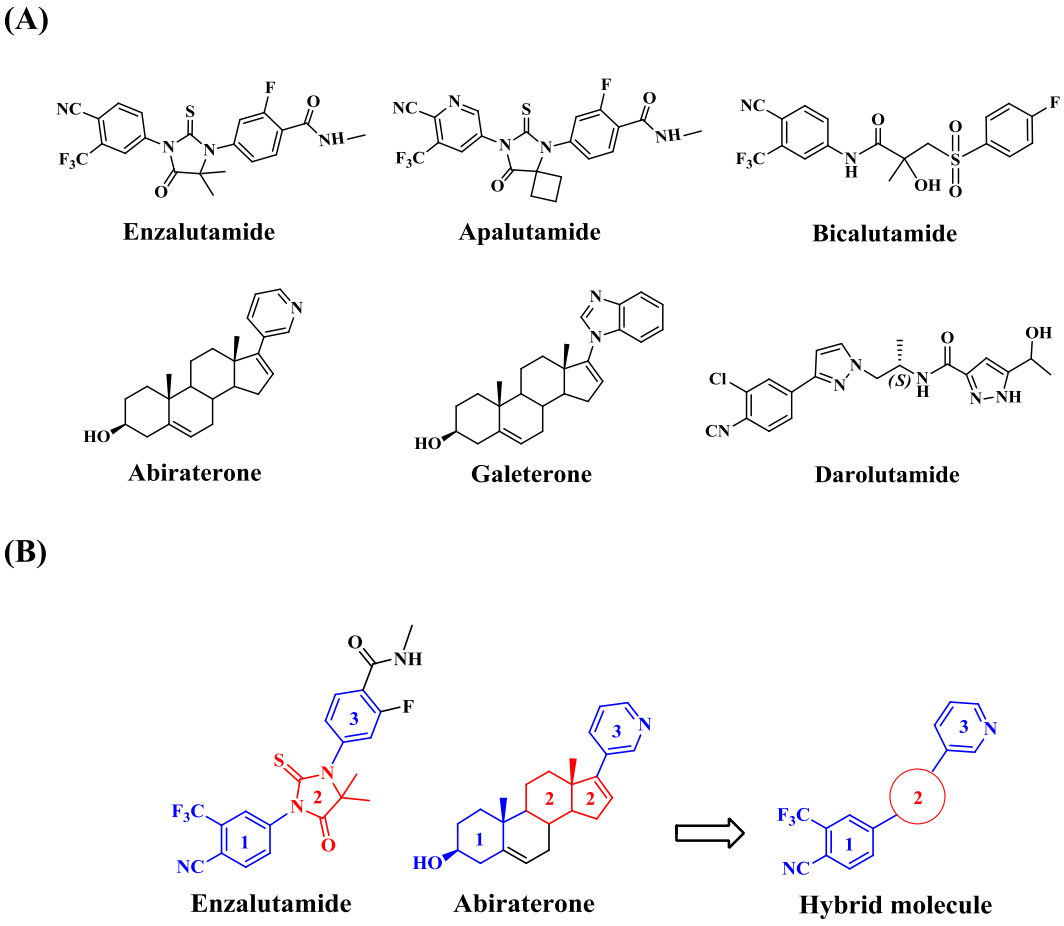



**Fig. 1.** (A) Examples of antiandrogen. **(B)** Design of novel compounds.

three-ring structure and the ring 1 and ring 3 of Enzalutamide match well with those of Abiraterone. Thus, the resulting hybrid molecules were expected to maintain activity on AR or CYP17 enzyme. Encouragingly, several compounds deriving from Pregnenolone or Galeterone with similar three-ring scaffold have been reported to potently inhibit the activities of AR or CYP17 enzyme [35,36]. Therefore, to develop novel type of compounds we started our research from hybrid molecule bearing a bicyclic linker at the position of ring 2.

To obtain desired compounds **1a-k**, intermediate **3** was synthesized following literature procedure [37] as shown in Scheme 1. The subsequent nucleophilic substitution reaction of 4-Fluoro-2-(trifluoromethyl) benzonitrile with corresponding amine (**2**–**4**) followed by Suzuki coupling yielded compounds **1a-k**. In addition, compound **4b** was successfully prepared by a two-step procedure. Nucleophilic substitution reaction using 4-Fluoro-2-(trifluoromethyl) benzonitrile (**8**) and 3-Iodophenol provided corresponding diphenyl ether (**9**), which underwent Suzuki coupling to achieve compound **4b**.

Scheme 2 describes the general procedure for the synthesis of diphenylamine analogues (**2a-h, 3a-n, 4a, 4c-e**). Diazotization of arylamine (**10**) by nitrous acid generated *in situ* and the substitution of N2 group by iodide resulted in product **12**. Next, intermediates **17**–**20** were obtained from **12** or commercially available **11** through palladium-catalyzed cross coupling and *N*-alkylation reactions. Finally, various ring C were introduced by Suzuki coupling or Cu-catalyzed *C*-N coupling reactions (**2a-h**, **3a-n**). The desired compound **4a** was synthesized using 4-((5-bromo-2-methylphenyl) amino)-2-chlorobenzonitrile (**15**) and 3,5-dimethylisoxazole-4-boronic acid pinacol ester via Suzuki coupling reaction. To achieve compounds **4c-e**, intermediate **22** was prepared from commercially available 5-Bromo-2-methylaniline (**21**) through Suzuki coupling reaction. The subsequent palladium-catalyzed *C*-N coupling afforded desired compounds **4e** and intermediate **23**, which underwent *N*-alkylation reaction to yield compounds **4d** and **4c**, respectively.

## 3. Results and discussion

### 3.1. Discovery of compound *4a*

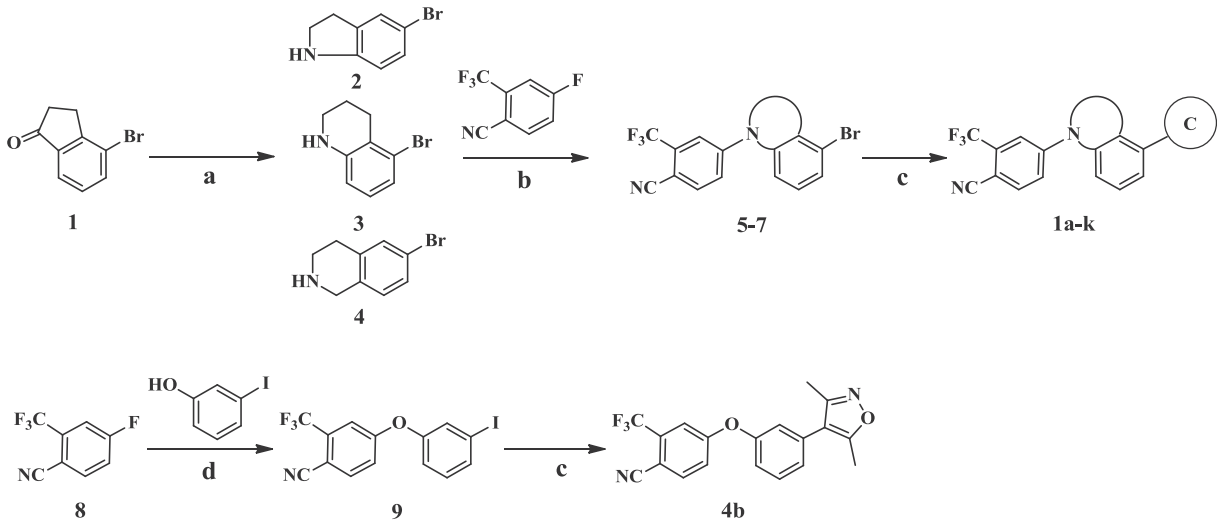
All compounds were firstly evaluated in LNCaP/AR human prostate cancer cells [17] via a CCK-8 cell proliferation assay (SAB, CP002). Compounds with $IC_{50}$ value lower than 1 μM were next tested in AR-overexpressing VCaP cells [38] or 22Rv1 cells bearing H874Y-mutated AR [39,40]. To rule out the non-AR-mediated toxicity, AR-negative human prostate cancer cells (PC-3) and non-cancer originated human hepatocyte cells (L-02) were also used.

To develop novel inhibitors, we initially designed compounds with tetrahydroisoquinoline, indoline and tetrahydroquinoline fragments (**1a-k**). As presented in Table 1, compounds bearing tetrahydroquinoline scaffold (**1h-j**) showed superior activity against LNCaP/AR cells, although poor inhibitory activities toward VCaP and 22Rv1 cells were observed.

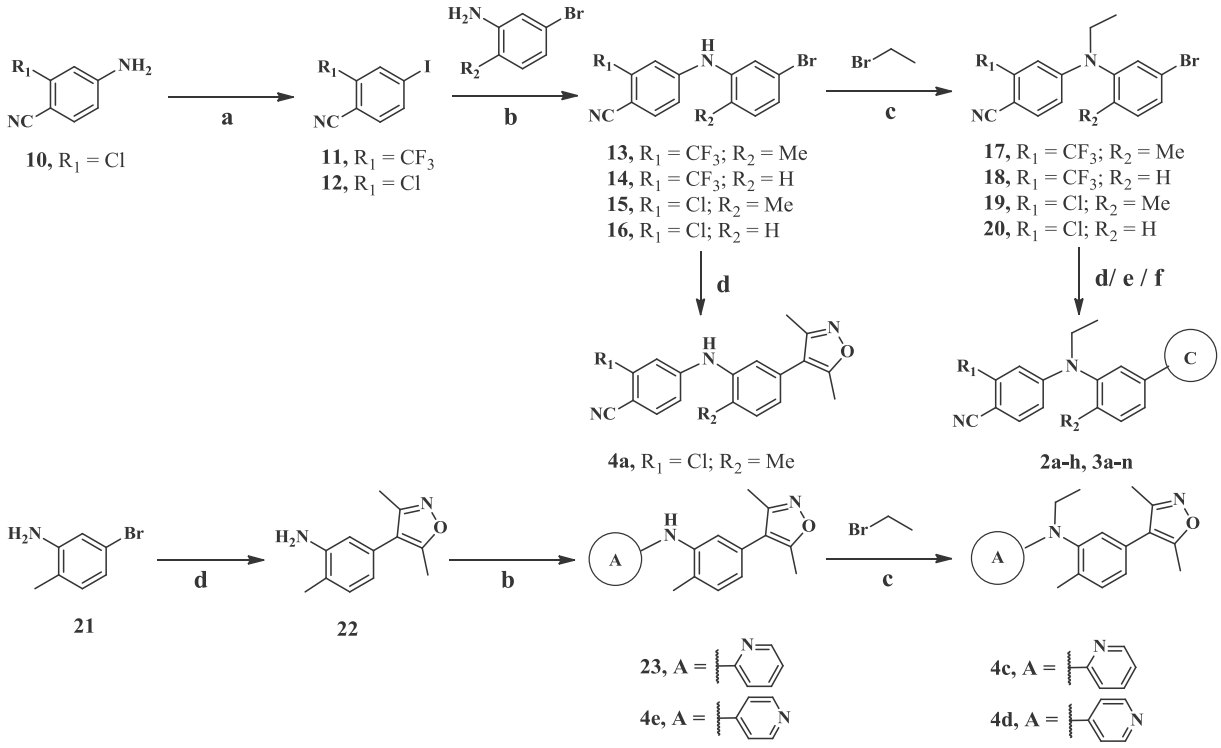
Then we simplified the tetrahydroquinoline scaffold by opening its piperidine ring. The resulting diphenylamine analogues were synthesized and evaluated. As presented in Table 2, replacing the $CF_3$ group (**2a, 2b**) with Cl (**2c, 2d**) caused a moderate increase in activities against LNCaP/AR. Comparing compounds **2b**, **2d**, **2f** with **2a**, **2c**, **2e**, the introduction of methyl group at $R_2$ position had little effect on the inhibitory activity toward LNCaP/AR cells. In addition, the phenyl substituted analogues **2g** and **2h** exhibited significantly decreased activity against LNCaP/AR with $IC_{50}$ values of 1.29 μM and 2.78 μM, respectively. This result indicates the heteroaromatic substituent on ring B is of great importance. Noteworthily, compound **2f** bearing a methyl group at $R_2$ position exhibited improved potency toward VCaP cells with an $IC_{50}$ value of 3.92 μM, suggesting that the *N*-ethyl substituted diphenylamine scaffold was worthy of further optimization.

Next, we kept Cl and methyl group as substituents at $R_1$ and $R_2$ positions and optimized ring C to achieve compounds **3a-n** (Table 3). Among them, five compounds (**3c, 3d, 3e, 3i, 3j**) exhibited favorable antiproliferative activity with $IC_{50}$ values ranging from 1.03 to 5.38 μM toward VCaP and 22Rv1 cells. And 3,5-dimethylisoxazole (**3e**) was identified as the preferred ring C, which was chosen as the terminal heteroaromatic ring for further modification.

As presented in Table 4, replacing N atom (**4a**) with O atom (**4b**) at $R_3$ position led to obvious decrease in LNCaP/AR inhibitory activity. Compound **4c** bearing 2-pyridyl group for ring A was found to be more potent than compound with 4-pyridyl group (**4d**). Importantly, compound **4a** featuring NH group as $R_3$ substituent exhibited more desirable anticancer activity toward VCaP cells ($IC_{50} = 0.70$ μM) than *N*-ethyl substituted compound **3e**




**Scheme 1.** Synthesis of compounds **1a-k** and **4b**. Reagents and conditions: (a) $H_2NOH$-HCl, MeOH, reflux, 1 h; $AlH(Bu\text{-}i)_2$, DCM, 0°C-rt, overnight; (b) NaH, DMF, 0°C-rt, 2 h; (c) Suzuki coupling, boronic acid/ester, $Pd(PPh_3)_4$, $K_2CO_3$, $H_2O$, 1,4-Dioxane, 90 °C, 6 h; (d) $K_2CO_3$, DMSO, 110 °C, 2 h.




**Scheme 2.** Synthesis of compounds **2a-h**, **3a-n**, **4a** and **4c-e**. Reagents and conditions: (a) $H_2SO_4$, $NaNO_2$, $H_2O$, KI, MeCN, 0°C-rt, overnight; (b) $Pd_2(dba)_3$, DavePhos, $Cs_2CO_3$, MeCN, reflux, 5 h; (c) Bromoethane, NaH, DMF, 0°C-rt, 2 h; (d) Suzuki coupling, boronic acid/ester, $Pd(PPh_3)_4$, $K_2CO_3$, $H_2O$, 1,4-Dioxane, 90 °C, 6 h; (e) Benzimidazole/imidazole, CuI, $Cs_2CO_3$, DMF, 120 °C, 40 h; (f) Pyrazole, CuI, NaH, DMSO, 120 °C, overnight.

($IC_{50} = 1.20\ \mu M$) and better cell selectivity than 2-pyridyl substituted compound **4c**.

### 3.2. Compound *4a* antagonizes wild-type AR and the AR-F876L mutant

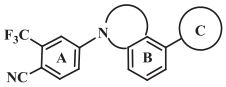
To investigate whether our compounds suppress AR-regulated gene transcription in prostate cancer cells, qRT-PCR analysis was performed on VCaP cells. As illustrated in Fig. 2A, compounds **2f, 3d, 3i, 4a** and **4e** caused significant inhibition of R1881-induced PSA and FKBP5 mRNA expression at a concentration of 5 μM. And the most potent compound **4a** reduced PSA and FKBP5 mRNA expression in a concentration-dependent manner (Fig. 2B). Thus, given its favorable cell selectivity and potent anti-cancer activities against different PC cells (Fig. 2C), compound 4a was selected for further studies. Next, the direct interaction between 4a and AR-LBD was demonstrated via a competitive AR binding assay. Compound 4a was measured in competition with radio ligand [$^3$H] R1881 in cytosolic lysates from LNCaP cells using endogenous hormone progesterone and synthetic androgen R1881 as standards. As shown in Fig. 2D, R1881 and progesterone displayed strong binding potency to AR-LBD with $IC_{50}$ values of 4.6 nM and 13.7 nM respectively, which indicated the feasibility of this assay system. Satisfyingly, compound **4a** ($IC_{50} = 641.3$ nM) was found ~8-fold more effective than Enzalutamide ($IC_{50} = 5336.0$ nM), suggesting that compound **4a** antagonizes AR by targeting the ligand binding domain. However, the binding affinity of Enzalutamide was lower than that in other papers [17,18,33], possibly because of the different source of AR protein and different radioactive ligand used.

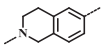
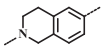
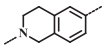
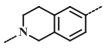
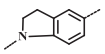
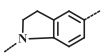
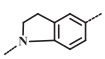
The emergence of mutant ARs have been reported to be linked to resistance to first- and second-generation AR antagonists. A missense mutation (F876L) in AR-LBD was identified to mediate Enzalutamide resistance [28]. Therefore, the effect of compound **4a** on AR-F876L mutant was next measured via dual luciferase reporter assay using AR luciferase reporter system (CCS-1019L; QIAGEN). As shown in Fig. S1, R1881 significantly induced the expression of luciferase even at a concentration of 0.3 nM. Satisfyingly, compound 4a was found to suppress 1 nM R1881-induced AR-F876L transactivation in a dose-dependent manner (Fig. 3A). As expected, treatment with high concentration (10 μM and 20 μM) of Enzalutamide caused obvious increase in AR-F876L transcription compared to those of low concentration (0.1 μM and 1 μM), which was in accord with Moilanen's report [41]. On the other hand, both of Enzalutamide and compound **4a** potently suppressed the transcriptional activity of wild-type AR (wt-AR) (Fig. 3B). Furthermore, to measure the effect of compound 4a on AR nuclear translocation, WPMY-1 cells were transfected with enhanced green fluorescence protein (EGFP) tagged ARs. As shown in Fig. 3C–D, compound 4a prevented wt-AR and AR-F876L mutant from translocating into the nucleus in the presence of 0.5 nM R1881. In comparison to 4a, treatment with Enzalutamide increased the nuclear translocation of AR-F876L mutant but not wt-AR. These results confirm that compound **4a** effectively suppress the activity of wt-AR and the AR-F876L mutant *in vitro*, indicating that compound **4a** has the potential to overcome Enzalutamide resistance.

### 3.3. Molecular modeling

To investigate the possible binding mode of compound **4a**, molecular docking simulation was performed using Autodock 4.0 [42]. Compounds were docked into the active site of AR protein crystal structures (PDB ID: 3V49) [43]. As shown in Fig. 4B, the cyano group of compound **4a** formed a key hydrogen bond interaction with Arg752, which was similar to that of Enzalutamide. The methyl group on benzene ring of **4a** formed hydrophobic interactions with the side chains of Phe764, Leu704, Met780. And the

**Table 1**
*In vitro* antiproliferative activities of **1a-k**.[a].




| Compd | B | C | IC50 (μM) | | | | |
|---|---|---|---|---|---|---|---|
| | | | LNCaP/AR | VCaP | 22Rv1 | PC-3 | L-02 |
| Enza | _ | _ | 0.19 | 50%@30 | >30 | >30 | 17.10 |
| 1a |  |  | 0.76 | 2.53 | 4.79 | NT | NT |
| 1b |  |  | 0.98 | 3.50 | 6.94 | NT | NT |
| 1c |  |  | 1.67 | NT | NT | NT | NT |
| 1d |  |  | 0.98 | >30 | 25.90 | 25.10 | NT |
| 1e |  |  | 1.24 | NT | NT | NT | NT |
| 1f |  |  | 21.00 | NT | NT | NT | NT |
| 1g |  |  | 11.50 | NT | NT | NT | NT |
| 1h |  |  | 0.12 | 13.50 | 12.30 | 7.97 | 10.50 |
| 1i |  |  | 0.07 | >30 | 15.00 | 28.70 | 6.38 |
| 1j |  |  | 0.24 | >30 | 10.39 | 9.80 | NT |
| 1k |  |  | 1.42 | >30 | NT | NT | NT |

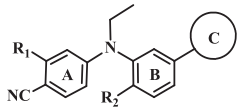
[a] $IC_{50}$, 50% inhibitory concentration. $IC_{50}$ values are the mean value of at least two experiments with duplicate measurements. The deviations were less than 40%. 50% @30, 50% inhibition at 30 μM. NT, not tested.

3-methyl substituent on isoxazole moiety of compound **4a** had contact with the hydrophobic pocket formed by the residues of Leu873, His874 and Met742. Differently, Enzalutamide formed additional hydrogen bond interactions through its nitrogen and fluorine atoms with the side chains of Gln711 and His874 (Fig. 4A). And the sigma-pi interaction between benzene ring of Enzalutamide and Met742 was also observed. Therefore, the distinctive binding modes of compound **4a** and Enzalutamide with AR might contribute to their different inhibitory abilities toward the AR-F876L mutant and 22Rv1 cells bearing H874Y-mutated AR *in vitro*.

### 3.4. Compound *4a* has little effect on CYP17A1 hydroxylase

Since compound **4a** is the hybrid of Abiraterone and Enzalutamide, it is expected to still inhibit the catalytic activity of CYP17A1 enzyme. Thus, we measured the effect of **4a** on the production of 17α-hydroxyprogesterone (17-OHP) from progesterone, which was catalyzed by CYP17A1. As shown in Fig. 5, Abiraterone completely suppressed the formation of 17-OHP at a concentration of 10 μM. And nearly 50% inhibition of CYP17A1 hydroxylase was also observed by Galeterone at the same concentration. Interestingly, out of our expectation, compound **4a** slightly blocked the production of 17-OHP, which didn't reach statistical significance

**Table 2**
*In vitro* antiproliferative activities of **2a-h**.[a].




| Compd | $R_1$ | $R_2$ | C | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | LNCaP/AR | VCaP | 22Rv1 | PC-3 | L-02 |
| Enza | _ | _ | _ | 0.19 | 50%@30 | >30 | >30 | 17.10 |
| 2a | $CF_3$ | H |  | 0.58 | >30 | 19.00 | >30 | NT |
| 2b | $CF_3$ | Me |  | 0.56 | >30 | 16.30 | >30 | NT |
| 2c | Cl | H |  | 0.33 | >30 | >30 | >30 | 23.20 |
| 2d | Cl | Me |  | 0.32 | >30 | >30 | >30 | 15.00 |
| 2e | Cl | H |  | 0.36 | >30 | >30 | >30 | NT |
| 2f | Cl | Me |  | 0.40 | 3.92 | >30 | >30 | NT |
| 2g | Cl | H |  | 1.29 | NT | NT | NT | NT |
| 2h | Cl | Me |  | 2.78 | >30 | >30 | NT | NT |

[a] $IC_{50}$, 50% inhibitory concentration. $IC_{50}$ values are the mean value of at least two experiments with duplicate measurements. The deviations were less than 40%. 50% @30, 50% inhibition at 30 μM. NT, not tested.

(P = 0.0672).

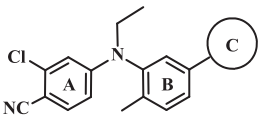
### 3.5. Antitumor effect of compound *4a* in vivo

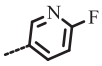
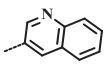
To assess the potency of compound **4a** *in vivo*, the pharmacokinetic study was conducted in Sprague-Dawley rats. The plasma level of **4a** was measured after a single oral dose of 30 mg/kg. Based on the concentration-time curve (Fig. 6A), PK parameters of compound **4a** were calculated. As summarized in Fig. 6B, the maximum plasma concentration ($C_{max}$ = 2320.0 ng/ml) was achieved at 0.5 h after administration. Acceptable half-life ($T_{1/2}$ = 4.4 h) and the area under the curve ($AUC_{0-t}$ = 4041.9 ng*h/ml) were observed. Thus, we next evaluated the antitumor efficacy of compound **4a** *in vivo*. The BALB/c nude male mice were subcutaneously injected with VCaP cells. When the average tumor volume reached approximately 150 $mm^3$, the mice were castrated or sham-operated. When tumors regrew to 200–350 $mm^3$, oral treatments with compound **4a**, Enzalutamide or vehicle were initiated once a day for 40 days. Given its faster tumor growth than castrated groups, the sham-operated mice were administered with vehicle for only 15 days and the tumors were collected solely. As shown in Fig. 6C, the tumor growth was inhibited by compound **4a** with 44.7% tumor growth inhibition (TGI), which was equal to that of Enzalutamide (43.0%). The tumor weights of each group were respectively recorded at the end of treatment (Fig. 6D). And no significant weight loss was observed during the treatment (Fig. S2).

## 4. Conclusions

In conclusion, a new series of nonsteroidal AR antagonists deriving from the hybridization of Enzalutamide and Abiraterone

**Table 3**
*In vitro* antiproliferative activities of **2d**, **3a-n**.[a].

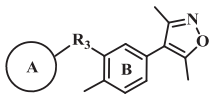



| Compd | C | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | LNCaP/AR | VCaP | 22Rv1 | PC-3 | L-02 |
| Enza | – | 0.19 | 50%@30 | >30 | >30 | 17.10 |
| 2d |  | 0.32 | >30 | >30 | >30 | 15.00 |
| 3a |  | 0.29 | 2.34 | >30 | 15.70 | NT |
| 3b |  | 0.70 | NT | >30 | 15.30 | NT |
| 3c |  | 0.32 | 3.19 | 5.38 | 9.03 | 5.78 |
| 3d |  | 0.17 | 1.67 | 4.33 | 7.29 | 6.23 |
| 3e |  | 0.19 | 1.20 | 2.99 | 4.84 | 8.67 |
| 3f |  | 0.48 | 4.01 | >30 | >30 | NT |
| 3g |  | 0.49 | >30 | >30 | >30 | NT |
| 3h |  | 0.38 | NT | 22.00 | >30 | NT |
| 3i |  | 0.33 | 1.27 | 2.00 | 11.00 | 12.70 |
| 3j |  | 0.48 | 2.90 | 1.03 | 3.58 | 1.73 |
| 2b |  | 0.40 | 3.92 | NT | NT | NT |
| 3k |  | 0.41 | >30 | 16.50 | >30 | NT |
| 3l |  | 1.98 | 25.30 | NT | 3.57 | NT |
| 3 m |  | 0.39 | 8.56 | 3.04 | 8.16 | NT |
| 2h |  | 2.78 | >30 | >30 | NT | >30 |
| 3n |  | 2.55 | 20.00 | NT | NT | NT |

[a] $IC_{50}$, 50% inhibitory concentration. $IC_{50}$ values are the mean value of at least two experiments with duplicate measurements. The deviations were less than 40%. 50% @30, 50% inhibition at 30 μM. NT, not tested.

were designed and synthesized. Among them, compound **4a** bearing a diphenylamine scaffold was identified as the most potent compound with better inhibitory activities than Enzalutamide against AR-overexpressing VCaP cells and 22Rv1 cells bearing

**Table 4**
*In vitro* antiproliferative activities of **3e**, **4a-e**.[a].




| Compd | A | $R_3$ | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|---|
| | | | LNCaP/AR | VCaP | 22Rv1 | PC-3 | L-02 |
| Enza | – | – | 0.19 | 50%@30 | >30 | >30 | 17.10 |
| 3e |  |  | 0.19 | 1.20 | 2.99 | 4.84 | 8.67 |
| 4a |  |  | 0.43 | 0.70 | 3.50 | 23.30 | 11.30 |
| 4b[b] |  |  | 1.67 | NT | NT | NT | NT |
| 4c |  |  | 0.63 | 0.87 | 5.43 | 4.80 | 4.42 |
| 4d |  |  | 2.88 | 2.01 | NT | NT | NT |
| 4e |  |  | 3.70 | 1.97 | NT | NT | NT |

[a] $IC_{50}$, 50% inhibitory concentration. $IC_{50}$ values are the mean value of at least two experiments with duplicate measurements. The deviations were less than 40%. 50% @30, 50% inhibition at 30 μM. NT, not tested.
[b] Compound without methyl group on ring B.

H874Y-mutated AR. The direct interaction between compound **4a** and AR-LBD suggested that **4a** antagonized AR by targeting the ligand binding domain. Importantly, compound **4a** efficiently suppressed the activities of wt-AR and the AR-F876L mutant that confers resistance to Enzalutamide. The antitumor activity of **4a** was also proved in castration-resistant VCaP xenograft model *in vivo*. Therefore, these results make compound **4a** promising lead compound for the treatment of Enza-resistant CRPC. Further efforts have focused on the structural optimization of compound **4a** to improve the pharmacokinetic profile and antitumor activity *in vivo*.

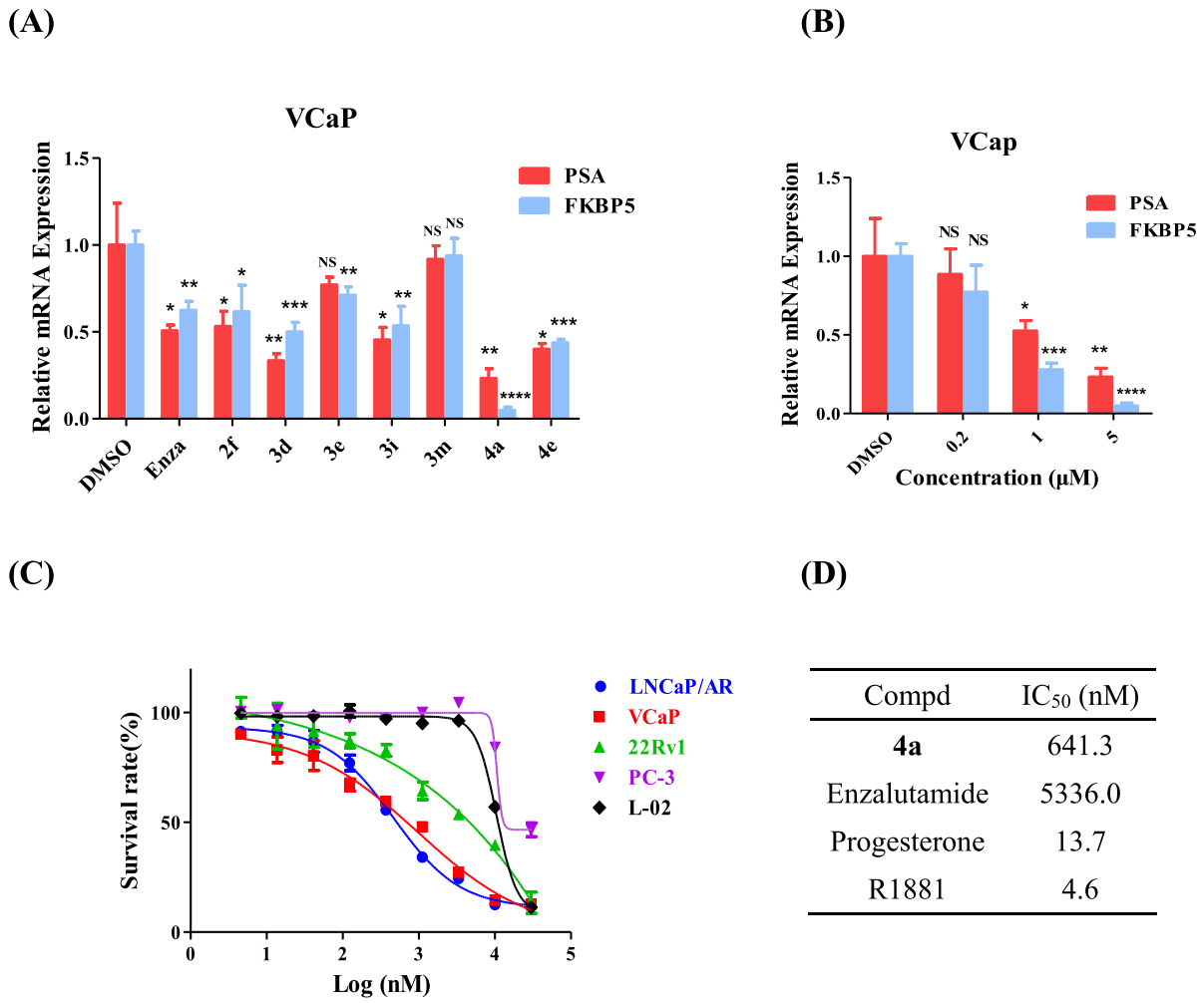
## 5. Experimental section

### 5.1. Cell culture

LNCaP/AR cells were gift from Kang Cheng Biotech (KC BIO, Sichuan, China). Other cells used were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). LNCaP/AR, 22Rv1 (TCHu100) and L-02 (GNHu6) cells were cultured in RPMI-1640, VCaP (TCHu220) and WPMY-1 (GNHu36) cells in DMEM, PC-3 (SCSP-532) in a mixture of DMEM and F12 (1:1) nutrient medium supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) penicillin/streptomycin (HyClone, SV30010). Cells were grown in an incubator with 5% $CO_2$ at 37 °C.

### 5.2. CCK-8 cell proliferation assay

The cells were seeded in 96-well plate at a density of $2 \times 10^4$ cells for VCaP and $1–3 \times 10^3$ cells per well for other cells. After 24 h, an equal volume of medium containing various concentrations of compounds was added to each well (9-point and 3-fold serial dilution starting at 30 μM). Cells were treated for 6 days and then CCK-8 reagent (SAB, CP002) was added. After an additional 1–4 h incubation, the absorbance value (OD) was measured




| Compd | $IC_{50}$ (nM) |
|---|---|
| **4a** | 641.3 |
| Enzalutamide | 5336.0 |
| Progesterone | 13.7 |
| R1881 | 4.6 |

**Figure 2.** (A, B) qRT-PCR analysis for 0.2 nM R1881-induced PSA and FKBP5 mRNA expression in VCaP cells treated with **(A)** compounds at a concentration of 5 μM; **(B)** compound **4a** at different concentrations for 24 h. The relative mRNA expression was normalized to GAPDH. Data were expressed as the mean ± SD (n = 3). * indicates significance as $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared to samples treated with DMSO by two-tailed *t*-test. NS, no significant difference. **(C)** Growth inhibitory curves of compound **4a** in different cell lines. **(D)** Binding affinity of **4a** to AR-LBD. $IC_{50}$, 50% inhibitory concentration. 1 nM radioligand [$^3$H] R1881 and LNCaP cytosol were used.

at 450 nm via spectrophotometry (Thermo Multiskan MK3). $IC_{50}$ was the mean value of at least two independent experiments with duplicate measurements and calculated by GraphPad Prism 5 software.

### 5.3. QPCR

Cells were maintained in 6-well plates in DMEM supplemented with 5% charcoal stripped FBS and 1% penicillin-streptomycin for 48 h and then treated with combination of antiandrogen and 0.2 nM R1881 (Nanjing Chemlin Chemical Industrial Co., Ltd., CAS: 965-93-5). RNA was extracted using TRIzol reagent (Invitrogen, 15596026) and cDNA was subsequently synthesized using reverse conversion kit (abm, G492). Diluted cDNA and gene-specific primers were mixed with EvaGreen qPCR MasterMix (abm, MasterMix-S) and the transcripts were measured by CFX96™ Real-Time PCR Detection Systems (BIO-RAD). Primers were purchased from GeneCopoeia Inc (PSA: HQP009633, FKBP5: HQP057374). The relative expression of PSA or FKBP5 was quantified and normalized to GAPDH (GeneCopoeia, HQP006940). The positive control, Enzalutamide, was a gift from Hinova Pharmaceuticals Inc. (Sichuan, China).

### 5.4. Competitive AR binding assay

The assay was performed using [$^3$H] R1881 (PerkinElmer, Cat: NET590250UC, Lot: 2133648) and LNCaP cytosol containing wild-type AR and T877A mutant. Transfer 1 μl of serial dilution reference compounds and testing compounds to assay plate (8-point and 4-fold serial dilution starting at 10 μM for **4a** and Enza or starting at 1 μM for Progesterone and R1881.1 μl of 2 mM Progesterone was transferred to assay plate for nonspecific binding and DMSO was used for total binding. Then 100 μl of cell cytosol stocks and 100 μl of radio ligand (final concentration was 1 nM) were added into the plate. The plate was sealed and incubated at 4 °C for 24 h. When binding completed, 100 μl of radioligand adsorption buffer was added, which was shook at 4 °C for 15 min. Then, the plate was centrifuged for 20 min at 4600 rpm, 4 °C. 100 μl of supernatant was transferred into scint-tube (PerkinElmer, Cat: 6000192) followed by addition of 2 ml Ultima Gold cocktail (PerkinElmer, Cat: 6013329, Lot: 77–16371). TriCap was used for scintillation counting. Calculate the "Inhibition [% Control]" using the equation: %Inh = (1-Background subtracted Assay value/Background subtracted HC value) *100 (Accomplished by WuXi Apptec Co., Ltd., China)

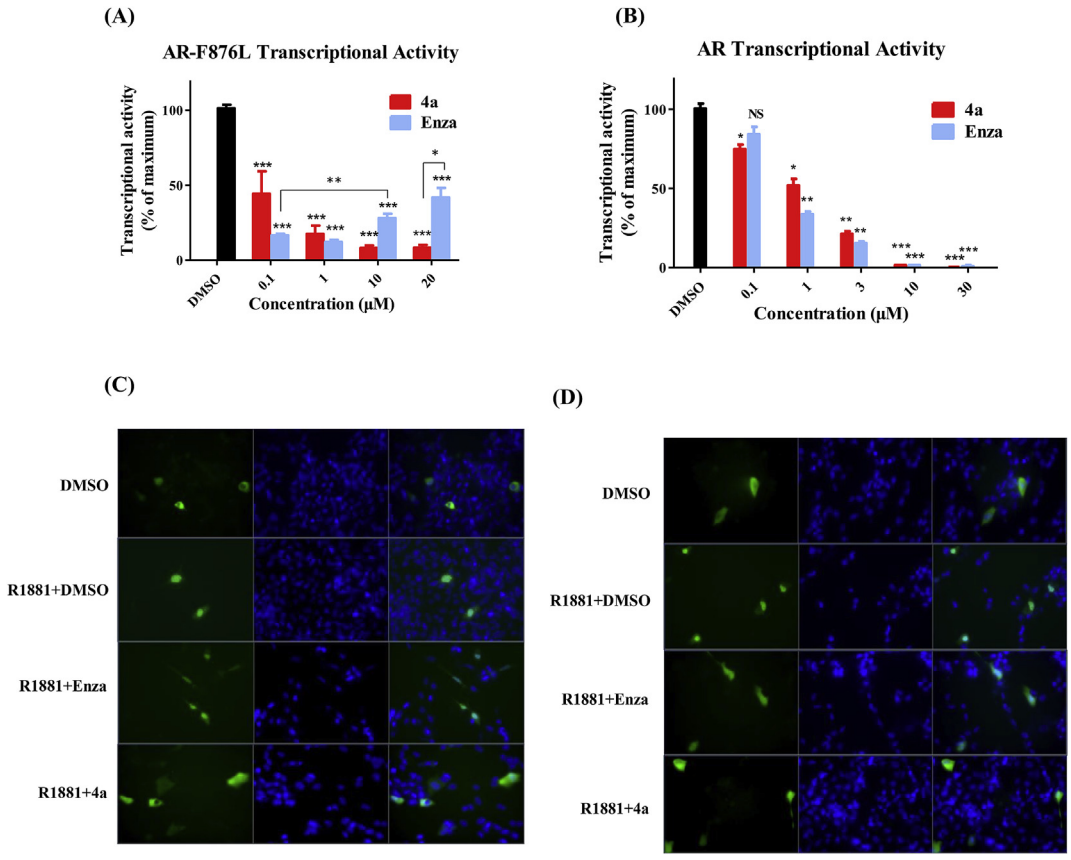



**Fig. 3.** (A) Compound **4a** inhibited R1881-induced transactivation of AR-F876L. PC-3 cells maintained in medium supplemented with charcoal stripped FBS were treated with compound and 1 nM R1881 for 24 h. The Firefly luciferase signal was normalized to Renilla luciferase signal. Data were expressed as the mean ± SEM (n = 3). **(B)** Compound **4a** and Enzalutamide inhibited the transcriptional activity of wt-AR. HEK293 cells maintained in medium supplemented with dialyzed FBS were treated with compound in the presence of 2 nM testosterone for 24 h. **(C, D)** Nuclear localization of transfected **(C)** AR-F876L mutant and **(D)** wt-AR in WPMY-1 cells treated with vehicle or 10 μM compound in the presence or absence of 0.5 nM R1881 for 24 h. The nuclei were labeled with Hoechst 33342 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 5.5. Dual luciferase reporter assay

AR-negative PC-3 cells were grown in 96-well plates in a mixture of DMEM and F12 (1:1) nutrient medium supplemented with 5% charcoal stripped FBS. Within 24 h, cells were transiently cotransfected with 50 ng F876L AR plasmid (established by Sangon Biotech Co., Ltd., Shanghai, China.) and 50 ng reporter plasmid (QIAGEN, CCS-1019L) per well using EndoFectin$^{TM}$-Max transfection reagent (GeneCopoeia, EF003) according to the manufacturer's instructions. 24 h after transfection, cells were treated with combination of compound and 1 nM R1881 for another 24 h. Firefly and Renilla luciferase activities were measured by the Dual Luciferase Kit (Promega, E1910) according to the manufacturer's instructions. The luminescence was recorded on a configurable multimode microplate reader (BioTek, Synergy H1) and the Firefly luciferase signal was normalized to that of the Renilla as relative luciferase units.

### 5.6. Wt-AR reporter assay

HEK293 cells were seeded into 96-well plate in 89% DMEM nutrient medium (Gibco, 31053028) supplemented with 10% Dialyzed FBS (Biological Industries, 04-011-1A) and 1% GlutaMax. At the same time, cells were transiently cotransfected with 5 ng AR plasmid (Origene, RC235415) and 100 ng reporter plasmid (Promega, E1360) per well using transfection reagent (Promega, E2311) according to the manufacturer's instructions. 24 h after transfection, cells were treated with combination of compound and 2 nM testosterone for another 24 h. Luciferase activity was measured by the addition of 100 μl Steady-Glo reagent per well (Promega, E2550) and the signal was read on Envision (Accomplished by WuXi Apptec Co., Ltd., China)

### 5.7. AR nuclear translocation

WPMY-1 cells were grown in 48-well plate in DMEM nutrient medium supplemented with 5% charcoal stripped FBS. Within 6 h, cells were transiently cotransfected with 200 ng AR-F876L EGFP plasmid (established by Sangon Biotech Co., Ltd., Shanghai, China.) per well using EndoFectin$^{TM}$-Max transfection reagent (GeneCopoeia, EF003) according to the manufacturer's instructions. The second day, cells were treated with combination of compound and 0.5 nM R1881 for another 24 h. Hoechst 33342 (Sigma, B-2261), the fluorescent stain for DNA, was added into each well according to the manufacturer's instructions. After an additional 30 min incubation at room temperature, nutrient medium was removed and the cells were washed with PBS buffer solution twice. Visualization performed on Zeiss Observer D1 fluorescence microscope (AX10 cam HRC).

### 5.8. Molecular modeling

The possible binding modes of compound **4a** and Enzalutamide with androgen receptor were analyzed by Autodock 4.0 [42]. The X-

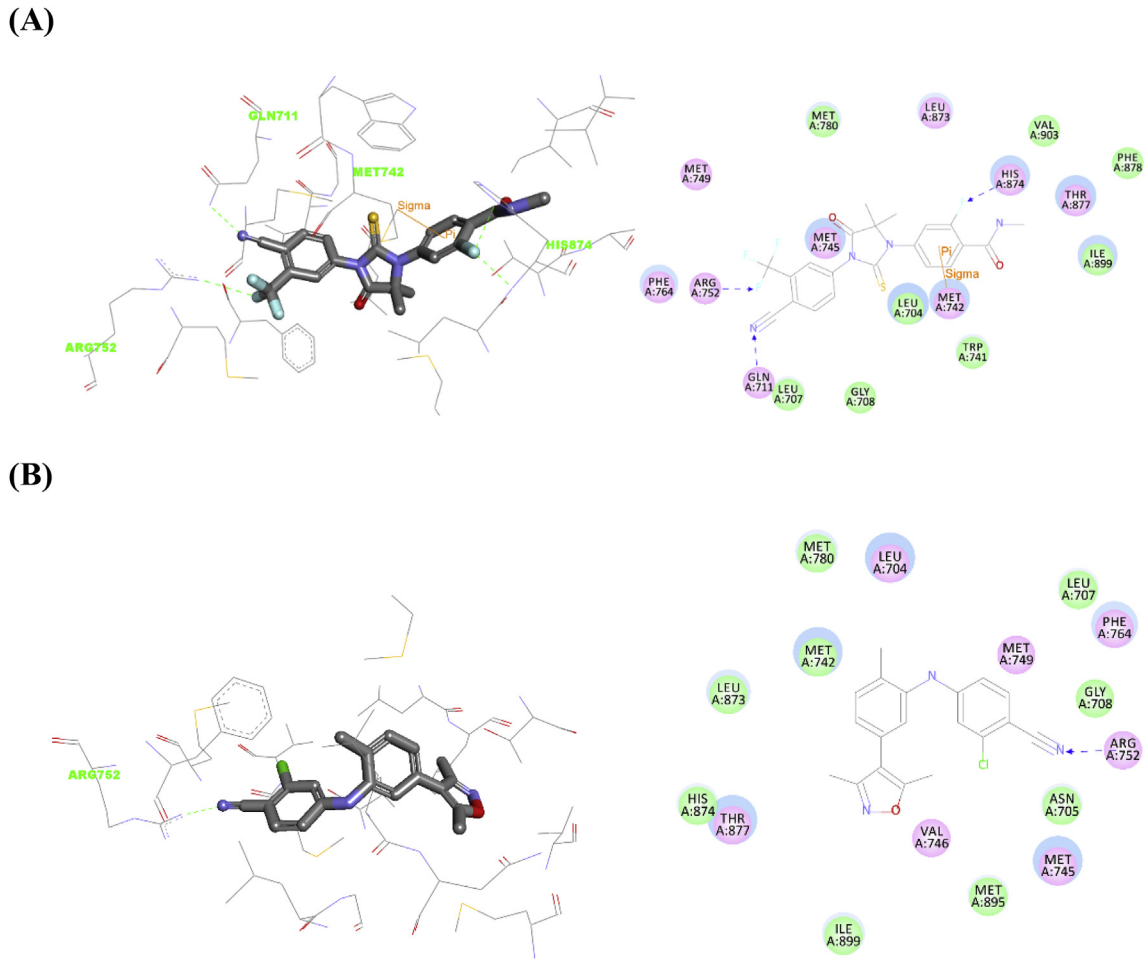



**Fig. 4.** The predicted binding modes and 2D diagram of **(A)** Enzalutamide and **(B)** compound **4a** in complex with AR (PDB ID: 3V49).

ray crystal structure of AR (PDB ID:3V49) [43] was obtained from Protein Database online (www.rcsb.org). The binding site of the ligand was chosen as active site and the grid dimensions were set to $20 \times 20 \times 20$ Å$^3$. A total of 20 conformations of each compound were generated for molecular docking. The other parameters were as default.

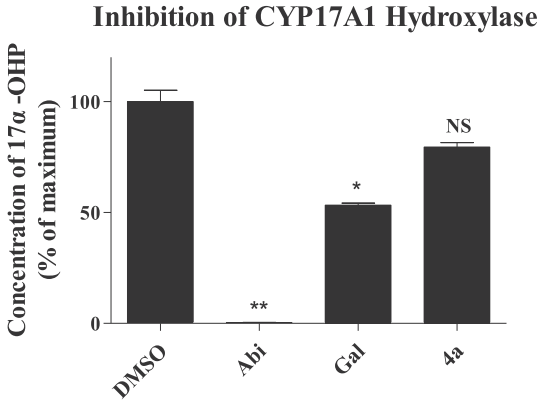



**Fig. 5.** Inhibition of CYP17A1 hydroxylase. 10 μM compounds were used. Data were expressed as the mean ± SD (n = 2). * indicates the significance as $p < 0.05$ and ** $p < 0.01$ by two-tailed *t*-test compared to samples treated with DMSO. NS, no significant difference. 17-OHP, 17α-hydroxy progesterone.

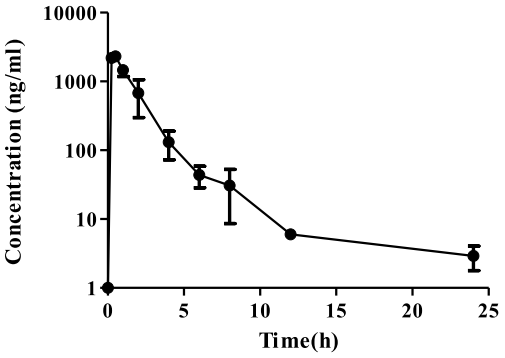
### 5.9. CYP17A1 hydroxylase assay

The catalytic activity of CYP17 enzyme was evaluated by measuring the production of 17α-hydroxyprogesterone. The reaction was carried out in a 50 μl final volume containing 1 pmol lysate of CYP17A1 (Origene, LY400030), 10 μM compound or 0.5% DMSO, 50 μM Progesterone (MedChem Express, MCE) and 1 mM NADPH (Roche) in buffer (50 mM Tris, pH 7.4 and 5 mM $MgCl_2$). The mixture was incubated at 37 °C for 30 min, which was quenched with 30 μl 20% trichloroacetic acid. The production of 17α-hydroxyprogesterone was analyzed by LC-MS/MS (Shimadzu LC-20AD; API 4000). Abiraterone (E1203570010) and Galeterone (AK323649) were purchased from Sun Chemical Technology (Shanghai) Co., Ltd. (Accomplished by Sichuan XPiscoric Inc.)

### 5.10. Pharmacokinetic studies

Compound was formulated in PEG200/DMA (9:1, v/v) for a single dose of 30 mg/kg in male SD rats (N = 3). Blood samples were withdrawn at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h from retrobulbar vein and stored on ice (0–4 °C). Heparinized plasma was obtained by centrifugation (8000 rpm for 5 min at 4 °C) and then stored at −20 °C. Compound was separated from plasma samples via protein precipitation with methol. The concentration of plasma drug was determined by LC-MS/MS (Shimadzu LC-20AD; API 4000). The pharmacokinetic parameters were analyzed by non-compartmental method using WinNonlin 5.2 (Accomplished by Sichuan XPiscoric Inc.).

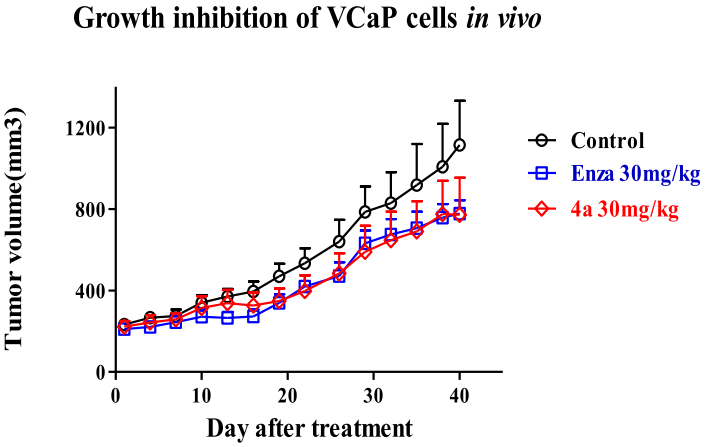
(A)




(B)

| Compd | **4a** |
|---|---|
| $T_{1/2}$ (h) | 4.4 |
| $T_{max}$ (h) | 0.5 |
| $C_{max}$ (ng/ml) | 2320.0 |
| $AUC_{0-t}$ (ng*h/ml) | 4041.9 |
| $AUC_{0-\infty}$(ng*h/ml) | 4058.4 |

(C)




(D)

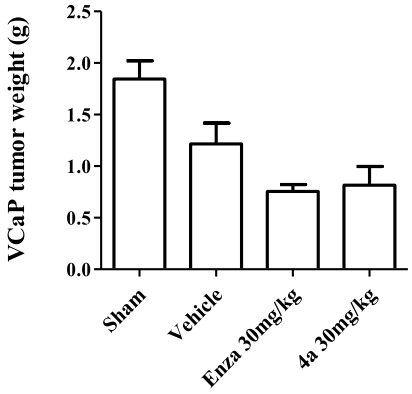



**Fig. 6.** **(A)** The concentration-time curve of compound **4a** in rats. **(B)** Pharmacokinetic parameters of compound **4a**. Compound was formulated in 10% DMA and 90% PEG200 for a single oral dose of 30 mg/kg. **(C)** Growth inhibitory effects of **4a** and Enzalutamide on CRPC xenografts. **(D)** Individual VCaP tumor weight of each group. The tumor volume and weight were expressed as the mean ± sem (N = 8–10).

### 5.11. In vivo xenograft study

BALB/c nude male mice (5 weeks old from HFK Bioscience Co. Ltd., Beijing, China) were subcutaneously injected with $1 \times 10^7$ VCaP cells in 150 μl mixture of DMEM medium and Matrigel (BD, 354234) (1:1). When the average tumor volume reached 150–200 $mm^3$, the mice were castrated or sham-operated under Nembutal anesthesia and then randomized into groups (n = 8–10). When castrated tumor regrew to 200–350 $mm^3$, oral treatments with **4a** (30 mg/kg, qd), Enzalutamide (30 mg/kg, qd) or vehicle were initiated and continued for 40 days. For all *in vivo* studies, PEG200/DMA (9:1, v/v) was used as a vehicle. The body weight and tumor volume were monitored twice weekly and the tumor volume was calculated according to the formula $W^2 \times L/2$ ($mm^3$), wherein W was the short diameter and L was the long diameter. Percentage of tumor growth inhibition (TGI) was calculated as $[1 - (T - T_0)/(C - C_0)] \times 100$, where T and $T_0$ were the mean tumor volumes on the final day and first day of treatment for the test groups; and likewise C and $C_0$ were the mean tumor volume of vehicle group. All animal experiments have been approved by Institutional Animal Care and Treatment Committee of Sichuan University in China (IACUC number: 20100318).

### 5.12. Synthetic procedures and characterization

#### 5.12.1. General chemistry

Reagents and solvents were obtained from commercial sources and used as received. Flash column chromatography was carried out using Biotage Isolera One apparatus with Agela flash column silica-CS. NMR spectra were recorded on Bruker AMX 400 spectrometer (Bruker Company, Germany, $^1H = 400$ MHz, $^{13}C = 101$ MHz). Chemical shifts were reported in parts per million (ppm) and referenced to an internal standard of tetramethylsilane (TMS) or residual deuterated solvent (DMSO-$d_6$, 2.50 ppm for $^1H$ NMR and 39.52 ppm for $^{13}C$ NMR; $CDCl_3$, 7.26 ppm for $^1H$ NMR and 77.16 ppm for $^{13}C$ NMR). Purity of prepared compounds was determined by high-performance liquid chromatography (HPLC) analysis, performed on Dionex ultimate 3000 HPLC instrument using Waters e2695 Series chromatographs and Waters xBridge column (5 μm, 4.6 mm × 150 mm). All key compounds were at least 95% pure. HRMS spectra were recorded on Q-TOF Premier mass spectrometer (Micromass, Manchester, UK).

#### 5.12.2. General procedure a for the synthesis of ***1a-k***

To a solution of 4-Bromo-1-indanone (1.05 g, 5.0 mmol) in

MeOH (14.0 ml) hydroxylamine hydrochloride (1.4 g, 20.0 mmol) was added. The resulting mixture was heated to reflux for 1 h and then cooled to room temperature. The mixture was concentrated under vacuum. DCM (15.0 ml) and 50% $NaHCO_3$ (15.0 ml) were added. The mixture was vigorously stirred for another 20min and the organic phase was separated, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The obtained white solid (5.0 mmol) in DCM (30.0 ml) at 0 °C was treated with AlH(Bu-*i*)$_2$ (20.0 ml, 30.0 mmol, 1.5 M in toluene) dropwise and stirred for 30 min. The reaction mixture was warmed to room temperature, stirred for another 12 h and quenched with cold water and extracted with DCM. The organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Pure 5-bromo-tetrahydroquinoline (**3**) was obtained as a yellow solid (600.0 mg, yield 56.6%) after flash column chromatography using a solvent gradient of 5–25% ethyl acetate in hexanes.

The mixture of 60% dispersion of NaH (80.0 mg, 2.0 mmol) in mineral oil and 5-bromo-tetrahydroquinoline (**3**) (212.0 mg, 1.0 mmol) in dry DMF (4.0 ml) was stirred at 0 °C for 30 min. 4-Fluoro-2-(trifluoromethyl) benzonitrile (378.2 mg, 2.0 mmol) was added and the mixture was warmed to room temperature. After 2 h, the reaction mixture was quenched with cold water and extracted with ethyl acetate. The organic phase was washed with water twice and then dried over anhydrous $Na_2SO_4$, filtered, and concentrated under vacuum. Pure 4-(5-bromo-3,4-dihydroquinolin −1(2H)-yl)-2-(trifluoromethyl)benzonitrile (**6**) was obtained as a yellow solid (100.0 mg, yield 26.2%) after flash column chromatography using a solvent of 10% ethyl acetate in hexanes. For the synthesis of intermediate **5** and **7**, commercially available **2** and **4** were used respectively by the procedure described for intermediate **6**.

Under $N_2$ atmosphere, a mixture of **6** (100.0 mg, 0.26 mmol), Pd(pph$_3$)$_4$ (30.0 mg, 0.026 mmol), 2.0 M aq $Na_2CO_3$ (0.29 ml, 0.78 mmol) and 1-Methyl-1H-pyrazole-5-boronic acid pinacol ester (108.2 mg, 0.52 mmol) in 1,4-Dioxane (0.65 ml) was heated to 90 °C and stirred for 6 h. The reaction mixture was cooled, diluted with ethyl acetate, washed with water, dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Purification on silica using a solvent gradient of 10–30% ethyl acetate in hexanes yielded the desired compound **1j** (77.0 mg, 77.5%). Compounds **1a-k** were prepared according to general procedure as described for compound **1j** using corresponding aryl bromide **2**–**4** and the appropriate boronic acid or boronic acid pinacol ester. The characterization data for compounds **1a-k** were provided below.

*5.12.2.1. 4-(6-(pyridin-3-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-2-(trifluoromethyl)benzonitrile, **1a**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.62 (s, 1H), 7.92–7.82 (m, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.48 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.45 (s, 1H), 7.39 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 2.5 Hz, 1H), 7.00 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.60 (s, 2H), 3.73 (t, *J* = 5.9 Hz, 2H), 3.11 (t, *J* = 5.8 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl3) δ 151.58, 148.66, 148.21, 137.00, 136.04, 135.55, 134.30, 134.00, 132.75, 127.31, 126.87, 125.61, 124.06, 123.59, 117.03, 114.33, 110.05, 100.03, 95.62, 48.51, 44.55, 29.05. HRMS (ESI) *m*/*z* for C22H17F$_3$N3 [M+H]$^+$, calcd 380.1296, found 380.1366. HPLC analysis: MeOH-$H_2O$ (70:30), 7.25 min, 96.10% purity.

*5.12.2.2. 4-(6-(pyridin-4-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-2-(trifluoromethyl)benzonitrile, **1b**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 5.3 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.52 (dd, *J* = 11.2, 6.9 Hz, 4H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 2.2 Hz, 1H), 7.00 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.61 (s, 2H), 3.73 (t, *J* = 5.9 Hz, 2H), 3.12 (t, *J* = 5.8 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.50, 150.28, 147.80, 137.26, 136.05, 135.60, 134.37, 134.05, 133.80, 127.37, 126.78, 125.44, 124.13, 116.98, 114.38, 110.09, 95.74, 48.55, 44.53, 29.04. HRMS (ESI) *m*/*z* for C22H17F$_3$N3 [M+H]$^+$, calcd 380.1296, found 380.1366. HPLC analysis: MeOH-$H_2O$ (70:30), 7.41 min, 94.01% purity.

*5.12.2.3. 4-(6-(pyrimidin-5-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-2-(trifluoromethyl)benzonitrile, **1c**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.96 (s, 2H),7.67 (d, *J* = 8.8 Hz, 1H), 7.57–7.32 (m, 3H), 7.17 (d, *J* = 1.7 Hz, 1H), 7.01 (dd, *J* = 8.7, 2.0 Hz, 1H), 4.62 (s, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.13 (t, *J* = 5.7 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 157.63, 154.85, 151.53, 136.07, 134.40, 134.08, 133.80, 133.38, 127.69, 126.75, 125.42, 124.02, 121.38, 116.93, 114.43, 110.19, 95.90, 48.51, 44.48, 29.02. HRMS (ESI) *m*/*z* for C21H16F$_3$N4 [M+H]$^+$, calcd 381.1249, found 381.1243. HPLC analysis: MeOH-$H_2O$ (70:30), 4.70 min, 97.98% purity.

*5.12.2.4. 4-(6-(quinolin-3-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-2-(trifluoromethyl)benzonitrile, **1d**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.18 (d, *J* = 1.5 Hz, 1H), 8.32 (s, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.66–7.47 (m, 3H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.17 (s, 1H), 7.01 (dd, *J* = 8.8, 1.9 Hz, 1H), 4.63 (s, 2H), 3.75 (t, *J* = 5.8 Hz, 2H), 3.15 (t, *J* = 5.7 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.58, 149.71, 147.45, 137.04, 136.04, 135.66, 134.36, 134.05, 133.22, 132.76, 129.58, 129.28, 128.00, 127.41, 127.18, 127.11, 125.88, 124.15, 121.45, 117.03, 114.35, 110.12, 95.69, 48.54, 44.57, 29.10. HRMS (ESI) *m*/*z* for C26H19F$_3$N$_3$ [M+H]$^+$, calcd 430.1453, found 430.1491. HPLC analysis: MeOH-$H_2O$ (70:30), 18.66 min, 94.30% purity.

*5.12.2.5. 4-(5-(pyridin-3-yl)indolin-1-yl)-2-(trifluoromethyl)benzonitrile, **1e**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.83 (d, *J* = 1.7 Hz, 1H), 8.63–8.49 (m, 1H), 7.92–7.80 (m, 1H), 7.73 (t, *J* = 13.4 Hz, 1H), 7.54 (d, *J* = 2.2 Hz, 1H), 7.50 (s, 1H), 7.46–7.30 (m, 4H), 4.13 (t, *J* = 8.3 Hz, 2H), 3.30 (t, *J* = 8.3 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 148.13, 147.90, 146.78, 143.95, 136.03, 134.43, 134.11, 133.78, 133.50, 131.70, 126.49, 124.39, 123.61, 121.14, 117.75, 116.42, 113.42, 110.61, 98.89, 52.08, 28.04. HRMS (ESI) *m*/*z* for C21H15F$_3$N$_3$ [M+H]$^+$, calcd 366.1140, found 366.1187. HPLC analysis: MeOH-$H_2O$ (70:30), 7.69 min, 99.47% purity.

*5.12.2.6. 4-(5-(pyrimidin-5-yl)indolin-1-yl)-2-(trifluoromethyl)benzonitrile, **1f**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.94 (s, 2H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.55 (d, *J* = 2.0 Hz, 1H), 7.51 (s, 1H), 7.47–7.32 (m, 3H),4.15 (t, *J* = 8.4 Hz, 2H), 3.32 (t, *J* = 8.3 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 157.06, 154.36, 146.62, 144.80, 136.06, 134.47, 134.15, 133.91, 127.80, 126.48, 124.14, 123.80, 121.07, 118.03, 116.29, 113.64, 110.72, 99.38, 52.22, 27.86. HRMS (ESI) *m*/*z* for C21H18F$_3$N$_4$O [M + CH$_3$OH + H]$^+$, calcd 399.1433, found 399.1431. HPLC analysis: MeOH-$H_2O$ (70:30), 4.82 min, 99.84% purity.

*5.12.2.7. 4-(5-(quinolin-3-yl)indolin-1-yl)-2-(trifluoromethyl)benzonitrile, **1g**.* The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 2.2 Hz, 1H), 8.27 (d, *J* = 2.0 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.82–7.67 (m, 2H), 7.64 (s, 1H), 7.62–7.52 (m, 3H), 7.42 (s, 1H), 7.40 (s, 1H), 4.15 (t, *J* = 8.3 Hz, 2H), 3.34 (t, *J* = 8.3 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 149.62, 147.14, 146.76, 143.96, 136.04, 134.44, 134.12, 133.63, 133.25, 132.36, 131.71, 129.22, 127.89, 127.11, 126.78, 124.61, 123.85, 121.13, 117.78, 116.43, 113.44, 110.71, 98.91, 52.20, 27.95. HRMS (ESI) *m*/*z* for C25H17F$_3$N$_3$ [M+H]$^+$, calcd 416.1296, found 416.1356. HPLC analysis: MeOH-$H_2O$ (70:30), 19.93 min,

96.98% purity.

*5.12.2.8. 4-(5-(pyridin-3-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-(trifluoromethyl)benzonitrile,* ***1h***. The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.63 (dd, $J$ = 4.8, 1.6 Hz, 2H), 7.67 (m, 2H), 7.51 (d, $J$ = 2.3 Hz, 1H), 7.43–7.37 (m, 1H), 7.34 (dd, $J$ = 8.7, 2.4 Hz, 1H), 7.30–7.26 (m, 1H), 7.26–7.19 (m, 1H), 7.00 (dd, $J$ = 7.2, 1.5 Hz, 1H), 3.68 (t, $J$ = 6.6 Hz, 2H), 2.70–2.55 (m, 2H), 2.03–1.91 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.16, 149.93, 148.54, 140.74, 138.48, 136.59, 136.26, 135.76, 134.10, 133.72, 130.25, 126.57, 125.01, 123.10, 120.67, 119.62, 116.62, 115.33, 98.89, 48.25, 25.10, 24.58. HRMS (ESI) *m/z* for $C_{22}H_{16}F_3N_3$ Na $[M+Na]^+$, calcd 402.1194, found 402.1166. HPLC analysis: MeOH-$H_2O$ (70:30), 8.01 min, 99.47% purity.

*5.12.2.9. 4-(5-(pyridin-4-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-(trifluoromethyl)benzonitrile,* ***1i***. The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.66 (d, $J$ = 3.7 Hz, 2H), 7.94 (d, $J$ = 8.5 Hz, 1H), 7.57 (s, 1H), 7.50 (d, $J$ = 8.3 Hz, 1H),7.42 (d, $J$ = 4.2 Hz, 2H), 7.36 (d, $J$ = 7.8 Hz, 1H),7.28 (t, $J$ = 7.7 Hz, 1H), 7.04 (d, $J$ = 7.2 Hz, 1H), 3.72 (t, $J$ = 6.0 Hz, 2H), 2.59 (t, $J$ = 5.6 Hz, 2H), 1.88 (m, $J$ = 5.5 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.23, 149.83, 148.41, 140.77, 139.61, 135.78, 134.20, 133.70, 129.61, 126.71, 124.25, 123.86, 121.01, 119.69, 116.48, 115.35, 99.12, 48.27, 25.02, 24.52. HRMS (ESI) *m/z* for C22H17$F_3N_3$ $[M+H]^+$, calcd 380.1296, found 380.1364. HPLC analysis: MeOH-$H_2O$ (70:30), 8.27 min, 99.56% purity.

*5.12.2.10. 4-(5-(1-methyl-1H-pyrazol-5-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-(trifluoromethyl)benzonitrile,* ***1j***. The title compound was obtained following general procedure A. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, $J$ = 8.7 Hz, 1H), 7.56 (d, $J$ = 1.7 Hz, 1H), 7.51 (d, $J$ = 1.9 Hz, 1H), 7.35 (dd, $J$ = 8.6, 2.2 Hz, 1H), 7.29 (d, $J$ = 8.1 Hz, 1H), 7.21 (t, $J$ = 7.8 Hz, 1H), 6.98 (d, $J$ = 7.4 Hz, 1H), 6.24 (d, $J$ = 1.7 Hz, 1H), 3.73 (s, 3H), 3.69 (t, $J$ = 6.5 Hz, 2H), 2.58–2.43 (t, 2H), 2.07–1.91 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.15, 141.32, 140.75, 138.59, 135.72, 134.22, 133.79, 131.38, 130.99, 126.43, 125.29, 121.16, 120.04, 116.37, 115.64, 106.78, 99.52, 48.38, 36.78, 24.76, 24.15. HRMS (ESI) *m/z* for $C_{21}H_{17}F_3N_4$Na $[M+Na]^+$, calcd 405.1303, found 405.1315. HPLC analysis: MeOH-$H_2O$ (70:30), 7.83 min, 98.50% purity.

*5.12.2.11. 4-(5-(3,5-dimethylisoxazol-4-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-(trifluoromethyl)benzonitrile,* ***1k***. The title compound was obtained following general procedure A**.** $^1$H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, $J$ = 8.7 Hz, 1H), 7.51 (d, $J$ = 2.3 Hz, 1H), 7.34 (dd, $J$ = 8.7, 2.4 Hz, 1H),7.26 (dd, $J$ = 8.0, 0.8 Hz, 2H),7.19 (t, $J$ = 7.8 Hz, 1H), 6.86 (dd, $J$ = 7.4, 1.1 Hz, 1H), 3.79–3.53 (m, 2H), 2.56–2.37 (t, 2H), 2.29 (s, 3H), 2.15 (s, 3H), 2.04–1.91 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.70, 159.29, 151.19, 140.80, 135.77, 134.08, 133.73, 131.78, 129.73, 126.66, 125.81, 120.65, 119.72, 116.48, 115.32, 115.22, 98.96, 48.17, 24.71, 24.54, 11.51, 10.61. *HRMS (ESI) m/z for* $C_{22}H_{18}F_3N_3ONa$ $[M+Na]^+$, calcd 420.1300, found 420.1286. HPLC analysis: MeOH-$H_2O$ (70:30), 9.317 min, 99.32% purity.

*5.12.3. General procedure B for the synthesis of* ***2a-h***, ***3b-l*** *and* ***3n***

A solution of 4-amino-2-chlorobenzonitrile (**10**) (1.6 g, 10.5 mmol) in MeCN (60.0 ml) and water (18.0 ml) was cooled to 0 °C. Sulfuric acid (1.8 ml) and 2.0 M aqueous sodium nitrite (840.0 mg, 6.0 ml) was slowly added, respectively. Thereafter potassium iodide (3.3 g, 20.0 mmol) dissolved in 5.0 ml of water was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred overnight. The organic phase was evaporated, the resulting suspension was dissolved in ethyl acetate and washed three times with 10% aqueous $NaHSO_3$. The organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Purification on silica using a solvent of 10% ethyl acetate in hexanes yielded the intermediate **12** as a white solid (1.71 g, yield 62.5%).

Under $N_2$ atmosphere, a mixture of intermediate **12** (263.5 mg, 1.0 mmol), 3-bromoaniline (172.0 mg, 1.0 mmol), $Cs_2CO_3$ (325.8 mg, 1.0 mmol), $Pd_2(dba)_3$ (45.8 mg, 0.05 mmol) and Davephos (31.5 mg, 0.08 mmol) in MeCN (6.0 ml) was heated to reflux for 5 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The resulting mixture was filtered off and the solution was concentrated under vacuum to give a crude product **16** that was purified by column chromatography on silica using a solvent of 10% ethyl acetate in hexanes. The desired compound **16** was obtained as a yellow solid (220.0 mg, yield 72%). For the synthesis of intermediate **13**–**15**, compound **12** or commercially available **11** and corresponding aromatic amine were used respectively following the procedure described for **16**.

60% dispersion of NaH (35.0 mg, 0.875 mmol) in mineral oil was added portion-wise to a solvent of **16** (110.0 mg, 0.35 mmol) in dry DMF (4.0 ml) at 0 °C. The reaction mixture was stirred for 30 min and then warmed to room temperature. Bromoethane (76.3 mg, 0.7 mmol) was added. After an additional 2 h, the mixture was quenched with water and extracted with ethyl acetate. The organic phase was washed twice with water, dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Pure product **20** was obtained as a yellowish green solid (90.0 mg, yield 77.0%) after flash column chromatography using a solvent of 10% ethyl acetate in hexanes. For the synthesis of **17**–**19**, intermediate **13**–**16** were used by the procedure described for intermediate **20**, respectively.

A suspension of **20** (133.5 mg, 0.4 mmol), imidazole (36.8 mg, 0.54 mmol), $Cs_2CO_3$ (260.6 mg, 0.8 mmol) and CuI (17.5 mg, 0.09 mmol) in DMF (2.0 ml) was degassed under a stream of nitrogen over 10 min and then heated to 120 °C and stirred for 40 h. The resulting mixture was cooled to room temperature, diluted with ethyl acetate, washed twice with water, dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Purification on silica using a solvent gradient of 10–50% ethyl acetate in hexanes yielded the desired compound **2c** (40.0 mg, yield 31.2%) as a white solid. Compounds **2a, 2b, 2d** and **3m** were prepared according to general procedure B as described for compound **2c** using corresponding aryl bromide (**17**–**19**).

Compounds **2e-h, 3b-l** and **3n** were obtained from **19** or **20** and appropriate boronic acid via Suzuki coupling reaction as described in general procedure A for compound **1a-k**. The characterization data for compounds **2a-h** and **3b-n** are provided below.

*5.12.3.1. 4-((3-(1H-imidazol-1-yl)phenyl)(ethyl)amino)-2-(trifluoromethyl)benzonitrile,* ***2a***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.64–7.53 (m, 2H), 7.38 (dd, $J$ = 8.1, 1.2 Hz, 1H), 7.29 (s, 1H), 7.25–7.16 (m, 3H), 7.02 (d, $J$ = 2.3 Hz, 1H), 6.85 (dd, $J$ = 8.8, 2.4 Hz, 1H), 3.87 (q, $J$ = 7.1 Hz, 2H), 1.31 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 150.44, 146.22, 139.32, 135.93, 134.49, 134.14, 132.05, 130.90, 126.14, 123.85, 121.13, 120.00, 119.83, 116.59, 116.00, 111.64, 96.86, 47.23, 12.28. HRMS (ESI) *m/z* for C19H16F3N4 $[M+H]^+$, calcd 357.1247, found 357.1299. HPLC analysis: MeOH-$H_2O$ (70:30), 4.05 min, 99.56% purity.

*5.12.3.2. 4-((5-(1H-imidazol-1-yl)-2-methylphenyl)(ethyl)amino)-2-(trifluoromethyl)-benzonitrile,* ***2b***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.33 (s, 1H), 7.81 (d, $J$ = 8.4 Hz, 2H), 7.70 (dd, $J$ = 8.3, 2.2 Hz, 1H), 7.62 (t, $J$ = 5.5 Hz, 1H), 7.58 (d, $J$ = 8.3 Hz, 1H), 7.11 (s, 1H), 6.80 (d, $J$ = 26.2 Hz, 2H), 3.83 (s, 2H), 2.07 (s, 3H), 1.20 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 150.19, 143.34, 137.12, 136.13, 135.41, 134.50, 134.32, 133.51, 130.75, 123.78, 121.79, 120.98, 118.06, 116.85,

114.31, 109.50, 95.91, 46.45, 17.50, 12.37. HRMS (ESI) *m/z* for C20H18F3N4 $[M+H]^+$, calcd 371.1405, found 371.1462. HPLC analysis: MeOH-$H_2O$ (70:30), 4.76 min, 98.28% purity.

*5.12.3.3. 4-((3-(1H-imidazol-1-yl)phenyl)(ethyl)amino)-2-chlorobenzonitrile,* ***2c****.* The title compound was obtained following general procedure B. 1H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.57 (t, $J = 8.0$ Hz, 1H), 7.41 (d, $J = 8.8$ Hz, 1H), 7.35 (dd, $J = 8.0$, 1.2 Hz, 1H), 7.29 (s, 1H), 7.25–7.12 (m, 3H), 6.77 (d, $J = 2.4$ Hz, 1H), 6.62 (dd, $J = 8.8$, 2.4 Hz, 1H), 3.82 (q, $J = 7.1$ Hz, 2H), 1.29 (t, $J = 7.1$ Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 151.41, 146.36, 139.31, 137.95, 135.55, 134.60, 131.80, 130.87, 126.07, 119.92, 119.51, 117.98, 117.13, 114.56, 112.72, 100.98, 47.06, 12.47. HRMS (ESI) *m/z* for C18H16ClN4 [M+H]+, calcd 323.0985, found 323.1052. HPLC analysis: MeOH-$H_2O$ (70:30), 4.51 min, 97.31% purity.

*5.12.3.4. 4-((5-(1H-imidazol-1-yl)-2-methylphenyl)(ethyl)amino)-2-chlorobenzonitrile,* ***2d****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.32 (s, 1H), 7.80 (s, 1H), 7.67 (dd, $J = 8.3$, 2.3 Hz, 1H), 7.64–7.46 (m, 3H), 7.10 (s, 1H), 6.68 (s, 1H), 6.46 (d, $J = 7.5$ Hz, 1H), 3.77 (s, 1H), 2.07 (s, 2H), 1.18 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl3) δ 151.26, 143.54, 138.31, 137.20, 136.18, 135.35, 134.72, 133.40, 130.63, 122.06, 120.93, 118.09, 117.37, 112.55, 110.72, 99.88, 46.40, 17.44, 12.61. HRMS (ESI) *m/z* for C19H18ClN4 $[M+H]^+$, calcd 337.1142, found 337.1145. HPLC analysis: MeOH-$H_2O$ (70:30), 4.70 min, 96.49% purity.

*5.12.3.5. 2-Chloro-4-(ethyl(3-(pyrimidin-5-yl)phenyl)amino)benzonitrile,* ***2e****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.95 (s, 2H), 7.63 (t, $J = 7.8$ Hz, 1H), 7.58–7.47 (m, 1H), 7.40 (dd, $J = 5.3$, 3.5 Hz, 2H), 7.35–7.27 (m, 1H), 6.74 (d, $J = 2.4$ Hz, 1H), 6.60 (dd, $J = 8.9$, 2.5 Hz, 1H), 3.83 (q, $J = 7.1$ Hz, 2H), 1.30 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl3) δ 157.99, 154.89, 151.67, 145.92, 138.03, 136.70, 134.48, 133.51, 131.38, 127.93, 125.95, 125.37, 117.27, 114.08, 112.18, 100.46, 47.13, 12.44. HRMS (ESI) *m/z* for C19H16ClN4 $[M+H]^+$, calcd 335.0985, found 335.1060. HPLC analysis: MeOH-$H_2O$ (70:30), 4.44 min, 99.88% purity.

*5.12.3.6. 2-Chloro-4-(ethyl(2-methyl-5-(pyrimidin-5-yl)phenyl)amino)benzonitrile,* ***2f****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.94 (s, 2H), 7.54 (s, 2H), 7.39 (d, $J = 8.7$ Hz, 1H), 7.34 (s, 1H), 6.54 (s, 1H), 6.38 (d, $J = 6.5$ Hz, 1H), 3.73 (s, 2H), 2.18 (s, 3H), 1.31 (t, $J = 6.7$ Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 157.77, 154.72, 151.45, 143.52, 138.29, 137.84, 134.81, 134.42, 133.21, 133.12, 127.66, 126.52, 117.42, 112.46, 110.69, 99.65, 46.31, 17.62, 12.40. HRMS (ESI) *m/z* for C20H18ClN4 $[M+H]^+$, calcd 349.1142, found 349.1220. HPLC analysis: MeOH-$H_2O$ (70:30), 5.25 min, 99.88% purity.

*5.12.3.7. 4-([1,1′-biphenyl]-3-yl(ethyl)amino)-2-chlorobenzonitrile,* ***2g****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.62–7.34 (m, 9H), 7.21–7.10 (m, 1H), 6.72 (t, $J = 3.1$ Hz, 1H), 6.56 (dt, $J = 6.8$, 3.4 Hz, 1H), 3.81 (dq, $J = 14.2$, 7.1 Hz, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.98, 144.99, 143.71, 139.99, 137.97, 134.59, 130.64, 128.96, 127.83, 127.07, 126.45, 126.42, 125.99, 117.47, 113.66, 111.91, 99.65, 47.04, 12.25. HRMS (ESI) *m/z* for C21H17ClN2Na $[M+Na]^+$, calcd 355.0978, found 355.0979. HPLC analysis: MeOH-$H_2O$ (70:30), 4.83 min, 95.97% purity.

*5.12.3.8. 2-Chloro-4-(ethyl(4-methyl-[1,1′-biphenyl]-3-yl)amino)benzonitrile,* ***2h****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.56 (td, $J = 8.4$, 1.6 Hz, 3H), 7.50–7.38 (m, 3H), 7.39–7.29 (m, 3H), 6.55 (s, 1H), 6.37 (d, $J = 8.2$ Hz, 1H), 3.71 (s, 2H), 2.14 (s, 3H), 1.29 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.75, 142.83, 141.28, 139.78, 138.17, 135.46, 134.64, 132.44, 128.94, 127.70, 127.66, 126.88, 126.69, 117.73, 112.28, 110.66, 99.00, 46.27, 17.50, 12.27. HRMS (ESI) *m/z* for C22H19ClN2Na $[M+Na]^+$, calcd 369.1134, found 369.1119. HPLC analysis: MeOH-$H_2O$ (70:30), 5.25 min, 96.10% purity.

*5.12.3.9. 2-Chloro-4-(ethyl(2-methyl-5-(1H-pyrazol-4-yl)phenyl)amino)benzonitrile,* ***3b****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 2H), 7.45 (dd, 1H), 7.36 (d, $J = 8.4$ Hz, 2H), 7.22 (d, 1H), 6.54 (s, 1H), 6.36 (d, $J = 7.6$ Hz, 1H), 3.70 (s, 2H), 2.10 (s, 3H), 1.28 (t, $J = 11.6$, 4.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.70, 142.84, 138.18, 134.70, 132.53, 131.18, 127.25, 126.26, 125.44, 117.68, 116.73, 112.28, 110.63, 108.71, 106.05, 99.04, 46.33, 17.39, 12.41. HRMS (ESI) *m/z* for C19H18ClN4 $[M+H]^+$, calcd 337.1142, found 337.1210. HPLC analysis: MeOH-$H_2O$ (70:30), 6.48 min, 99.82% purity.

*5.12.3.10. 2-Chloro-4-(ethyl(2-methyl-5-(3-methyl-1H-pyrazol-4-yl)phenyl)amino)benzonitrile,* ***3c****.* The title compound was obtained following general procedure B. 1H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.45–7.31 (m, 3H), 7.12 (d, $J = 1.1$ Hz, 1H), 6.54 (s, 1H), 6.37 (d, $J = 8.1$ Hz, 1H), 3.68 (s, 2H), 2.45 (s, 3H), 2.12 (s, 3H), 1.28 (t, $J = 7.1$ Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 151.73, 142.58, 138.17, 134.70, 134.26, 133.52, 132.32, 130.76, 129.95, 127.89, 127.08, 119.05, 117.70, 112.29, 110.62, 98.98, 46.22, 17.39, 12.35, 11.72. HRMS (ESI) *m/z* for C20H19ClN4 [M+H]+, calcd 351.1298, found 351.1365. HPLC analysis: MeOH-H2O (70:30), 8.35 min, 98.10% purity.

*5.12.3.11. 2-Chloro-4-(ethyl(2-methyl-5-(1-methyl-1H-pyrazol-5-yl)phenyl)amino)benzonitrile,* ***3d****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.60 (d, $J = 8.9$ Hz, 1H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.51 (dd, $J = 7.9$, 1.7 Hz, 1H), 7.46 (d, $J = 1.9$ Hz, 1H), 7.38 (d, $J = 1.5$ Hz, 1H), 6.69 (s, 1H), 6.45 (d, $J = 1.9$ Hz, 2H), 3.86 (s, 3H), 3.78 (s, 2H), 2.11 (s, 3H), 1.17 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 152.17, 142.76, 142.12, 138.36, 137.35, 136.73, 135.57, 132.78, 130.48, 129.30, 128.34, 117.84, 112.26, 111.73, 106.35, 97.80, 46.27, 38.04, 17.53, 12.53. HRMS (ESI) *m/z* for C20H20ClN4 $[M+H]^+$, calcd 351.1298, found 351.1375. HPLC analysis: MeOH-$H_2O$ (70:30), 18.75 min, 99.25% purity.

*5.12.3.12. 2-Chloro-4-((5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)(ethyl)amino)benzoin-trile,* ***3e****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, $J = 7.9$ Hz, 1H), 7.38 (d, $J = 8.9$ Hz, 1H), 7.21 (dd, $J = 7.8$, 1.7 Hz, 1H), 6.98 (d, $J = 1.6$ Hz, 1H), 6.53 (s, 1H), 6.36 (d, $J = 8.1$ Hz, 1H), 3.70 (s, 2H), 2.42 (s, 3H), 2.28 (s, 3H), 2.15 (s, 3H), 1.28 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.27, 158.31, 151.60, 142.74, 138.22, 135.91, 134.76, 132.57, 130.33, 129.65, 128.64, 117.57, 115.61, 112.38, 110.59, 99.30, 46.15, 17.48, 12.24, 11.60, 10.80. HRMS (ESI) *m/z* for C21H21ClN3O $[M+H]^+$, calcd 366.1295, found 366.1331. HPLC analysis: MeOH-$H_2O$ (70:30), 9.81 min, 95.97% purity.

*5.12.3.13. 2-Chloro-4-(ethyl(5-(furan-3-yl)-2-methylphenyl)amino)benzonitrile,* ***3f****.* The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.55–7.46 (m, 1H), 7.46–7.39 (m, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 7.19 (d, $J = 1.5$ Hz, 1H), 6.72–6.63 (m, 1H), 6.53 (s, 1H), 6.35 (d, $J = 8.0$ Hz, 1H), 3.70 (s, 2H), 2.10 (s, 3H), 1.28 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.69, 143.92, 142.76, 138.57, 138.17, 135.15, 134.64, 132.56, 132.44, 126.40, 125.53, 125.45, 117.67, 112.28, 110.65, 108.53, 98.98, 46.27, 17.35, 12.39. HRMS (ESI) *m/z* for $C_{20}H_{17}ClN_2ONa$ $[M+Na]^+$, calcd 359.0927, found 359.0927. HPLC analysis: MeOH-H2O (70:30), 18.74 min, 99.94% purity.

*5.12.3.14. 2-Chloro-4-(ethyl(5-(furan-2-yl)-2-methylphenyl)amino) benzonitrile,* ***3g***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, $J$ = 8.0, 1.7 Hz, 1H), 7.46 (d, $J$ = 1.4 Hz, 1H), 7.40 (d, $J$ = 1.6 Hz, 1H), 7.37 (s, 1H), 7.35 (d, $J$ = 2.0 Hz, 1H), 6.64 (d, $J$ = 3.3 Hz, 1H), 6.53 (s, 1H), 6.48 (dd, $J$ = 3.3, 1.8 Hz, 1H), 6.36 (d, $J$ = 7.5 Hz, 1H), 3.65 (s, 2H), 2.10 (s, 3H), 1.28 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz) δ 152.89, 151.67, 142.67, 142.31, 138.23, 135.61, 134.77, 132.47, 131.11, 124.53, 123.50, 117.83, 112.39, 111.88, 110.68, 105.40, 99.10, 46.30, 17.63, 12.43. HRMS (ESI) $m/z$ for C20H17ClN2ONa $[M+Na]^+$, calcd 359.0927, found 359.0920. HPLC analysis: MeOH-$H_2O$ (70:30), 25.03 min, 99.44% purity.

*5.12.3.15. 2-Chloro-4-(ethyl(2-methyl-5-(thiophen-2-yl)phenyl) amino)benzonitrile,* ***3h***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.55 (dd, $J$ = 7.9, 1.8 Hz, 1H), 7.42–7.27 (m, 5H), 7.08 (dd, $J$ = 4.8, 3.9 Hz, 1H), 6.54 (s, 1H), 6.36 (d, $J$ = 7.7 Hz, 1H), 3.68 (s, 2H), 2.10 (s, 3H), 1.29 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.63, 143.03, 142.71, 138.14, 135.72, 134.71, 134.52, 132.53, 128.15, 126.42, 125.43, 125.15, 123.37, 117.69, 112.33, 110.70, 99.18, 46.29, 17.49, 12.41. HRMS (ESI) $m/z$ for C20H18ClN2S $[M+H]^+$, calcd 353.0801, found 353.0880. HPLC analysis: MeOH-$H_2O$ (70:30), 12.35 min, 99.22% purity.

*5.12.3.16. 2-Chloro-4-(ethyl(2-methyl-5-(pyridin-3-yl)phenyl) amino)benzonitrile,* ***3i***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.83 (d, $J$ = 1.6 Hz, 1H), 8.61 (d, $J$ = 3.7 Hz, 1H), 7.93–7.78 (m, 1H), 7.54 (dd, $J$ = 7.9, 1.8 Hz, 1H), 7.47 (d, $J$ = 7.9 Hz, 1H), 7.37 (dd, $J$ = 6.2, 2.7 Hz, 2H), 7.32 (d, $J$ = 1.7 Hz, 1H), 6.55 (s, 1H), 6.37 (d, $J$ = 8.5 Hz, 1H), 3.72 (s, 2H), 2.16 (s, 3H), 1.30 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.60, 148.85, 148.08, 143.12, 138.23, 137.95, 136.63, 135.33, 134.76, 134.14, 132.84, 127.77, 126.70, 123.65, 117.60, 112.36, 110.67, 99.39, 46.33, 17.52, 12.38. (ESI) $m/z$ for C21H18ClN3Na $[M+Na]^+$, calcd 370.1087, found 370.1086. HPLC analysis: MeOH-$H_2O$ (70:30), 8.13 min, 98.99% purity.

*5.12.3.17. 2-Chloro-4-(ethyl(2-methyl-5-(pyridin-4-yl)phenyl) amino)benzonitrile,* ***3j***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, $J$ = 4.8 Hz, 2H), 7.60 (dd, $J$ = 7.9, 1.9 Hz, 1H), 7.50 (s, 2H), 7.48 (s, 1H), 7.37 (d, $J$ = 8.7 Hz, 2H), 6.54 (s, 1H), 6.37 (d, $J$ = 8.1 Hz, 1H), 3.72 (s, 2H), 2.17 (s, 3H), 1.30 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.56, 150.42, 146.90, 143.18, 138.25, 138.21, 137.84, 134.77, 132.90, 127.72, 126.54, 121.32, 117.55, 112.38, 110.67, 99.43, 46.32, 29.71, 17.61, 12.44. (ESI) $m/z$ for C21H19ClN3 $[M+H]^+$, calcd 348.1189, found 348.1259. HPLC analysis: MeOH-$H_2O$ (70:30), 8.07 min, 99.07% purity.

*5.12.3.18. 2-Chloro-4-(ethyl(5-(6-fluoropyridin-3-yl)-2-methylphenyl)amino)benzonitrile,* ***3k***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.96 (t, $J$ = 6.8 Hz, 1H), 7.46 (dd, $J$ = 21.1, 13.1 Hz, 2H), 7.38 (d, $J$ = 8.7 Hz, 1H), 7.27 (s, 1H), 7.12–6.89 (m, 1H), 6.54 (s, 1H), 6.38 (s, 1H), 3.72 (s, 2H), 2.18 (s, 3H), 1.30 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.69, 145.89, 145.74, 143.32, 139.69, 138.37, 136.93, 134.90, 133.75, 133.04, 127.80, 126.73, 117.66, 112.52, 110.78, 109.95, 109.57, 99.56, 46.47, 17.64, 12.58. (ESI) $m/z$ for C21H17ClFN3Na $[M+Na]^+$, calcd 388.0993, found 388.0975. HPLC analysis: MeOH-$H_2O$ (70:30), 10.36 min, 99.87% purity.

*5.12.3.19. 2-Chloro-4-(ethyl(2-methyl-5-(quinolin-3-yl)phenyl) amino)benzonitrile,* ***3l***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.16 (d, $J$ = 2.3 Hz, 1H), 8.29 (d, $J$ = 2.1 Hz, 1H), 8.14 (d, $J$ = 8.4 Hz, 1H), 7.88 (d, $J$ = 7.8 Hz, 1H), 7.74 (ddd, $J$ = 8.4, 6.9, 1.4 Hz, 1H), 7.68 (dd, $J$ = 7.9, 1.9 Hz, 1H), 7.59 (dt, $J$ = 16.4, 4.7 Hz, 1H), 7.52 (d, $J$ = 8.0 Hz, 1H), 7.46 (d, $J$ = 1.8 Hz, 1H), 7.39 (d, $J$ = 8.9 Hz, 1H), 6.58 (s, 1H), 6.41 (d, $J$ = 8.5 Hz, 1H), 3.77 (d, $J$ = 11.8 Hz, 2H), 2.18 (s, 3H), 1.32 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.63, 149.47, 147.51, 143.24, 138.26, 138.04, 136.65, 134.78, 133.08, 132.92, 132.49, 129.65, 129.31, 128.01, 127.98, 127.93, 127.24, 126.95, 117.59, 112.40, 110.71, 99.36, 46.40, 17.56, 12.48. (ESI) $m/z$ for C25H20ClN3H $[M+H]^+$, calcd 398.1346, found 398.1413. HPLC analysis: MeOH-$H_2O$ (70:30), 20.52 min, 97.63% purity.

*5.12.3.20. 4-((5-(1H-benzo[d]imidazol-1-yl)-2-methylphenyl)(ethyl) amino)-2-chloro benzonitrile,* ***3m***. The title compound was obtained form **19** and benzimidazole following general procedure B as described for compound **2c**. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.88 (dd, $J$ = 6.2, 2.8 Hz, 1H), 7.58 (d, $J$ = 8.2 Hz, 1H), 7.50 (d, $J$ = 2.0 Hz, 1H), 7.48 (d, $J$ = 2.0 Hz, 1H), 7.42 (d, $J$ = 8.8 Hz, 1H), 7.39–7.32 (m, 2H), 7.30 (d, $J$ = 2.1 Hz, 1H), 6.59 (d, $J$ = 1.7 Hz, 1H), 6.50–6.34 (m, 1H), 3.75 (d, $J$ = 6.7 Hz, 2H), 2.21 (s, 3H), 1.31 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.28, 143.82, 142.00, 138.38, 136.81, 136.05, 134.89, 133.61, 124.60, 123.98, 123.50, 123.06, 120.88, 117.35, 112.63, 110.82, 110.15, 100.05, 100.00, 46.40, 17.59, 12.48. (ESI) $m/z$ for C23H20ClN4 $[M+H]^+$, calcd 387.1298, found 387.1392. HPLC analysis: MeOH-$H_2O$ (70:30), 10.73 min, 99.27% purity.

*5.12.3.21. 2-Chloro-4-(ethyl(4-methyl-2′,3′,4′,5′-tetrahydro-[1,1′-biphenyl]-3-yl)amino) benzonitrile,* ***3n***. The title compound was obtained following general procedure B. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.43–7.30 (m, 2H), 7.28 (s, 1H), 7.09 (d, $J$ = 1.7 Hz, 1H), 6.50 (s, 1H), 6.32 (d, $J$ = 7.5 Hz, 1H), 6.21–6.08 (m, 1H), 3.67 (d, $J$ = 62.9 Hz, 2H), 2.53–2.28 (m, 2H), 2.29–2.14 (m, 2H), 2.06 (s, 3H), 1.85–1.73 (m, 2H), 1.65 (dtd, $J$ = 12.5, 6.3, 2.8 Hz, 2H), 1.26 (t, $J$ = 7.1 Hz, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.83, 142.66, 142.15, 138.08, 135.35, 134.62, 134.38, 131.69, 125.36, 125.19, 124.49, 117.80, 112.17, 110.59, 98.68, 46.21, 27.25, 25.85, 22.94, 22.06, 17.32, 12.34. (ESI) $m/z$ for C22H23ClN2Na $[M+Na]^+$, calcd 373.1447, found 373.1454. HPLC analysis: MeOH-$H_2O$ (70:30), 10.13 min, 96.89% purity.

*5.12.3.22. 2-Chloro-4-(ethyl(2-methyl-5-(1H-pyrazol-1-yl)phenyl) amino)benzonitrile,* ***3a***. The intermediate **19** was prepared following general procedure B. To a solution of 1H-pyrazole (54.4 mg, 0.8 mmol) in dry DMSO (1.0 ml) was added 60% NaH (80.0 mg, 2.0 mmol) portion-wise at 0 °C. The mixture was warmed to room temperature and stirred for 30 min. Thereafter CuI (7.6 mg, 0.04 mmol) and **19** (140.0 mg, 0.4 mmol) were added. The mixture was degassed under a stream of nitrogen over 10 min and was stirred overnight at 120 °C. The resulting mixture was cooled to room temperature, diluted with ethyl acetate and washed twice with water. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0–30% ethyl acetate in hexanes to give **3a** as a yellow oil (80.0 mg, yield 59%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, $J$ = 2.5 Hz, 1H), 7.75 (d, $J$ = 1.6 Hz, 1H), 7.47–7.40 (m, 2H), 7.29 (d, $J$ = 2.0 Hz, 1H), 7.23 (d, $J$ = 8.2 Hz, 1H), 6.91 (s, 1H), 6.55–6.44 (m, 1H), 6.30 (d, $J$ = 7.5 Hz, 1H), 3.72 (s, 2H), 2.07 (s, 3H), 1.27 (t, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.45, 143.81, 143.77, 141.74, 135.87, 135.27, 133.24, 132.22, 131.19, 129.78, 120.27, 118.44, 111.41, 107.94, 106.87, 91.89, 46.36, 17.43, 12.42. HRMS (ESI) $m/z$ for C19H18ClN4 $[M+H]^+$, calcd 337.1142, found 337.1134. HPLC analysis: MeOH-$H_2O$ (70:30), 9.72 min, 94.71% purity.

*5.12.3.23. 2-Chloro-4-((5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)amino)benzonitrile,* ***4a***. The title compound was

obtained from **15** to 3,5-dimethylisoxazole-4-boronic acid pinacol ester via Suzuki coupling reaction as described in general procedure A for compound **1a-k**. $^1$H NMR (400 MHz, DMSO) δ 8.73 (s, 1H), 7.63 (d, $J$ = 8.6 Hz, 1H), 7.41 (d, $J$ = 7.6 Hz, 1H), 7.22 (s, 1H), 7.17 (d, $J$ = 7.5 Hz, 1H), 6.89 (s, 1H), 6.77 (d, $J$ = 8.3 Hz, 1H), 2.40 (s, 3H), 2.23 (s, 6H). $^{13}$C NMR (101 MHz, DMSO) δ 165.54, 158.56, 151.65, 138.57, 137.05, 135.85, 132.62, 132.28, 129.05, 126.46, 125.25, 117.76, 115.81, 113.79, 112.97, 98.89, 17.88, 11.83, 10.97. (ESI) $m/z$ for $C_{19}H_{16}ClN_3ONa$ $[M+Na]^+$, calcd 360.0880, found 360.0854. HPLC analysis: MeOH-$H_2O$ (70:30), 6.92 min, 99.58% purity.

*5.12.3.24. 4-(3-(3,5-dimethylisoxazol-4-yl)phenoxy)-2-(trifluoromethyl)benzonitrile,* ***4b****.* A mixture of 4-fluoro-2-(trifluoromethyl)benzonitrile (**8**) (378.0 mg, 2.0 mmol), 3-iodophenol (440.0 mg, 2.0 mmol) and $K_2CO_3$ (414.6 mg, 3.0 mmol) in DMSO (4.0 ml) was heated to 110 °C and stirred for 2 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. Purification on silica using a solvent of 10% ethyl acetate in hexanes yielded the product **9** (670.0 mg, yield 86.1%) as a white solid. The title compound was obtained from **9** to 3,5-dimethylisoxazole-4-boronic acid pinacol ester through Suzuki coupling reaction as described in general procedure A for compound **1a-k**. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, $J$ = 8.5 Hz, 1H), 7.53 (t, $J$ = 7.8 Hz, 1H), 7.38 (s, 1H), 7.19 (d, $J$ = 7.1 Hz, 2H), 7.08 (d, $J$ = 7.6 Hz, 1H), 6.99 (s, 1H), 2.42 (s, 3H), 2.28 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.62, 161.24, 158.29, 154.40, 136.81, 135.30, 134.97, 133.31, 130.98, 126.51, 120.91, 119.99, 119.32, 115.91, 115.60, 115.32, 103.53, 11.65, 10.81. (ESI) $m/z$ for $C_{19}H_{13}F_3N_2O_2Na$ $[M+Na]^+$, calcd 381.0827, found 381.0818. HPLC analysis: MeOH-$H_2O$ (70:30), 7.33 min, 98.78% purity.

*5.12.4. General procedure C for the synthesis of* ***4c-e***

5-bromo-2-methylaniline (**21**) and 3,5-dimethylisoxazole-4-boronic acid pinacol ester underwent Suzuki coupling reaction as described in general procedure A for compound **1a-k** to give intermediate **22.** Under $N_2$ atmosphere, a mixture of 4-iodopyridine (123.0 mg, 0.6 mmol), 22 (101.0 mg, 0.5 mmol), $Cs_2CO_3$ (292.5 mg, 1.25 mmol), $Pd_2(dba)_3$ (23.0 mg, 0.025 mmol) and Davephos (15.7 mg, 0.04 mmol) in MeCN (3.0 ml) was heated to reflux for 5 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The mixture was filtered off and the solution was concentrated under vacuum to give a crude product **4e** that was purified by column chromatography on silica using a solvent of 10% MeOH in DCM. The compound **4e** was obtained as a yellow oil (100.0 mg, 71.7%). For the synthesis of intermediate **23**, commercially available 2-iodopyridine were used following the procedure described for **4e**.

Compound **4c** and **4d** were successfully prepared using **23** or **4e** through *N*-alkylation reaction as described in general procedure B for compound **17**–**19.** The characterization data for compounds **4c-e** are provided below.

*5.12.4.1. N-(5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)-N-ethylpyridin-2-amine,* ***4c****.* The title compound was obtained following general procedure C. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.21 (ddd, $J$ = 5.0, 1.9, 0.8 Hz, 1H), 7.40 (d, $J$ = 7.8 Hz, 1H), 7.32–7.26 (m, 1H), 7.15 (dd, $J$ = 7.8, 1.8 Hz, 1H), 7.06 (d, $J$ = 1.8 Hz, 1H), 6.56 (ddd, $J$ = 7.0, 5.0, 0.8 Hz, 1H), 6.02 (d, $J$ = 8.6 Hz, 1H), 3.94 (s, 2H), 2.41 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 1.24 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.12, 158.56, 157.96, 147.97, 143.47, 136.92, 136.68, 132.06, 130.47, 129.78, 127.71, 115.95, 112.31, 107.60, 43.95, 17.61, 13.22, 11.66, 10.88. (ESI) $m/z$ for C19H21N$_3$*ONa* $[M+Na]^+$, calcd 330.1582, found 330.1618. HPLC analysis: MeOH-$H_2O$ (55:45), 8.38 min, 99.93% purity.

*5.12.4.2. N-(5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)-N-ethylpyridin-4-amine,* ***4d****.* The title compound was obtained following general procedure C. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, $J$ = 6.4 Hz, 2H), 7.43 (d, $J$ = 7.9 Hz, 1H), 7.20 (dd, $J$ = 7.8, 1.8 Hz, 1H), 7.01 (d, $J$ = 1.8 Hz, 1H), 6.36 (d, $J$ = 5.4 Hz, 2H), 3.71 (s, 2H), 2.42 (s, 3H), 2.28 (s, 3H), 2.16 (s, 3H), 1.28 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.24, 158.43, 153.11, 149.07, 142.69, 136.13, 132.36, 130.22, 129.82, 128.48, 115.70, 107.15, 45.50, 17.48, 12.36, 11.68, 10.86. (ESI) $m/z$ for C19H22N$_3$O $[M+H]^+$, calcd 308.1685, found 308.1775. HPLC analysis: MeOH-$H_2O$ (70:30) with 0.1% $Et_3N$, 4.01 min, 94.25% purity.

*5.12.4.3. N-(5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)pyridin-4-amine,* ***4e****.* The title compound was obtained following general procedure C. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.27 (dd, $J$ = 4.9, 1.5 Hz, 2H), 7.35 (d, $J$ = 7.8 Hz, 1H), 7.17 (d, $J$ = 1.7 Hz, 1H), 7.09–6.97 (m, 1H), 6.67 (dd, $J$ = 4.9, 1.5 Hz, 2H), 6.00 (s, 1H), 2.41 (s, 3H), 2.29 (s, 3H), 2.28 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.22, 158.54, 151.48, 149.96, 138.01, 132.00, 131.83, 129.38, 126.22, 124.62, 115.97, 109.28, 17.70, 11.65, 10.87. (ESI) $m/z$ for $C_{17}H_{18}N_3O$ $[M+H]^+$, calcd 280.1372, found 280.1444. HPLC analysis: MeOH-$H_2O$ (70:30) with 0.1% $Et_3N$, 4.93 min, 97.75 purity.

## Author contributions

J.Y and L.Z contributed equally to this work. The manuscript was written by J.Y. The design of compounds was performed by J.Y. The synthesis of compounds was performed by J.Y., L.Z., C.C. Biological evaluation was accomplished by J.Y., P.Z. and F.Z. The molecular modeling was performed by G.Y. And X. L provided important suggestions to this work. All authors have given approval to the final version of the manuscript.

## Conflicts of interest

The authors declare no competing financial interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.03.041.

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