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Design and synthesis of novel androgen receptor antagonists via molecular modeling

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ABSTRACT

Several androgen receptor (AR) antagonists are clinically prescribed to treat prostate cancer. Unfortunately, many patients become resistant to the existing AR antagonists. To overcome this, a novel AR antagonist candidate called DIMN was discovered by our research group in 2013. In order to develop compounds with improved potency, we designed novel DIMN derivatives based on a docking study and substituted carbons with heteroatom moieties. Encouraging in vitro results for compounds **1b**, **1c**, **1e**, **3c**, and **4c** proved that the new design was successful. Among the newly synthesized compounds, **1e** exhibited the strongest inhibitory effect on LNCaP cell growth (IC_{50} = 0.35 μM) and also acted as a competitive AR antagonist with selectivity over the estrogen receptor (ER) and the glucocorticoid receptor (GR). A docking study of compound **1e** fully supported these biological results. Compound **1e** is considered to be a novel, potent and AR-specific antagonist for treating prostate cancer. Thus, our study successfully applied molecular modeling and bioisosteric replacement for hit optimization. The methods here provide a guide for future development of drug candidates through structure-based drug discovery and chemical modifications.

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1. Introduction

The androgen receptor (AR) belongs to the nuclear receptor family of ligand-activated transcriptional factors. Testosterone and dihydrotestosterone (DHT) are the most common ligands of AR.[1–3] Since the AR plays a critical role in prostate cancer, many AR antagonists have been used clinically to treat prostate cancer.[4,5] However, long term treatment of AR antagonists often leads to drug-resistance and mutations in the AR receptor are considered to be the main reason.[6–10] As reported, AR antagonists prevent formation of the activation function 2 (AF2) site essential for recruitment of AR coactivators by displacing helix 12 (H12).[11] Mutations in AR usually allow H12 to reposition to form AF2 again even in the presence of an antagonist.[12] For example, the first nonsteroidal AR antagonist flutamide (Fig. 1) acts as an agonist when the T877A mutation is present in AR.[13]

Several of the AR antagonists administered clinically are derivatives of flutamide.[14,15] A novel AR antagonist enzalutamide (MDV3100) was approved by the U.S. Food and Drug Administration (FDA) in 2012. The drug was investigated based on the research of flutamide.[16] Due to the structural similarity, flutamide-like drugs are expected to possess similar physicochemical and pharmacological limitations as flutamide. Thus, considering this fact, more and more research groups have focused on the development of AR antagonists with a novel scaffold to treat prostate cancer.[17–19]

Our research group has a long-standing interest in discovery of novel AR antagonists for treating prostate cancer. In a previous study, we successfully discovered nicotinamide derivative 6-(3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-(6-methylpyridin-2-yl) nicotinamide (DIMN) (Fig. 1) as a novel class of nonsteroidal AR antagonist for the treatment of prostate cancer.[20,21] DIMN was anticipated to interact with AR as shown in Figure 2. On one side of the ligand-binding domain (LBD), the amide group and N of pyridine of DIMN form several hydrogen bonds with Arg752, Phe764, and Gln711 of AR (Fig. 2B). On the other end, the isoquinoline ring approaches Met895 of H12 and possibly pushes the helix out of the LBD to attain an open conformation of AR.

AR perfectly accommodates linear DIMN. However, a large space of the hydrophobic cone-shaped cavity surrounded by Trp741, Met742, His874, Met895, Ile898, Ile899, and Val903 above the isoquinoline ring remains unoccupied (Fig. 2A). Thus, we hypothesized that introduction of a long linear hydrophobic group at the end of the isoquinoline ring, which can fit into this cavity, may enhance binding affinity and increase displacement of H12.

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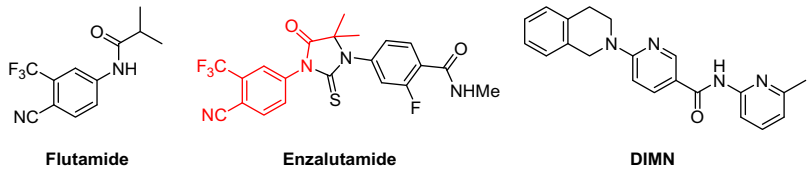


Figure 1. Known AR antagonists.

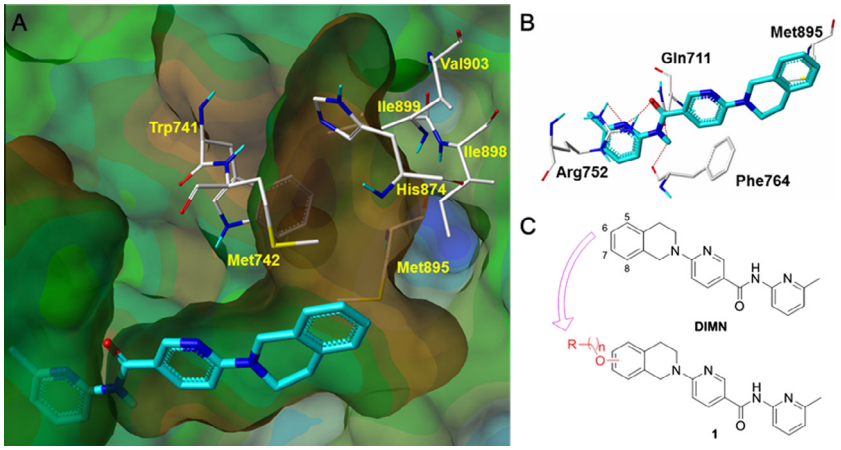


Figure 2. Design of novel derivatives of DIMN. (A) & (B) The binding mode of DIMN in complex with AR. Hydrogen bonds are represented as red dashed lines. Hydrophobicity (lipophilicity) is represented as textured colors where dark brown is more hydrophobic and bright green is less hydrophobic. (C) Design of new DIMN derivatives.

Therefore, novel derivatives of DIMN that contain an additional linear group on the isoquinoline ring were designed (Fig. 2C).

Several compounds were designed and applied to the docking study. Most of the newly designed compounds exhibited better docking scores than DIMN. Among the compounds subjected to docking, derivatives with an additional group at the 6-position of the isoquinoline ring exhibited the best docking results. The binding model of one newly designed compound, **1b**, is illustrated in Figure 3. The amide moiety of **1b** interacted with Arg752, Phe764, and Gln711 of AR via several hydrogen bonds. An additional hydrogen bond was formed between the ethereal O of the isoquinoline ring and Thr877. As expected, the *n*-Pr moiety occupied the hydrophobic cavity. These two new interactions may increase the binding affinity between the compound **1b** and the AR. With these encouraging docking results in hand, novel DIMN derivatives with linear alkyl substituents were synthesized.

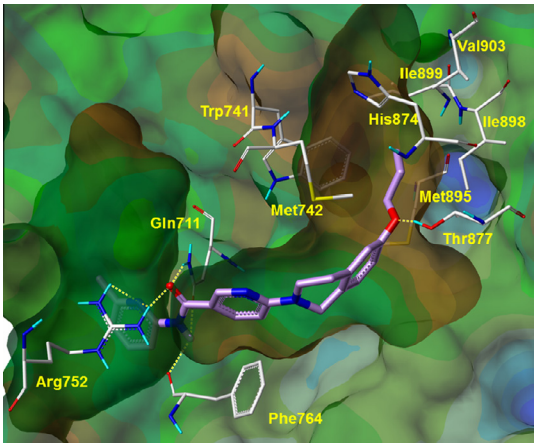


Figure 3. The binding mode of compound **1b** in complex with AR. Hydrogen bonds are represented as yellow dashed lines. Hydrophobicity (lipophilicity) is represented as textured colors where dark brown is more hydrophobic and bright green is less hydrophobic.

In addition to the development of DIMN derivatives with linear substituents, preparation of bioisosteres of DIMN was considered to be beneficial. Previous studies show that compounds with a nicotinamide moiety exhibited much greater activity than those with a benzamide functional group (**2**, Fig. 4). Coincidentally, another research group showed that replacing pyridinone with pyrimidinone improved activity.[22] On the basis of these findings, we hypothesized that an additional N in the core ring may improve bioactivity. Therefore, changing the pyridine ring to a pyrazine and a pyrimidine may gain bioisosteric effects and enhance hydrophilicity. For this reason, compounds **3a** and **4a** were designed and applied to the docking study (Fig. 4).

The docking study demonstrated that **3a** and **4a** had higher docking scores than those of DIMN. A complex of **3a** and AR showed that the amide moiety interacted with Phe764, Arg752, and Gln711 through five hydrogen bonds (Fig. 5A). The N of methyl pyridine associated with AR via hydrogen bonding to Arg752. The N from the pyrazine ring created another hydrogen bond with Gln711. This demonstrated that introducing an additional N to the pyrazine ring is beneficial. The isoquinoline ring faced Met895 to repel H12 and attain an open conformation. Compound **4a** also exhibited interactions with AR similar to DIMN (Fig. 5B). The amide moiety interacted with Phe764, Arg752, and Gln711 of AR via several hydrogen bonds. The isoquinoline ring was oriented in a position to push H12 further away from the ligand binding pocket. According to these results, several additional compounds belonging to these two new series of pyrazinamides and pyrimidinamides were synthesized.

2. Chemistry

The detailed synthesis procedure for nicotinamides **1a–h** is described in Scheme 1.[20] Chloronicotinic acid **5** was refluxed with

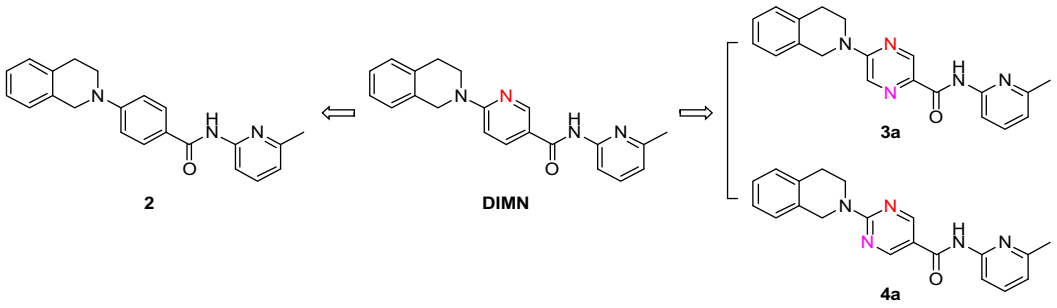


Figure 4. Design of a new series of compounds **3a** and **4a**.

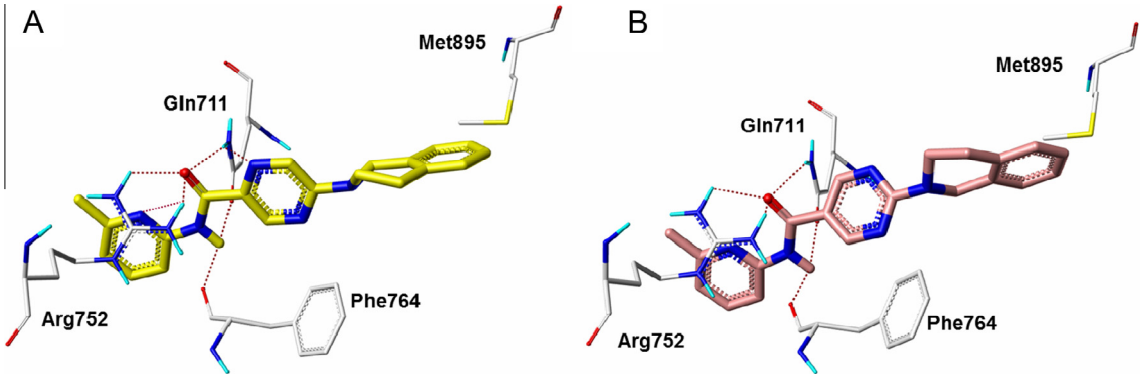
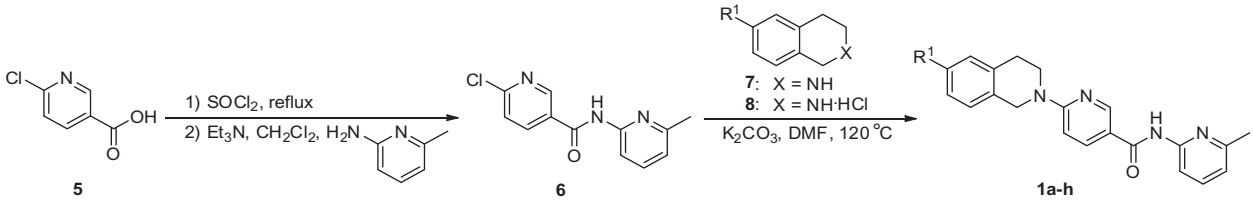


Figure 5. The binding mode of compound **3a** (A) and **4a** (B) in complex with AR. Hydrogen bonds are represented as red dashed lines.



Scheme 1. The synthesis of nicotinamides **1a**–**h**.

$SOCl_2$ followed by treatment with 2-amino-6-methylpyridine to yield amide **6**. The final compounds **1a**–**h** (Table 1) were obtained by a S_NAr reaction of compound **6** with different isoquinolines in the presence of K_2CO_3.

Synthesis of pyrazinamides **3a**–**l** is outlined in Scheme 2. Pyrazine carboxylic acid **10** was obtained after de-esterification of commercially available methylpyrazinecarboxylate **9**. The remaining synthetic steps to produce pyrazinamides **3a**–**l** were similar to the methods used for nicotinamides **1a**–**h**; amidation of acid **10** with different amines followed by an S_NAr reaction with isoquinoline afforded compounds **3a**–**l** (Table 2).

For synthesizing pyrimidinamides **4a**–**f**, the chloropyrimidine carboxylic acid **17** was prepared by the method described in Scheme 3.[23] Compound **13** was obtained from ester **12** in the presence of NaOEt. Subsequent cyclization of compound **13** with urea was applied to afford compound **14** which was then reduced to yield hydroxyl pyrimidine **15**. The chlorination and further de-esterification of compound **15** gave the chloropyrimidine carboxylic acid **17**. With the precursor in hand, the same synthetic route used for nicotinamides **1a**–**h** was used to afford pyrimidinamides **4a**–**f** (Table 3).

To improve aqueous solubility of DIMN based AR antagonists, carboxylic acids **4g**–**h** and sodium salt **4i** were prepared as illustrated in Scheme 4. Compounds **4g**–**h** were obtained via de-esterification of compounds **4e**–**f**. Treatment of acid **4h** with *n*-BuOH and NaOH afforded sodium salt **4i**.

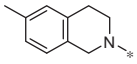
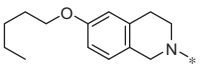
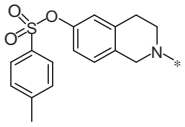
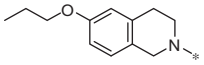
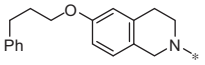
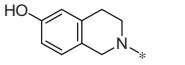
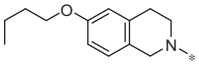
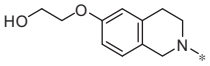
3. Results and discussion

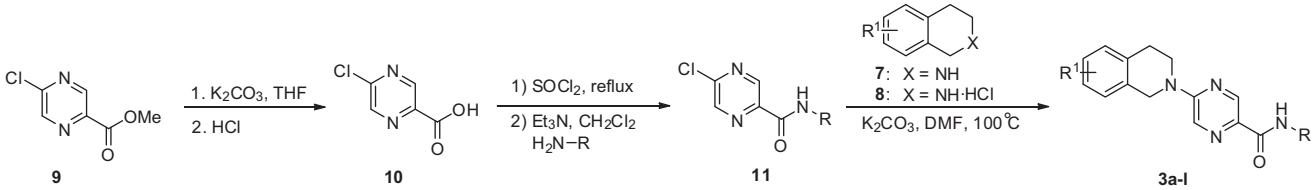
3.1. Cell growth inhibition

In total, 29 compounds were synthesized based on the design. Prior to verifying the probability of synthesized compounds as new AR antagonists, the potential cytotoxic effects of the test compounds in mouse myoblast C2C12 cells (normal cells) were investigated at a concentration of 10 μM using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Fig. 6A). Additionally, inhibition of LNCaP cell (prostate cancer cells) proliferation was also investigated (Fig. 6B). Most of the newly synthesized compounds did not exhibit cytotoxicity in C2C12 cells. In contrast, some of them exhibited potent inhibition of prostate cancer cell growth compared to DIMN.

Six compounds (**1b**, **1c**, **1e**, **1h**, **3c**, and **4c**) with potent cytotoxicity in prostate cancer cells but no cytotoxicity in normal cells were selected for further studies. The half maximal inhibitory concentration (IC_{50}) of the six selected compounds on prostate cancer

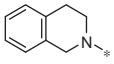
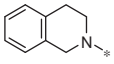
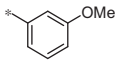
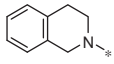
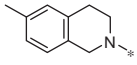
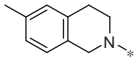
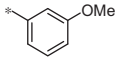
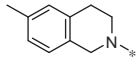
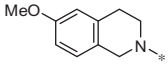
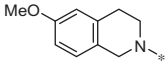
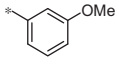
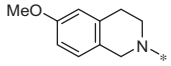
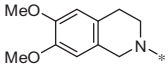
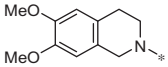
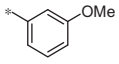
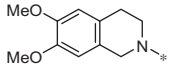
Table 1
Structures of nicotinamides **1a–h**

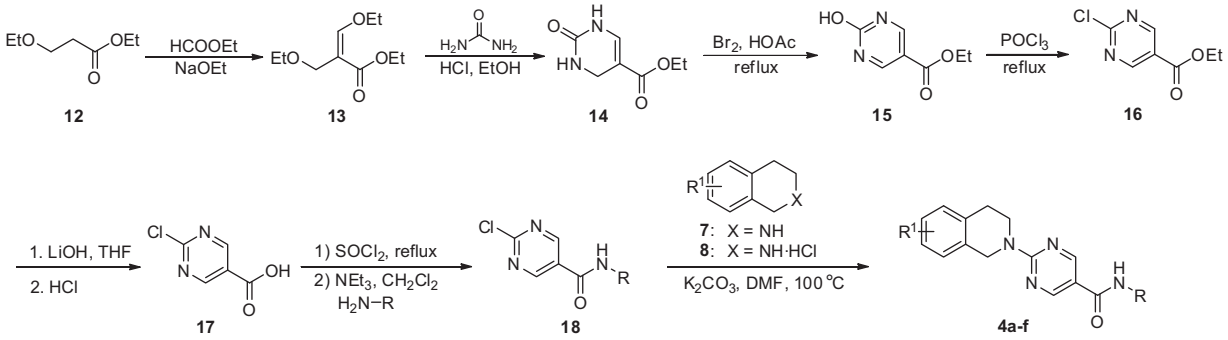
Compd		Compd		Compd	
1a		**1d**		**1g**	
1b		**1e**		**1h**	
1c		**1f**			



Scheme 2. The synthesis of pyrazinamides **3a–l**.

Table 2
Structures of pyrazinamides **3a–l**

Compd	R		Compd	R		Compd	R	
3a			**3e**			**3i**		
3b			**3f**			**3j**		
3c			**3g**			**3k**		
3d			**3h**			**3l**		

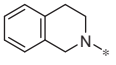
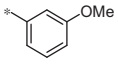
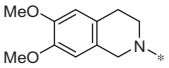
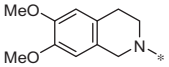
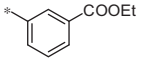
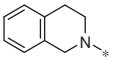
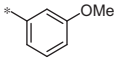
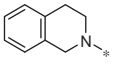
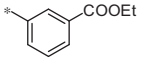
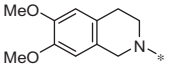


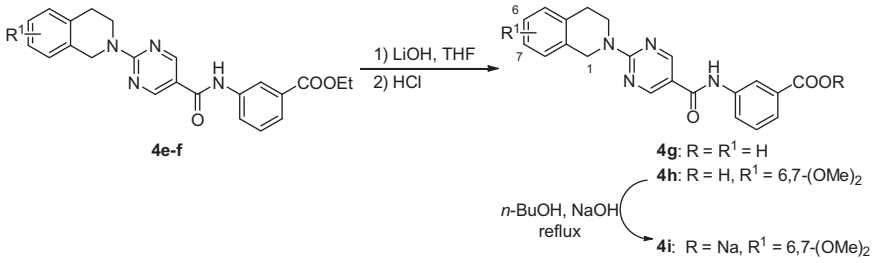
Scheme 3. The synthesis of pyrimidinamides **4a–f**.

cell (LNCaP) growth was further determined. The IC_{50} values of these compounds were lower than that of DIMN and BIC (Table 4). Compound **1e** was the most active among these compounds with the lowest IC_{50} (0.35 μM).

As expected, derivatives with long, linear, hydrophobic side chains, such as *n*-Pr (**1b**), *n*-Bu (**1c**), and $(CH_2)_3Ph$ (**1e**), exhibited potent bioactivity. The activity increased as the length of the side chain became longer; the *n*-Bu moiety (**1c**, IC_{50} = 0.41 μM) was more cytotoxic than the *n*-Pr moiety (**1b**, IC_{50} = 1.01 μM). In contrast, the long linear hydrophilic side chain $(CH_2)_2OH$ (**1f**) reduced the inhibition of cancer cell proliferation. When a bulky group such as a phenyl ring (**1e**, IC_{50} = 0.35 μM) was introduced at the end of

Table 3
Structures of pyrimidinamides **4a–f**

Compd	R		Compd	R	
4a			**4d**		
4b			**4e**		
4c			**4f**		



Scheme 4. The synthesis of pyrimidinamides **4g–i**.

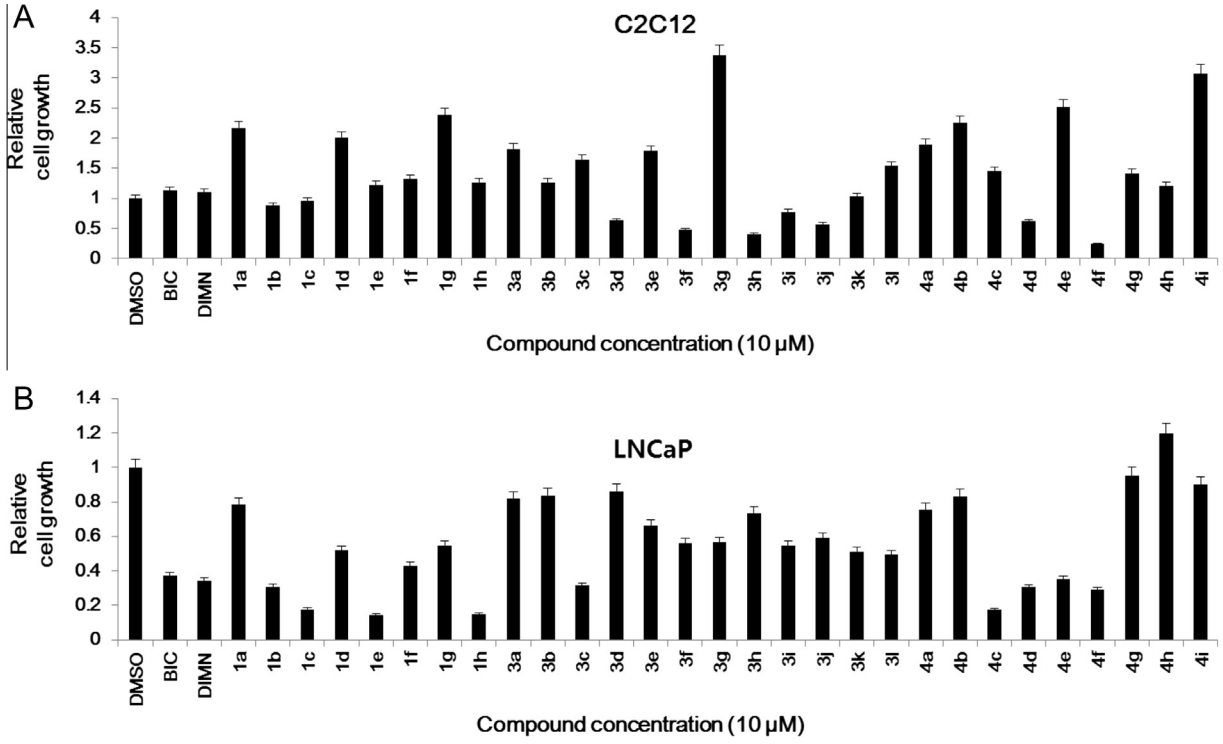


Figure 6. Cytotoxicity of nicotinamides, pyrazinamides, and pyrimidinamides in myoblast C2C12 (A) and prostate cancer LNCaP (B) cells. C2C12 myoblast and LNCaP prostate cancer cells were cultured in DMEM (for C2C12 cell) or RPMI1640 (for LNCaP cell) media with 10% FBS and were treated with the reference compounds bicalutamide (BIC), DIMN, and the 29 synthesized compounds (10 μM) for 3 days. The proliferation of LNCaP cells was measured by MTT assay.

alky chain, the activity improved compared to the unsubstituted alky chain. However, replacement of the alkyl linker with a sulfonate moiety, such as in compound **1g**, was detrimental. This result supported our hypothesis that introducing long, linear, hydrophobic chains to occupy the cavity increases bioactivity. Compounds **3a** and **4a**, which contain a pyrazine and a pyrimidine ring, respectively, did not exhibit anti-proliferative activity. However, compounds **3c** and **4c** showed significant in vitro results.

Interestingly, the inhibitory effect of **1b**, **1c**, **1e**, **1h**, and **3c** on proliferation of LNCaP cells was retained even after 5 days of treatment (Fig. 7). Compound **1e** exhibited the strongest inhibition of cancer cell growth (80%) after 5 days. The prolonged activity

Table 4
IC_{50} values of six selected compounds

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
1b	1.01	**3c**	0.49
1c	0.41	**4c**	0.58
1e	0.35	DIMN	4.46
1h	1.25	BIC	2.38

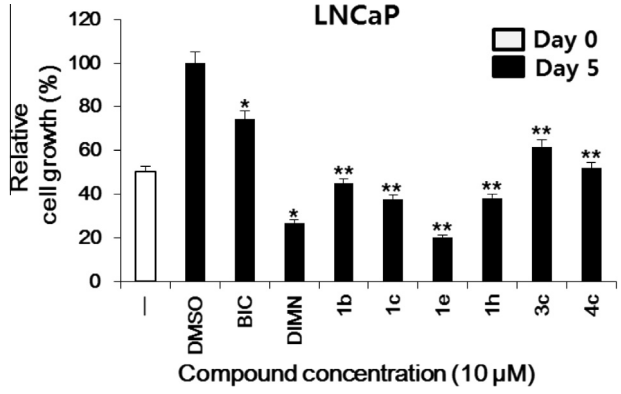


Figure 7. Inhibition effects of six selected compounds on cancer cell growth after 5 days. LNCaP prostate cancer cells were cultured in RPMI1640 media with 10% fetal bovine serum (FBS) and were treated with reference compounds BIC, DIMN, and compounds **1b**, **1c**, **1e**, **1h**, **3c**, and **4c** at a concentration of 10 μM for 5 days. The proliferation of LNCaP cells was measured by MTT assay. $^{*}p$ <0.05 compared to cells treated only with dimethylsulfoxide (DMSO); $^{**}p$ <0.05 compared to cells treated with the reference compounds (BIC, DIMN). All experiments were repeated at least three times. The averages and standard deviations (SD) of representative experiments are shown.

exhibited by the newer nicotinamides, pyrazinamides, and pyrimidinamides indicates that the cellular metabolism and excretion rate of the compounds are slow. The sustained effect of the derivatives may be advantageous since it would decrease the therapeutic dose, reduce the frequency of drug administration, and improve patient compliance.

3.2. AR antagonistic effect

To analyze whether the selected six compounds (**1b**, **1c**, **1e**, **1h**, **3c**, and **4c**) exhibit antagonistic activity against AR, a luciferase assay with the androgen reporter ARE-TATA-Luc, which contains the androgen response element (ARE) of the androgen target gene C3, was used (Fig. 8). The AR luciferase assays were examined in the presence of 0.3 nM DHT co-treatment. All of the six test compounds inhibited AR activity. Among the compounds tested, compound **1e** demonstrated the highest antagonistic effect, even with DHT co-treatment. Significant AR antagonistic activity of nicotinamides **1b**, **1c**, and **1e** supports our assumption that the lengthy, linear, and hydrophobic groups at the isoquinoline end are essential for increasing AR affinity by occupying the empty hydrophobic cavity and repelling H12. In addition, potent inhibition of AR activity and prostate cancer cell growth of compounds **1b**, **1c**, **1e**, **3c**, and **4c** demonstrates that these compounds prevent cancer cell growth primarily by blocking AR activity.

3.3. Selectivity against nuclear receptors

The antagonistic effect of the selected six compounds was tested on the estrogen receptor (ER) and the glucocorticoid receptor (GR). Even with co-treatment with estradiol (E2, ER agonist), most of the derivatives did not exhibit any antagonistic effect on ER activity, with the exception of compound **1h** (Fig. 9A). Furthermore, none of these six compounds showed antagonistic effects on GR activity even with co-treatment with dextromethorphan (DXM, GR agonist). However, compounds **1h** and **4c** may act as agonists of GR since both of them increased the biological response.

3.4. Docking study

Compound **1e**, which exhibited potent cytotoxicity in prostate cancer cells as well as strong AR-specific antagonistic effect, binds to AR as shown in Figure 10. Five hydrogen bonds were created between the amide moiety and Gln711, Arg752, and Phe764. The N of the pyridine ring interacted with Arg752 to form another hydrogen bond. The ethereal O, which formed a hydrogen bond with Thr877, played an essential role in anchoring the linear side chain into the cone-shaped cavity. The isoquinoline ring and the alkyl chain faced towards amino acids Met895, Ile898, and Ile899 of H12. The consequential hindrance between compound **1e** and H12 possibly leads to displacement of a part of H12 (Asp890-Ile899), which prevents formation of the AF2 site and antagonizes AR. This binding model is fully supported by the in vitro biological results for compound **1e**.

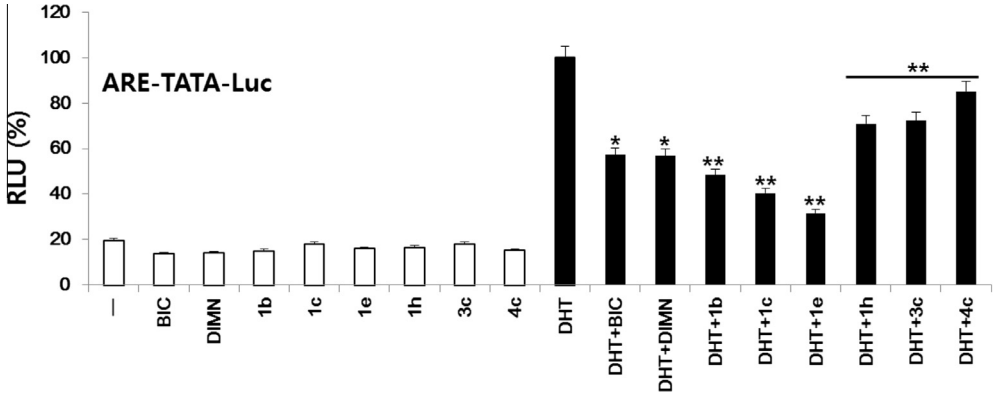


Figure 8. AR antagonistic activity of the six selected compounds. C2C12 myoblast cells were co-transfected with pCMV-β-gal (0.05 μg), an androgen luciferase reporter (AR, ARE-TATA-Luc, 0.2 μg). Cells were treated with the reference compounds BIC and DIMN (each 10 μM), and six selected compounds (**1b**, **1c**, **1e**, **1h**, **3c**, and **4c**, each 5 μM) in the presence of 0.3 nM DHT. Luciferase activities were measured and expressed as a percentage of AR activity in the presence of only 0.3 nM DHT. $^{*}p$ <0.05 compared to cells treated only with DHT; $^{**}p$ <0.05 compared to cells treated only with the reference compounds (BIC, DIMN) and DHT. All experiments were repeated at least three times. The averages and standard deviations (SD) of representative experiments are shown.

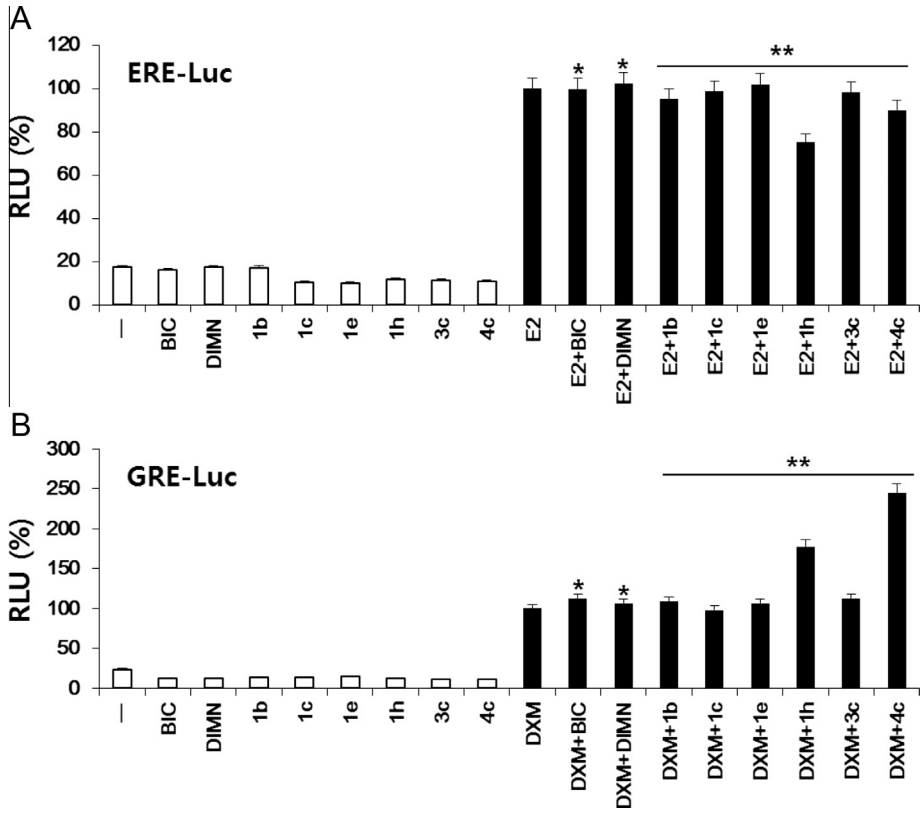


Figure 9. ER (A) and GR (B) antagonistic activity of six selected compounds. C2C12 myoblast cells were co-transfected with pCMV-β-gal (0.05 μg), an estrogen luciferase reporter (ERE-Luc, 0.2 μg, panel A), or a glucocorticoid luciferase receptor (GRE-Luc, 0.2 μg, panel B). Cells were treated with the reference compounds BIC, DIMN (each 10 μM), and compounds **1b**, **1c**, **1e**, **1h**, **3c**, and **4c** (each 5 μM) in the presence of 100 nM E2 and 100 nM dextromethorphan (DXM). Luciferase activities were measured and expressed as a percentage of ER or GR activity in the presence of 100 nM E2 or 100 nM DXM, respectively. All experiments were repeated at least three times. The averages and standard deviations (SD) of representative experiments are shown.

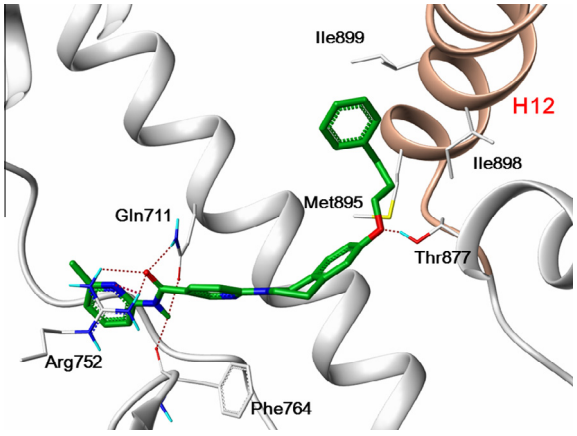


Figure 10. Binding model of compound **1e** in complex with AR. Hydrogen bonds are represented as red dashed lines. The ribbon of H12 is represented as red color.

4. Conclusion

Three new series of DIMN derivatives (nicotinamides, pyrazinamides, and pyrimidinamides) were designed and synthesized. Nicotinamides with lengthy alkoxy chain were designed on the basis of a docking study to orient them into the un-occupied hydrophobic cavity of the ligand binding pocket, while pyrazinamides and pyrimidinamides with an additional N were designed in response to previous reports indicating that heteroatomic bioisosteres display more potent biological responses. As expected, compounds (**1b**, **1c**, and **1e**) that contain long linear side chains on the isoquinoline ring suppressed prostate cancer cell growth better than DIMN. Compounds **3c** and **4c** also exhibited the desired cytotoxic results. The inhibitory effects of these compounds and compound **1h** on prostate cancer cell growth was sustained after five days, indicating a slow rate of metabolism and excretion from prostate cancer cell. Compounds **1b**, **1c**, **1e**, and **3c** exhibited potent AR antagonistic effects as well as good selectivity over ER and GR. The compound with the lowest IC_{50} value and most potent AR antagonistic effect, compound **1e**, was applied to the docking study. The biological results of compound **1e** were fully supported by the binding model. Our results indicate that the newer derivatives of DIMN are potent AR antagonists, and may be promising anticancer drugs that warrant further systematic optimization and pharmacological evaluation.

5. Experiments

5.1. Molecular modeling

For docking studies, Surflex-Dock in Sybyl version X 2.0 (Tripos Associates) was used, operating on Windows 7 on an HP computer (Intel Xeon 4, 2.8 GHz CPU, 1 GB memory). The structures of the AR antagonists were drawn in the Sybyl package with standard bond lengths and angles, and minimized using the conjugate gradient method. The Gasteiger-Huckel charge was applied for the minimization process, with a distance-dependent dielectric function. A preliminary docking study was carried out using the crystal structure of AR with EM5744 (PDB ID: 2PNU). The structure was polished as follows: all water molecules were removed from the

crystal structure and the ligand (EM5744) was extracted. The AR protein was then analyzed using the Protein Structure Preparation Tool in Sybyl. After adding hydrogens, the side-chain amides were also fixed and two bumping amino acids (such as Ser904 and Tyr972) were adjusted. Stage minimization was also applied with the AMBER FF99 force field. Then the protomol was generated.

Surflex-Dock performed flexible docking with newly designed AR antagonists as potential ligands. The maximum number of poses per ligand was 20.

5.2. Chemistry

Chemicals were purchased from Sigma–Aldrich Co. or Tokyo Chemical Industry Co. and utilized without further purification. Solvents were distilled prior to use. IR spectra were recorded on a JASCO-FT IR spectrometer using KBr pellets. Melting points were determined by the capillary method with a MEL-TEMP® 3.0 apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were collected on a Varian Unity Plus 300 MHz and Varian Unity Inova 500 FT spectrometers at the Korea Basic Science Institute. The NMR data are displayed as follows: chemical shifts (δ) are recorded as ppm, coupling constants (J) in hertz (Hz), integrity as the number of protons, and multiplicity as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), quintet, sextet, and m (multiplet). Mass spectra were obtained on a Waters 2695 Separations Module (LC) and Quatro Micro API triple-quadrupole tandem mass spectrometer (MS). Thin-layer chromatography (TLC) was carried out using plates coated with silica gel 60 F_{254}, purchased from Merck. Column chromatography was performed using Merck silica gel 60 (70–230 mesh).

5.2.1. Synthesis of nicotinamides 1a–h

5.2.1.1. 6-(6-Methyl-3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-(6-methylpyridin-2-yl)nicotinamide (1a). Compound **7a** (147 mg, 1.0 mmol), compound **6** (248 mg, 1.0 mmol), and potassium carbonate (414 mg, 3.0 mmol) were added into *N*,*N*-dimethylformamide, and the mixture was stirred at 120 °C for 1 day. After the reaction was completed, the mixture was extracted with EtOAc, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo. The residues were purified by flash column chromatography (EtOAc/hexane = 1:2) to give compound **1a** (177 mg, 49%) as a white solid. IR (cm^{-1}): 1673 (CO). Mp 88–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80 (d, J = 2.4 Hz 1H), 8.37 (br s, 1H), 8.15 (d, J = 8.1 Hz 1H), 8.02 (dd, J = 9.0, 2.4 Hz, 1H), 7.62 (t, J = 7.8 Hz 1H), 7.13–7.02 (m, 3H), 6.90 (d, J = 7.5 Hz, 1H), 6.66 (d, J = 9.0 Hz, 1H), 4.76 (s, 2H), 3.91 (t, J = 6.0 Hz, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.47 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.3, 159.8, 156.6, 151.0, 148.4, 138.7, 136.4, 136.3, 135.0, 130.6, 128.8, 127.2, 126.4, 119.0, 117.6, 111.0, 105.2, 64.3, 46.6, 42.5, 28.9, 25.3, 24.0, 21.0. MS (ESI): m/z 359 $(M+H)^+$.

5.2.1.2. *N*-(6-Methylpyridin-2-yl)-6-(6-propoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)nicotinamide (1b). The procedure described for the synthesis of compound **1a** was used with compound **8a** (120 mg, 0.6 mmol) and compound **6** (148 mg, 0.6 mmol) in the presence of potassium carbonate (248 mg, 1.8 mmol) to afford compound **1b** (177 mg, 73%) as a light yellow solid purified by flash column chromatography (EtOAc/hexane = 1:3). IR (cm^{-1}): 1652. Mp 95–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.82 (d, J = 2.4 Hz 1H), 8.74 (br s, 1H), 8.17 (d, J = 8.1 Hz 1H), 8.05 (dd, J = 9.0, 2.4 Hz, 1H), 7.63 (t, J = 7.5 Hz 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 7.5 Hz, 1H), 6.80–6.73 (m, 2H), 6.66 (d, J = 9.0 Hz, 1H), 4.73 (s, 2H), 3.94–3.88 (m, 4H), 2.95 (t, J = 6.0 Hz, 2H), 2.47 (s, 3H), 1.81 (sextet, J = 7.5 Hz, 2H), 1.03 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.0, 159.8, 157.6, 156.9, 152.1, 149.4, 138.6, 137.6, 136.7, 127.9, 126.3, 119.1, 118.0, 114.0, 113.2, 112.0, 105.6, 69.3, 46.2, 42.4, 28.7, 24.0, 22.5, 10.8. MS (ESI): m/z 403 $(M+H)^+$.

5.2.1.3. 6-(6-Butoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-(6-methylpyridin-2-yl)nicotinamide (1c). The procedure described for the synthesis of compound **1a** was used with compound **8b** (242 mg, 1.0 mmol) and compound **6** (247 mg, 1.0 mmol) in the presence of potassium carbonate (360 mg, 3.0 mmol) to afford compound **1c** (232 mg, 56%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:3). IR (cm^{-1}): 1670 (CO). Mp 138–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80 (s, 1H), 8.60 (br s, 1H), 8.18–8.15 (m, 1H), 8.03–8.00 (m, 1H), 7.58 (d, J = 9.0 Hz 1H), 7.11 (d, J = 9.0 Hz 1H), 6.89 (d, J = 9.0, 1H), 6.79–6.72 (m, 2H), 6.62 (d, J = 9.0 Hz, 1H), 4.70 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 3.88 (t, J = 6.0 Hz, 2H), 2.93 (t, J = 6.0 Hz, 2H), 2.44 (s, 3H), 1.78–1.71 (m, 2H), 1.55–1.45 (m, 2H), 1.01 (t, J = 6.0 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ: 136.4, 131.9, 130.0, 128.8, 123.1, 120.5, 110.7, 108.6, 108.5, 99.5, 97.6, 91.1, 89.7, 85.9, 85.1, 83.1, 77.3, 39.8, 18.4, 14.5, 3.4, 1.3. MS (ESI): m/z 417 $(M+H)^+$.

5.2.1.4. *N*-(6-Methylpyridin-2-yl)-6-(6-pentyloxy-3,4-dihydro-1*H*-isoquinolin-2-yl)nicotinamide (1d). The procedure described for the synthesis of compound **1a** was used with compound **8c** (256 mg, 1.0 mmol) and compound **6** (247 mg, 1.0 mmol) in the presence of potassium carbonate (360 mg, 3.0 mmol) to afford compound **1d** (173 mg, 40%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:3). IR (cm^{-1}): 1654 (CO). Mp 234–235 °C decompose (dec.). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.94 (br s, 1H), 8.83 (d, J = 2.7 Hz 1H), 8.18 (d, J = 8.4 Hz 1H), 8.06 (dd, J = 9.0, 2.7 Hz, 1H), 7.64 (t, J = 7.5 Hz 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.80–6.73 (m, 2H), 6.66 (d, J = 9.0 Hz, 1H), 4.73 (s, 2H), 3.95 (t, J = 6.9 Hz, 2H), 3.90 (t, J = 6.0 Hz, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.49 (s, 3H), 1.78 (quintet, J = 6.9 Hz, 2H), 1.47–1.35 (m, 4H), 0.93 (t, J = 6.9 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.3, 159.7, 157.9, 156.6, 151.0, 148.4, 141.5, 136.4, 127.4, 125.5, 119.0, 117.6, 111.0, 105.2, 68.0, 46.3, 45.3, 42.3, 28.9, 28.7, 28.1, 22.43, 22.40, 14.0, 13.99. MS (ESI): m/z 431 $(M+H)^+$.

5.2.1.5. *N*-(6-Methylpyridin-2-yl)-6-[6-(3-phenylpropoxy)-3,4-dihydro-1*H*-isoquinolin-2-yl]nicotinamide (1e). The procedure described for the synthesis of compound **1a** was used with compound **8d** (294 mg, 0.8 mmol) and compound **6** (194 mg, 0.8 mmol) in the presence of potassium carbonate (331 mg, 2.4 mmol) to afford compound **1e** (159 mg, 41%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:2). IR (cm^{-1}): 1671 (CO). Mp 93–95 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80 (d, J = 2.1 Hz 1H), 8.38 (br s, 1H), 8.15 (d, J = 8.4 Hz 1H), 8.02 (dd, J = 9.0, 2.4 Hz, 1H), 7.62 (t, J = 7.8 Hz 1H), 7.29–7.19 (m, 5H), 7.12 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 7.2 Hz, 1H), 6.79–6.72 (m, 2H), 6.65 (d, J = 9.0 Hz, 1H), 4.73 (s, 2H), 3.96 (t, J = 6.6 Hz, 2H), 3.90 (t, J = 6.0 Hz, 2H), 2.94 (t, J = 6.0 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.47 (s, 3H), 2.10 (quintet, J = 6.9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.3, 159.8, 157.8, 156.7, 151.0, 148.4, 141.4, 138.7, 136.48, 136.46, 128.5, 128.4, 127.4, 125.9, 125.7, 119.0, 117.7, 113.8, 113.0, 111.03, 105.2, 66.9, 64.3, 46.3, 42.4, 32.1, 30.8, 29.2, 25.3, 24.0. MS (ESI): m/z 479 $(M+H)^+$.

5.2.1.6. 6-[6-(2-Hydroxyethoxy)-3,4-dihydro-1*H*-isoquinolin-2-yl]-*N*-(6-methylpyridin-2-yl)nicotinamide (1f). The procedure described for the synthesis of compound **1a** was used with compound **8e** (183 mg, 0.8 mmol) and compound **6** (194 mg, 0.8 mmol) in the presence of potassium carbonate (331 mg, 2.4 mmol) to afford compound **1f** (249 mg, 77%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3670 (OH), 1672 (CO). Mp 184–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80 (d, J = 2.4 Hz 1H), 8.44 (br s, 1H), 8.16 (d, J = 8.4 Hz 1H), 8.03

(dd, J = 9.0, 2.4 Hz, 1H), 7.63 (t, J = 7.5 Hz 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.81–6.74 (m, 2H), 6.64 (d, J = 9.0 Hz, 1H), 4.71 (s, 2H), 4.10–4.07 (m, 2H), 3.99–3.96 (m, 2H),3.89 (t, J = 6.0 Hz, 2H), 2.94 (t, J = 6.0 Hz, 2H), 2.48 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.0, 159.8, 157.6, 156.9, 152.2, 149.4, 138.7, 137.6, 136.7, 127.9, 126.3, 119.1, 118.0, 114.0, 113.3, 112.0, 105.6, 69.9, 60.0, 46.2, 42.4, 28.7, 24.0. MS (ESI): m/z 405 $(M+H)^+$, 403 $(M-H)^-$.

5.2.1.7. 2-(5-((6-Methylpyridin-2-yl)carbamoyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinolin-6-yl 4-methylbenzenesulfonate (1g). The procedure described for the synthesis of compound **1a** was used with compound **8f** (321 mg, 0.94 mmol) and compound **6** (232 mg, 0.94 mmol) in the presence of potassium carbonate (338 mg, 2.8 mmol) to afford compound **1g** (93 mg, 19%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:2). IR (cm^{-1}): 1673 (CO). Mp 150–152 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80 (d, J = 3.0 Hz 1H), 8.38 (br s, 1H), 8.15 (d, J = 9.0 Hz 1H), 8.04 (dd, J = 9.0, 3.0 Hz, 1H), 7.74–7.72 (m, 2H), 7.62 (t, J = 6.0 Hz, 1H), 7.32 (d, J = 9.0 Hz, 2H),7.11 (d, J = 9.0 Hz, 1H), 6.90–6.88 (m, 2H), 6.78–6.75 (m, 1H), 6.67 (d, J = 9.0 Hz, 1H), 4.77 (s, 2H), 3.88 (t, J = 6.0 Hz, 2H), 2.93 (t, J = 6.0 Hz, 2H), 2.48 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.2, 159.6, 156.7, 150.9, 148.3, 148.0, 145.3, 138.7, 136.6, 132.5, 129.7, 128.4, 127.6, 122.1, 120.2, 119.1, 118.1, 111.0, 105.3, 46.3, 42.1, 28.8, 23.9, 21.7. MS (ESI): m/z 515 $(M+H)^+$.

5.2.1.8. 6-(6-Hydroxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-(6-methylpyridin-2-yl)nicotinamide (1h). The procedure described for the synthesis of compound **1a** was used with compound **24** (345 mg, 1.5 mmol) and compound **6** (372 mg, 1.5 mmol) in the presence of potassium carbonate (622 mg, 4.5 mmol) to afford compound **1h** (172 mg, 32%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:2). IR (cm^{-1}): 3386 (NH), 1672 (CO). Mp 213–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.75 (d, J = 2.4 Hz 1H), 8.47 (br s, 1H), 8.16 (d, J = 8.4 Hz 1H), 7.98 (dd, J = 9.0, 2.4 Hz, 1H), 7.64 (t, J = 7.8 Hz 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 7.5 Hz, 1H), 6.75–6.70 (m, 2H), 6.62 (d, J = 9.0 Hz, 1H), 4.70 (s, 2H), 3.86 (t, J = 6.0 Hz, 2H), 2.90 (t, J = 6.0 Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.0, 162.8, 159.8, 156.9, 156.2, 152.2, 149.4, 138.6, 137.5, 136.6, 127.8, 124.6, 119.1, 117.9, 114.9, 113.8, 112.0, 105.6, 46.2, 42.5, 36.2, 31.2, 28.65, 24.0. MS (ESI): m/z 361 $(M+H)^+$, 359 $(M-H)^-$.

5.2.2. Synthesis compounds 3a–l

5.2.2.1. 5-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrazine-2-carboxamide (3a). 1,2,3,4-Tetra-hydroisoquinoline (67 mg, 0.5 mmol), compound **11a** (125 mg, 0.5 mmol), and potassium carbonate (207 mg, 1.5 mmol) were added to *N*,*N*-dimethylformamide, and the mixture was stirred at 100 °C for 12 h. After the reaction was completed, the mixture was extracted with EtOAc, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo. The residues were purified by flash column chromatography (EtOAc/hexane = 1:3) to give compound **3a** (90 mg, 52%) as a white solid. IR (cm^{-1}): 1677 (CO). Mp 173–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.92 (s, 1H), 8.99 (s, 1H), 8.21 (d, J = 6.0 Hz, 1H), 8.05 (s, 1H), 7.63 (t, J = 9.0 Hz, 1H), 7.26–7.21 (m, 4H), 6.91 (d, J = 6.0 Hz, 1H), 4.85 (s, 2H), 3.97 (t, J = 6.0 Hz, 2H), 3.03 (t, J = 6.0 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5, 156.9, 154.9, 150.7, 143.2, 138.5, 134.8, 132.9, 132.2, 128.2, 127.3, 127.0, 126.7, 126.5, 119.0, 110.6, 46.4, 42.3, 28.7, 24.1. MS (ESI): m/z 346 $(M+H)^+$.

5.2.2.2. 5-(6-Methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrazine-2-carboxamide (3b). The procedure described for the synthesis of compound **3a** was used with compound **7a** (74 mg, 0.5 mmol) and compound **11a** (125 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3b** (146 mg, 41%) as a light yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 1677 (CO). Mp 142–143 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.92 (s, 1H), 8.98 (s, 1H), 8.21 (d, J = 6.0 Hz, 1H), 8.04 (s, 1H), 7.63 (t, J = 9.0 Hz, 1H), 7.15–7.12 (m, 1H), 7.08–7.04 (m, 2H), 6.91 (d, J = 6.0 Hz, 1H), 4.81 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.50 (s, 3H), 3.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5, 156.9, 154.9, 150.7, 143.2, 138.5, 136.7, 134.6, 132.1, 129.8, 128.8, 127.4, 127.3, 126.4, 118.9, 110.6, 46.2, 42.3, 28.7, 24.1, 21.0. MS (ESI): m/z 360 $(M+H)^+$.

5.2.2.3. 5-(6-Methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrazine-2-carboxamide (3c). The procedure described for the synthesis of compound **3a** was used with compound **7b** (82 mg, 0.5 mmol) and compound **11a** (125 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3c** (84 mg, 21%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3371 (NH), 1670 (CO). Mp 143–144 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.92 (s, 1H), 8.99 (s, 1H), 8.20 (d, J = 6.0 Hz, 1H), 8.03 (s, 1H), 7.63 (t, J = 9.0 Hz, 1H), 7.13 (t, J = 3.0 Hz, 1H), 6.91 (d, J = 6.0 Hz, 1H), 6.83–6.75 (m, 2H), 4.78 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.00 (t, J = 6.0 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5, 158.5, 156.9, 154.9, 150.7, 143.2, 138.51, 136.0, 132.1, 127.5, 127.4, 125.3, 124.8, 118.9, 113.2, 112.6, 110.6, 55.3, 45.9, 42.2, 29.0, 24.1. MS (ESI): m/z 376 $(M+H)^+$.

5.2.2.4. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrazine-2-carboxamide (3d). The procedure described for the synthesis of compound **3a** was used with 6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (115 mg, 0.5 mmol) and compound **11a** (125 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3d** (100 mg, 49%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:2). IR (cm^{-1}): 3366 (NH), 1680 (CO). Mp 188–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.93 (s, 1H), 8.98 (s, 1H), 8.21 (d, J = 6.0 Hz, 1H), 8.05 (s, 1H), 7.63 (t, J = 6.0 Hz, 1H), 6.91 (d, J = 6.0 Hz, 1H), 6.73 (s, 1H), 6.70 (s, 1H), 4.78 (s, 2H), 3.97 (t, J = 6.0 Hz, 2H), 3.89 (s, 6H), 2.94 (t, J = 6.0 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5, 156.9, 155.0, 150.7, 148.0, 147.9, 143.2, 138.5, 132.2, 127.3, 126.6, 124.5, 119.0, 111.2, 110.6, 109.3, 56.0, 56.03, 56.00, 46.2, 42.3, 28.1, 24.1. MS (ESI): m/z 406 $(M+H)^+$.

5.2.2.5. 5-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-*N*-(pyridin-2-yl) pyrazine-2-carboxamide (3e). The procedure described for the synthesis of compound **3a** was used with 1,2,3,4-tetra-hydroisoquinoline (40 mg, 0.3 mmol) and compound **11b** (70 mg, 0.3 mmol) in the presence of potassium carbonate (124 mg, 0.9 mmol) to afford compound **3e** (72 mg, 72%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3359 (NH), 1669 (CO). Mp 161–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.00 (s, 1H), 9.00 (s, 1H), 8.43–8.40 (m, 1H), 8.35–8.33 (m, 1H), 8.07 (s, 1H), 7.78–7.72 (m, 1H), 7.26–7.23 (m, 4H), 7.05–7.03 (m, 1H), 4.86 (s, 2H), 3.97 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 155.0, 151.4, 148.0, 143.2, 138.2, 134.8, 132.9, 132.1, 128.3, 127.4, 127.0, 126.7, 126.5, 119.5, 113.8, 46.4, 42.3, 28.7. MS (ESI): m/z 332 $(M+H)^+$.

5.2.2.6. 5-(6-Methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(pyridin-2-yl)pyrazine-2-carboxamide (3f). The procedure described for the synthesis of compound **3a** was used with compound **7a**

(44 mg, 0.3 mmol) and compound **11b** (70 mg, 0.3 mmol) in the presence of potassium carbonate (124 mg, 0.9 mmol) to afford compound **3f** (65 mg, 72%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3366 (NH), 1675 (CO). Mp 144–145 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.99 (s, 1H), 8.99 (s, 1H), 8.42–8.39 (m, 1H), 8.35–8.32 (m, 1H), 8.05 (s, 1H), 7.74–7.71 (m, 1H), 7.15–7.12 (m, 1H), 7.07–7.03 (m, 3H), 4.81 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 155.0, 151.4, 148.0, 143.2, 138.2, 136.7, 134.6, 132.0, 129.8, 128.8, 127.4, 126.4, 119.5, 113.8, 46.2, 42.3, 28.7, 21.0. MS (ESI): m/z 346 $(M+H)^+$.

5.2.2.7. 5-(6-Methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(pyridin-2-yl)pyrazine-2-carboxamide (3g). The procedure described for the synthesis of compound **3a** was used with compound **7b** (82 mg, 0.3 mmol) and compound **11b** (70 mg, 0.3 mmol) in the presence of potassium carbonate (124 mg, 0.9 mmol) to afford compound **3g** (45 mg, 42%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 1671 (CO). Mp 145–147 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 10.01 (s, 1H), 8.99 (s, 1H), 8.43–8.40 (m, 1H), 8.35–8.33 (m, 1H), 8.05 (s, 1H), 7.77–7.72 (m, 1H), 7.16 (d, J = 9.0 Hz, 1H), 7.05–7.03 (m, 1H), 6.83–6.80 (m, 1H), 6.76–6.75 (m, 1H), 4.78 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 3.00 (t, J = 6.0 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 162.6, 158.5, 154.9, 151.4, 148.0, 143.2, 138.2, 136.1, 132.0, 127.5, 127.4, 124.9, 120.0, 119.5, 114.0, 113.8, 113.3, 113.2, 112.6, 55.3, 45.9, 42.1, 29.0. MS (ESI): m/z 362 $(M+H)^+$.

5.2.2.8. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(pyridin-2-yl)pyrazine-2-carboxamide (3h). The procedure described for the synthesis of compound **3a** was used with 6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (69 mg, 0.3 mmol) and compound **11b** (70 mg, 0.3 mmol) in the presence of potassium carbonate (124 mg, 0.9 mmol) to afford compound **3h** (58 mg, 49%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 1653 (CO). Mp 179–182 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 10.00 (s, 1H), 9.00 (s, 1H), 8.42–8.39 (m, 1H), 8.35–8.33 (m, 1H), 8.07 (s, 1H), 7.78–7.72 (m, 1H), 7.06–7.03 (m, 1H), 6.73 (s, 1H), 6.70 (s, 1H), 4.79 (s, 2H), 3.98 (t, J = 6.0 Hz, 2H), 3.89 (s, 6H), 2.95 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 155.0, 151.4, 148.04, 148.01, 147.9, 143.2, 138.2, 132.0, 127.4, 126.5, 124.5, 119.5, 113.8, 111.2, 109.3, 56.03, 56.00, 46.2, 42.3, 28.1. MS (ESI): m/z 392 $(M+H)^+$.

5.2.2.9. 5-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-*N*-(3-methoxyphenyl)pyrazine-2-carboxamide (3i). The procedure described for the synthesis of compound **3a** was used with 1,2,3,4-tetra-hydroisoquinoline (67 mg, 0.5 mmol) and compound **11c** (132 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3i** (149 mg, 83%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3353 (NH), 1675 (CO). Mp 132–133 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 8.99 (s, 1H), 8.05 (s, 1H), 7.57 (t, J = 3.0 Hz, 1H), 7.28–7.23 (m, 5H), 7.19–7.16 (m, 1H), 6.70–6.66 (m, 1H), 4.85 (s, 2H), 3.96 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 3.03 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.1, 160.2, 154.9, 143.1, 139.3, 134.8, 132.9, 132.5, 129.6, 128.3, 127.1, 127.0, 126.7, 126.5, 111.7, 110.1, 104.9, 55.3, 46.3, 42.3, 28.7. MS (ESI): m/z 361 $(M+H)^+$.

5.2.2.10. *N*-(3-Methoxyphenyl)-5-(6-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)pyrazine-2-carboxamide (3j). The procedure described for the synthesis of compound **3a** was used with compound **7a** (74 mg, 0.5 mmol) and compound **11c** (132 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3j** (122 mg, 65%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3351 (NH), 1682 (CO). Mp 138–139 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 8.99 (s, 1H), 8.03 (s, 1H), 7.57 (s, 1H), 7.27–7.24 (m, 1H), 7.18–7.12 (m, 2H), 7.07–7.04 (m, 2H), 6.69–6.67 (s, 1H), 4.80 (s, 2H), 3.94 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.99 (t, J = 6.0 Hz, 2H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.1, 160.2, 154.9, 143.1, 139.3, 134.6, 132.4, 129.6, 128.8, 127.4, 127.1, 126.4, 111.7, 110.1, 104.9, 55.3, 46.2, 42.35, 28.6, 21.0. MS (ESI): m/z 375 $(M+H)^+$.

5.2.2.11. 5-(6-Methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(3-methoxyphenyl)pyrazine-2-carboxamide (3k). The procedure described for the synthesis of compound **3a** was used with compound **7b** (82 mg, 0.5 mmol) and compound **11c** (132 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3k** (72 mg, 37%) as a white solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 1682 (CO). Mp 136–138 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 8.99 (s, 1H), 8.03 (s, 1H), 7.57 (s, 1H), 7.27–7.24 (m, 1H), 7.18–7.13 (m, 2H), 6.82–6.80 (m, 1H), 6.76–6.75 (s, 1H), 6.69–6.67 (m, 1H), 4.78 (s, 2H), 3.95 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.00 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.1, 160.2, 158.6, 154.9, 143.1, 139.3, 136.1, 132.4, 129.6, 127.5, 127.1, 125.0, 113.2, 112.6, 111.7, 110.1, 104.9, 104.89, 55.34, 55.3. 45.9, 42.2, 29.0. MS (ESI): m/z 391 $(M+H)^+$.

5.2.2.12. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(3-methoxyphenyl)pyrazine-2-carboxamide (3l). The procedure described for the synthesis of compound **3a** was used with 6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (115 mg, 0.5 mmol) and compound **11c** (132 mg, 0.5 mmol) in the presence of potassium carbonate (207 mg, 1.5 mmol) to afford compound **3l** (119 mg, 57%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 3345 (NH), 1682 (CO). Mp 179–182 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 8.99 (s, 1H), 8.05 (s, 1H), 7.57 (s, 1H), 7.26–7.23 (m, 1H), 7.18–7.16 (m, 1H), 6.73 (br s, 1H), 6.70–6.67 (m, 2H), 4.78 (s, 2H), 3.97 (t, J = 6.0 Hz, 2H), 3.89 (s, 6H), 3.85 (s, 3H), 2.95 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.0, 160.2, 154.9, 148.0, 147.9, 143.1, 139.2, 132.4, 129.6, 127.1, 126.6, 124.6, 111.7, 111.2, 110.1, 109.4, 104.9, 56.04, 56.01, 55.3, 46.1, 42.3, 28.1. MS (ESI): m/z 421 $(M+H)^+$.

5.2.3. Synthesis of compounds 4a–f

5.2.3.1. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrimidine-5-carboxamide (4a). 1,2,3,4-Tetra-hydroisoquinoline (134 mg, 1.0 mmol), compound **18a** (124 mg, 0.5 mmol), and potassium carbonate (138 mg, 1.0 mmol) were added to *N*,*N*-dimethylformamide, and the mixture was stirred at 100 °C for 8 h. After the reaction was completed, the mixture was extracted with EtOAc, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo. The residues were purified by a recrystallization in 2-propanol to give compound **4a** (60 mg, 34%) as a white solid. IR (cm^{-1}): 3384 (NH), 1666 (CO). Mp 161–163 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.15 (s, 2H), 8.54 (d, J = 9.0 Hz, 1H), 7.94 (t, J = 9.0 Hz, 1H), 7.23–7.18 (m, 4H), 7.07 (d, J = 6.0 Hz, 1H), 5.03 (s, 2H), 4.17 (t, J = 6.0 Hz, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.68 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.3, 161.8, 158.5, 152.3, 149.8, 143.3, 134.8, 133.4, 128.5, 126.6, 126.5, 126.4, 119.2, 114.3, 113.0, 46.3, 42.0, 28.8, 21.2. MS (ESI): m/z 346 $(M+H)^+$.

5.2.3.2. 2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(6-methylpyridin-2-yl)pyrimidine-5-carboxamide (4b). The procedure described for the synthesis of compound **4a** was used with

6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (230 mg, 1.0 mmol) and compound **18a** (124 mg, 0.5 mmol) in the presence of potassium carbonate (138 mg, 1.0 mmol) to afford compound **4b** (104 mg, 51%) as a yellow solid purified by a recrystallization in 2-propanol IR (cm^{-1}): 1648 (CO). Mp 228–230 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 12.18 (s, 1H), 9.26 (s, 2H), 8.74 (d, J = 9.0 Hz, 1H), 8.10 (t, J = 9.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H), 6.70 (s, 1H), 6.67 (s, 1H), 4.98 (s, 2H), 4.17 (t, J = 6.0 Hz, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 2.89 (t, J = 6.0 Hz, 2H), 2.79 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.5, 161.8, 159.0, 150.1, 149.3, 147.7, 145.5, 126.5, 125.0, 119.0, 113.9, 113.6, 111.3, 109.2, 55.94, 55.9, 46.0, 42.07, 28.2, 19.8. MS (ESI): m/z 406 $(M+H)^+$.

5.2.3.3. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-*N*-(3-methoxyphenyl)pyrimidine-5-carboxamide (4c). The procedure described for the synthesis of compound **4a** was used with 1,2,3,4-tetra-hydroisoquinoline (134 mg, 1.0 mmol) and compound **18b** (131 mg, 0.5 mmol) in the presence of potassium carbonate (138 mg, 1.0 mmol) to afford compound **4c** (143 mg, 79%) as an off white solid purified by a recrystallization in 2-propanol. IR (cm^{-1}): 3285 (NH), 1640 (CO). Mp 152–153 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.83 (s, 2H), 7.66 (s, 1H), 7.40–7.39 (m, 1H), 7.26–7.17 (m, 5H), 6.71–6.68 (m, 1H), 4.99 (s, 2H), 4.13 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 2.96 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.1, 161.7, 160.1, 157.3, 138.9, 134.8, 133.4, 129.6, 128.5, 126.6, 126.4, 126.3, 116.1, 112.4, 110.5, 106.0, 55.2, 46.2, 41.8, 28.7. MS (ESI): m/z 361 $(M+H)^+$, 359 $(M-H)^-$.

5.2.3.4. 2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-(3-methoxyphenyl)pyrimidine-5-carboxamide (4d). The procedure described for the synthesis of compound **4a** was used with 6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (230 mg, 1.0 mmol) and compound **18b** (131 mg, 0.5 mmol) in the presence of potassium carbonate (138 mg, 1.0 mmol) to afford compound **4d** (172 mg, 82%) as a yellow solid purified by flash column chromatography (EtOAc/hexane = 1:1). IR (cm^{-1}): 1647 (CO). Mp 187–188 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.83 (s, 2H), 7.57 (s, 1H), 7.40–7.39 (m, 1H), 7.28–7.23 (m, 1H), 7.07–7.04 (m, 1H). 6.73–6.67 (m, 3H), 4.93 (s, 2H), 4.14 (t, J = 6.0 Hz, 2H), 3.88 (s, 6H), 3.83 (s, 3H), 2.89 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.0, 161.7, 160.1, 157.3, 147.7, 138.9, 129.6, 126.60, 125.3, 116.1, 111.3, 109.2, 106.0, 55.9, 55.2, 45.9, 41.9, 28.2. MS (ESI): m/z 421 $(M+H)^+$, 419 $(M-H)^-$.

5.2.3.5. Ethyl-3-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)pyrimidine-5-carboxamido)benzoate (4e). The procedure described for the synthesis of compound **4a** was used with 1,2,3,4-tetra-hydroisoquinoline (134 mg, 1.0 mmol) and compound **18c** (152 mg, 0.5 mmol) in the presence of potassium carbonate (138 mg, 1.0 mmol) to afford compound **4e** (81 mg, 40%) as an off white solid purified by a recrystallization in 2-propanol. IR (cm^{-1}): 1715 (CO), 1654 (CO). Mp 119–122 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.86 (s, 2H), 8.10–8.08 (m, 1H), 8.05–8.02 (m, 1H)., 7.87 (s, 1H), 7.83–7.80 (m, 1H), 7.41 (t, J = 9.0 Hz, 1H), 7.23–7.20 (m, 4H), 4.99 (s, 2H), 4.35 (q, J = 9.0 Hz, 2H), 4.14 (t, J = 6.0 Hz, 2H), 2.97 (t, J = 6.0 Hz, 2H), 1.39 (t, J = 9.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.2, 163.1, 161.8, 157.4, 137.9, 134.8, 133.4, 131.2, 129.1, 128.5, 126.6, 126.4, 125.5, 124.8, 121.1, 115.8, 61.2, 46.3, 41.9, 28.8, 14.2. MS (ESI): m/z 403 $(M+H)^+$, 401 $(M-H)^-$.

5.2.3.6. Ethyl-3-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pyrimidine-5-carboxamido)benzoate (4f). The procedure described for the synthesis of compound **4a** was used with 6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride (230 mg, 1.0 mmol) and compound **18c** (150 mg, 0.5 mmol) in the presence of potassium carbonate (138 mg, 1.0 mmol) to afford compound **4f** (141 mg, 61%) as a yellow solid purified by a recrystallization in 2-propanol. IR (cm^{-1}): 1715 (CO), 1637 (CO). Mp 151–153 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.86 (s, 2H), 8.10–8.09 (m, 1H), 8.05–8.02 (m, 1H)., 7.89 (s, 1H), 7.83–7.80 (m, 1H), 7.44 (t, J = 9.0 Hz, 1H), 6.70 (s, 1H), 6.67 (s, 1H), 4.92 (s, 2H), 4.39 (q, J = 9.0 Hz, 2H), 4.09 (t, J = 6.0 Hz, 2H), 3.87 (s, 6H), 2.88 (t, J = 6.0 Hz, 2H), 1.39 (t, J = 9.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.2, 163.3, 161.7, 157.5, 147.6, 138.0, 131.1, 129.1, 126.5, 125.4, 125.1, 128.4, 121.1, 115.7, 111.3, 109.2, 61.2, 55.9, 46.0, 41.9, 28.2, 14.2. MS (ESI): m/z 463 $(M+H)^+$, 461 $(M-H)^-$.

5.2.4. Synthesis of water soluble compounds

5.2.4.1. 3-(2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)pyrimidine-5-carboxamido)benzoic acid (4g). A solution of compound **4e** (56 mg, 0.14 mmol) in THF (10 mL) and EtOH (2 mL) was treated with 1 M NaOH solution (2.8 mL). The resulting reaction mixture was stirred at 25 °C for 12 h. Extra THF was removed in vacuo. The reaction mixture was then acidified to a pH of about 2 with concentrated HCl. Compound **4g** (52 mg, 99%) was obtained as a white solid by filtration. IR (cm^{-1}): 1645 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 13.0 (s, 1H), 10.29 (s, 1H), 8.96 (s, 2H), 8.35 (s, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.68–7.65 (m, 1H), 7.47 (t, J = 6.0 Hz, 1H), 7.30–7.20 (m, 4H), 4.97 (s, 2H), 4.08 (t, J = 6.0 Hz, 2H), 2.92 (t, J = 6.0 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.2, 163.0, 161.3, 158.2, 139.3, 134.8, 133.6, 131.32, 129.0, 128.5, 126.5, 126.5, 126.2, 124.4, 124.3, 121.0, 116.6, 46.0, 45.8, 28.0. MS (ESI): m/z 375 $(M+H)^+$, 373 $(M-H)^-$.

5.2.4.2. 3-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl) pyrimidine-5-carboxamido)benzoic acid (4h). The procedure described for the synthesis of compound **4g** was used with compound **4f** (90 mg, 0.2 mmol) and 1 M NaOH (4 mL, 0.4 mmol) to afford compound **4h** (64 mg, 74%) as an orange solid. IR (cm^{-1}): 1704 (CO), 1644 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 10.33 (s, 1H), 8.96 (s, 2H), 8.36 (s, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.47 (t, J = 9.0 Hz, 1H), 6.89 (s, 1H), 6.77 (s, 1H), 4.89 (s, 2H), 4.06 (t, J = 6.0 Hz, 2H), 3.74 (s, 3H), 3.72 (s, 3H), 2.92 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 167.2, 162.9, 161.2, 158.1, 147.44, 147.41, 139.3, 131.2, 128.9, 126.3, 125.2, 124.3, 121.0, 116.1, 111.9, 110.0, 55.6, 55.5, 45.5, 41.6, 27.5. MS (ESI): m/z 435 $(M+H)^+$, 433 $(M-H)^-$.

5.2.4.3. Sodium 3-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pyrimidine-5-carboxamido)benzoate (4i). A solution of compound **4h** (43 mg, 0.10 mmol) in *n*-BuOH (5 mL) was treated with NaOH (16 mg, 0.4 mmol). The resulting reaction mixture was stirred at 120 °C for 30 min. Then, the reaction mixture was cooled down, and the precipitate was filtered to afford compound **4i** (16 mg, 35%) as a brown solid. IR (cm^{-1}): 1653 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 10.19 (s, 1H), 8.97 (s, 2H), 8.11 (s, 1H), 7.83 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.22 (t, J = 9.0 Hz, 1H), 6.90 (s, 1H), 6.77 (s, 1H), 4.88 (s, 2H), 4.05 (t, J = 6.0 Hz, 2H), 3.74 (s, 3H), 3.72 (s, 3H), 2.81 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 169.6, 162.6, 161.3, 158.1, 141.1, 138.0, 134.8, 133.7, 128.4, 127.2, 126.5, 126.2, 124.5, 121.6, 120.8, 116.6, 79.2, 79.1, 60.4, 45.8, 28.0. MS (ESI): m/z 435 $(M-Na+2H)^+$.

5.2.5. Synthesis of intermediate compounds

5.2.5.1. 6-Chloro-*N*-(6-methylpyridin-2-yl)nicotinamide (6). A solution of 6-chloro-nicotinic acid **5** (1.57 g, 10 mmol) in $SOCl_2$ was heated to reflux overnight. The excess $SOCl_2$ was removed by vacuum distillation. The residue was dissolved in CH_2Cl_2 and carefully treated with a solution of 2-amino-6-methylpiridine

(5.4 g, 50 mmol) and NEt_3 (1.71 g, 17 mmol) in CH_2Cl_2. The mixture was stirred at room temperature (rt) overnight. After the reaction was completed, the mixture was extracted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo and the residue was recrystallized from 2-propanol to give compound **6** as a white solid (2.05 g, 83%). Mp: 107–115 °C. IR (cm^{-1}): 3309 (NH), 1665 (CO). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.95 (m, 1H,), 8.73 (s, 1H), 8.2–8.19 (m, 1H), 8.13 (d, J = 8.3 Hz, 1H), 7.76 (m, 1H), 7.46 (d, J = 8.3 Hz, 1H), 6.98 (d, J = 7.4 Hz, 1H), 2.46 (s, 3H). MS (ESI): m/z (248, MH^+).

5.2.5.2. 5-Chloropyrazine-2-carboxylic acid (10). A solution of methyl 5-chloropyrazine-2-carboxylate (345 mg, 2.0 mmol) in THF (10 mL) was treated with a solution of potassium carbonate (552 mg, 4.0 mmol) in water (5 mL). The resulting reaction mixture was stirred at 25 °C for 1 day. Extra THF was removed in vacuo. The reaction mixture was then acidified to a pH of about 2 with concentrated HCl. Compound **10** (216 mg, 68%) was obtained as a brown solid by filtration. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.97 (s, 1H), 8.19 (s, 1H).

5.2.5.3. 5-Chloro-*N*-(6-methylpyridin-2-yl)pyrazine-2-carboxamide (11a). A solution of compound **10** (216 mg, 1.36 mmol) in $SOCl_2$ was heated to reflux overnight. The excess $SOCl_2$ was removed by vacuum distillation. The residue was dissolved in CH_2Cl_2 and carefully treated with a solution of 2-amino-6-methylpiridine (739 mg, 6.83 mmol) and NEt_3 (275 mg, 2.7 mmol) in CH_2Cl_2. The mixture was stirred at rt overnight. After the reaction was completed, the mixture was extracted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo and the residue was recrystallized from 2-propanol to give compound **11a** as a yellow solid (181 mg, 53%). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.96 (br s, 1H), 9.28 (s, 1H), 8.60 (s, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.68 (t, J = 9.0 Hz, 1H), 6.99 (d, J = 9.0 Hz, 1H), 2.51 (s, 3H).

5.2.5.4. 5-Chloro-*N*-(pyridin-2-yl)pyrazine-2-carboxamide (11b). The procedure described for the synthesis of compound **11a** was used with compound **10** (1.4 g, 8.86 mmol) and 2-aminopyridine (4.1 g, 44.3 mmol) in the presence of NEt_3 (1.8 g, 17.7 mmol) to afford compound **11b** (1.03 g, 50%) as a brown solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.69 (br s, 1H), 9.28 (s, 1H), 8.60 (s, 1H), 8.41 (d, J = 9.0 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.68 (t, J = 9.0 Hz, 1H), 6.99 (d, J = 9.0 Hz, 1H).

5.2.5.5. 5-Chloro-*N*-(3-methoxyphenyl)pyrazine-2-carboxamide (11c). The procedure described for the synthesis of compound **11a** was used with compound **10** (1.58 g, 10.0 mmol) and *m*-anisidine (6.16 g, 50.0 mmol) in the presence of NEt_3 (2.02 g, 20.0 mmol) to afford compound **11c** (1.68 g, 64%) as a brown solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.49 (br s, 1H), 9.02 (s, 1H), 8.16 (s, 1H), 7.54–7.53 (m, 1H), 7.29–7.17 (m, 2H), 6.73–6.69 (m, 1H), 3.85 (s, 3H).

5.2.5.6. 5-Chloropyrazine-2-carboxylic acid (13). A solution of sodium ethoxide (136 mg, 2.0 mmol) in THF was treated with a mixture of 3-ehoxypropionate (146 mg, 1.0 mmol) and ethyl formate (148 mg, 2.0 mmol) at 20 °C, and stirred at rt for 1 h. The dimethyl sulfate (252 mg, 2.0 mmol) was then added to the mixture and heated to 50 °C for 2 h. The resulting reaction mixture was treated with H_2O and extracted with EtOAc. The organic layer was washed by brine and dried with Na_2SO_4. Extra EtOAc was removed in vacuo to afford the crude compound **13** (393 mg, quantitative) as a brown oil. The crude compound was used in the next step directly.

5.2.5.7. Ethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (14). A solution of compound **13** (393 mg, 2.0 mmol) in EtOH was treated with urea (100 mg, 1.6 mmol) at 30 °C, then HCl was added. The resulting reaction mixture was refluxed for 2 h. The mixture was cooled down, and the precipitate was filtered and washed with EtOH to give the compound **14** (153 mg, 45%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ δ: 8.82 (br s, 1H), 7.08 (d, J = 6.0 Hz, 1H), 7.01 (s, 1H), 4.07 (q, J = 6.0 Hz, 2H), 3.92 (s, 2H), 1.19 (t, J = 6.0 Hz, 3H).

5.2.5.8. Ethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (15). A solution of compound **14** (214 mg, 2.0 mmol) in HOAc was treated dropwise with a solution of bromine (639 mg, 4.0 mmol) in HOAc. The resulting reaction mixture was heated at 110 °C for 3 h. The mixture was cooled down and stirred at rt for 12 h. The precipitate was filtered and washed with acetone to give compound **15** (49 mg, 15%) as a brown solid. ^1H NMR (300 MHz, DMSO) δ: 8.69 (s, 2H), 4.25 (q, J = 6.0 Hz, 2H), 1.30 (t, J = 6.0 Hz, 3H).

5.2.5.9. Ethyl-2-chloropyrimidine-5-carboxylate (16). Compound **15** (168 mg, 1.0 mmol) was dissolved in $POCl_3$, and then refluxed for 8 h. The extra $POCl_3$ was removed by depressed pressure distillation. The residues were treated with ice, and the precipitate was filtered and washed by water to give compound **16** (180 mg, 56%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.16 (s, 2H), 4.46 (q, J = 6.0 Hz, 2H), 1.43 (t, J = 6.0 Hz, 3H).

5.2.5.10. 2-Chloropyrimidine-5-carboxylic acid (17). A solution of compound **16** (235 mg, 1.2 mmol) in THF (10 mL) was treated with 1 M NaOH solution (1.2 mL). The resulting reaction mixture was stirred at 25 °C for 2 h. HCl was added to adjust the pH to around 4. Extra THF was removed in vacuo. The reaction mixture was then again acidified to a pH of about 1 with concentrated HCl. Compound **17** (100 mg, 63%) was obtained as a white solid by filtration. ^1H NMR (300 MHz, DMSO) δ: 9.15 (s, 2H).

5.2.5.11. 2-Chloro-*N*-(6-methylpyridin-2-yl)pyrimidine-5-carboxamide (18a). A solution of compound **17** (628 mg, 4.0 mmol) in $SOCl_2$ was heated to reflux overnight. The excess $SOCl_2$ was removed by vacuum distillation. The residue was dissolved in CH_2Cl_2 and carefully treated with a solution of 2-amino-6-methylpiridine (2.16 g, 20.0 mmol) and NEt_3 (810 mg, 8.0 mmol) in CH_2Cl_2. The mixture was stirred at rt overnight. After the reaction was completed, the mixture was extracted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated in vacuo and the residue was recrystallized from 2-propanol to give compound **18a** as a yellow solid (618 mg, 62%). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.29 (s, 2H), 8.30 (d, J = 9.0 Hz, 1H), 7.87 (t, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 2.60 (s, 3H).

5.2.5.12. 2-Chloro-*N*-(3-methoxyphenyl)pyrimidine-5-carboxamide (18b). The procedure described for the synthesis of compound **18a** was used with compound **17** (474 mg, 3.0 mmol) and *m*-anisidine (1.85 g, 15.0 mmol) in the presence of NEt_3 (607 mg, 6.0 mmol) to afford compound **18b** (528 mg, 67%) as a brown solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.08 (s, 2H), 7.78 (s, 1H), 7.34–7.27 (m, 1H), 7.10–7.08 (m, 1H), 6.79–6.76 (m, 1H), 3.84 (s, 3H).

5.2.5.13. Ethyl-3-(2-chloropyrimidine-5-carboxamido)benzoate (18c). The procedure described for the synthesis of compound **18a** was used with compound **17** (786 mg, 4.97 mmol) and ethyl 3-aminobenzoate (4.1 g, 24.9 mmol) in the presence of NEt_3 (1.0 g, 9.95 mmol) to afford compound **18c** (567 mg, 37%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 9.15 (s, 2H), 8.60 (s, 1H), 8.07–8.05 (m, 1H), 7.10–7.08 (m, 1H), 7.47 (t, J = 6.0 Hz, 1H), 4.34 (q, J = 6.0 Hz, 2H), 1.38 (t, J = 6.0 Hz, 3H).

5.3. Biology

5.3.1. Plasmids and reagents

The mammalian expression plasmids for the mouse AR (pcDNA3.AR) and ARE-TATA-Luc reporter were described previously;[20,24] the human ER (pcDNA3.ER) and ERE-Luc were kindly provided by Dr. Keesook Lee (Chonnam National University, Korea).[25] Dihydrotestosterone (5-androstan-17-ol-3-one) was obtained from Perkin Elmer Life Sciences. Estradiol (E2) was purchased from Sigma–Aldrich.

5.3.2. Cell culture and transient transfection

C2C12 mouse myoblasts and LNCaP prostate cancer cells were cultured at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, for C2C12 cells) and Roswell Park Memorial Institute 1640 (RPMI1640, for LNCaP cells) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 g/mL streptomycin. DMEM, FBS, and the antibiotics were purchased from Life Technologies (Grand Island, NY, USA). Transient transfection was performed using the polyethyleneimine PEI (Polysciences, Warrington, PA, USA)-mediated method. Total amounts of transfected plasmids in each group were equalized by adding the empty vector.

5.3.3. Luciferase receptor assay

C2C12 cells were seeded on 24-well plates 1 day before transfection and then treated with chemicals (0.3 nM DHT, 100 nM E2) for 4 days, or in with no treatment for 1 day. ARE-TATA-Luc and ERE-Luc luciferase reporters contained the regulatory sequence of androgen and estrogen. Cells were transfected with a CMV promoter-driven β-galactosidase reporter (pCMV-β-gal), luciferase reporter, and the indicated combinations of expression plasmids. Thirty-six hours later, luciferase activities were measured using the Luciferase Reporter Assay Kit (Promega, E1501) and a luminometer, and normalized with the corresponding β-galactosidase activities for transfection efficiency. Experiments were performed in triplicates and repeated at least three times. The averages and standard deviations (SD) of representative experiments are shown.

5.3.4. Cell growth and proliferation assay

The cell growth and cytotoxicity rate of C2C12 and LNCaP cells were analyzed by MTT assay. For cell cytotoxicity, cells were cultured in DMEM (for C2C12 cells) and RPMI1640 (for LNCaP cells) supplemented with 10% FBS. 5×10^3 cells per well were seeded in 96-well plates (C2C12 and LNCaP). To calculate IC_{50}, LNCaP cells were treated with chemicals in the presence of 0.3 nM DHT and 100 nM E2 for 4 days. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole blue (MTT, Bioshop, Burlington, ON, Canada), is soluble in PBS. The solution was filter sterilized after adding MTT. MTT stock solution (5 mg/mL) was added to each culture being assayed to equal one-tenth the original culture volume and incubated for 2 h at 37 °C. At the end of the incubation period, the medium was removed and the converted dye was solubilized with acidic isopropanol (0.04–0.1 N HCl in absolute isopropanol). One set of wells with MTT but no cells was included as a negative control. All cell culture work was performed with aseptic technique. Absorbance of converted dye was measured at a wavelength of 570 nm using an ELISA plate reader. The averages and standard deviations (SD) of representative experiments are shown.

5.3.5. Statistical analysis

All experiments were performed with triplicate independent samples and were repeated at least three times giving qualitatively identical results. Results are expressed as mean ± standard error of the mean. Data were analyzed using Student's *t*-test (SPSS version 17.0 software; SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered to indicate a statistically significant difference.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.12.047.

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