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Design, synthesis, and biological evaluation of imidazoline derivatives as p53–MDM2 binding inhibitors

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ABSTRACT

Three series of novel imidazoline derivatives were designed, synthesized, and evaluated for their p53–MDM2 binding inhibitory activities, and anti-proliferation activities against PC3, A549, KB, and HCT116 cancer cell lines. Five of the tested compounds showed enhanced p53–MDM2 binding inhibitory potency and anti-proliferation activities in comparison with that of Nutlin-1. Flow cytometric analysis indicated that compound **7c**, one of the most potent p53–MDM2 binding inhibitors with a K_i value of 0.6 μM, showed its ability to arrest cell cycle progression.

1. Introduction

The close association between p53 and cancer has been recognized for decades.[1] p53, the tumor suppressor peptide, is a DNA damage responsive transcription factor that affects diverse cellular processes including transcription, DNA synthesis and repair, cell cycle arrest, senescence and apoptosis.[2] In approximately 50% human cancers p53 is in wild-type form,[3] while p53 can be inhibited by over-expression or amplification of MDM2 oncoprotein (murine double minute 2, also frequently referred to as HDM2 in human) through an auto-regulatory negative feedback loop.[4] Since more types of cancers are tolerant to elevated levels of wild-type p53,[5] the reactivation of p53 by antagonizing p53–MDM2 interaction might offer a new therapeutic strategy for cancer therapy.

Many series of compounds have been developed as p53–MDM2 binding inhibitors in recent decades.[5–10] Among those, 2,4,5-triphenyl imidazoline derivatives, or Nultins, are one series of anti-proliferative compounds targeted to p53–MDM2 binding. Nutlin-3 (Fig. 1),[8] a potent molecule in this series, has been demonstrated promising bioactivity in vitro and in vivo. It can bind to MDM2 in the p53-binding pockets and reactivate the p53 pathway in cancer cells, leading to cell cycle arrest, apoptosis, and growth inhibition of human tumor xenografts in nude mice.

In our previous study, a pharmacophore model was set up based on the information of p53–MDM2 binding structure and structures of known p53–MDM2 binding inhibitors. The pharmacophore model revealed that three hydrophobic groups on the core structure were necessary for a good p53–MDM2 binding inhibitor, and the introduction of hydrogen acceptor groups might also improve the inhibitory activities.[11]

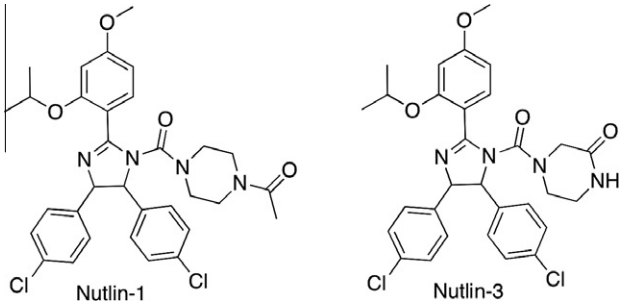

Figure 1. The structure of Nutlin-**1** and Nutlin-3.

Here, we reported the design, synthesis, and biological evaluation of three series of novel Nutlin analogs, aiming to better understand the structure–activity relationships (SARs) and to discover more potent p53–MDM2 binding inhibitors on the basis of above findings. Imidazoline scaffold of Nutlins was retained as the core structure, 2,4,5-triphenyl on imidazoline ring was modified, and the modification N-1 side chain of Nutlins was also carried out. For the first series of Nutlin analogs, the ester bonds were introduced to the N-1 side chain. For the second series, 2-phenyl group

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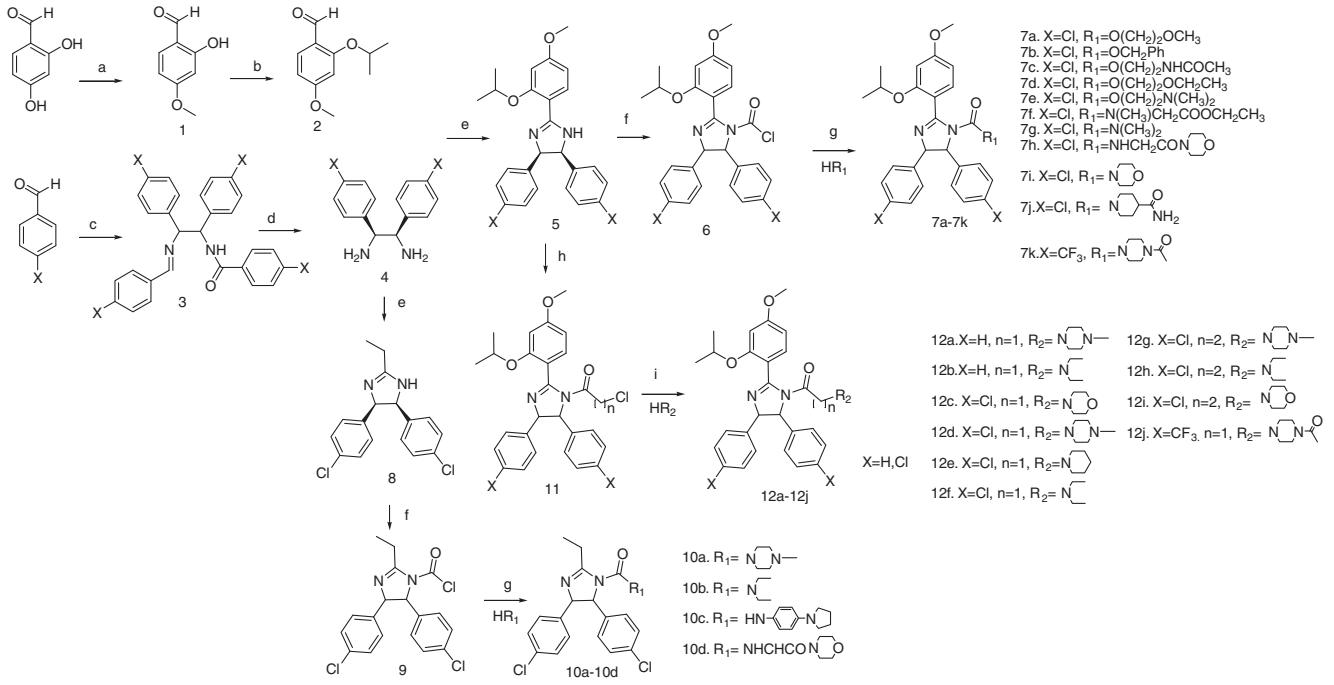


Scheme 1. Reagents and conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 3 h; (b) *i*-PrBr, K_2CO_3, acetone, 40 °C, 5 h; (c) CH_3COONH_4, reflux, 3 h; (d) H_2SO_4, 120 °C, 3 h; (e) NBS, CH_2Cl_2, rt, 24 h; (f) BTC, CH_2Cl_2, TEA; (g) R_1H, TEA, CH_2Cl_2; (h) chloroacetyl chloride or chloropropionyl chloride, acetone; (i) R_2H, TEA, CH_2Cl_2.

was cleaved and replaced by ethyl group. Also, the alky linker was introduced to N-1 to link the core structure with different heterocycles to form the third series. All synthesized compounds were evaluated in vitro for their p53–MDM2 binding inhibitory activities, part of the compounds were tested for their anti-proliferative activities against several human cancer cell lines, and the effects on cell cycle were investigated for the most potent compound, **7c**.

2. Results and discussion

2.1. Chemistry

The synthetic routes for the imidazoline derivatives are summarized in Scheme 1. Etherealization of 2,4-dihydroxy-benzaldehyde with dimethyl sulfate yielded 2-hydroxy-4-methoxybenzaldehyde **1**, which was substituted with isopropyl to give 2-isopropoxy-4-methoxybenzaldehyde **2**. On the other hand, 4-substituted benzaldehydes were converted into diamines **4** according to the known methods with minor modifications.[12] Condensation of **4** with 2-isopropoxy-4-methoxybenzaldehyde **2** in the presence of *N*-bromosuccinimide (NBS) afforded imidazoline intermediate **5**.[13] The hydrobromide salt of **5** was crystallized from methanol and ethanol by vapor diffusion, enabling a high-resolution X-ray crystal structure to be obtained that established the absolute stereochemistry as (*R*, *S*) (Fig. 2).

Acylation of **5** with bis(trichloromethyl)carbonate, chloroacetyl chloride or chloropropionyl chloride yielded **6** and **11**, which were reacted with corresponding alcohols or secondary amines to give target compounds **7a–k** and **12a–j**.

Besides, condensation of **4** with propyl aldehyde in the presence of NBS yielded imidazoline intermediate **8**. Then, acylation of **8** with bis(trichloromethyl)carbonate (BTC) in the presence of base (triethylamine) to give *N*-formyl chloride **9**, which formed 2-ethyl imidazoline analogs **10a–d** by treating with appropriate amines.

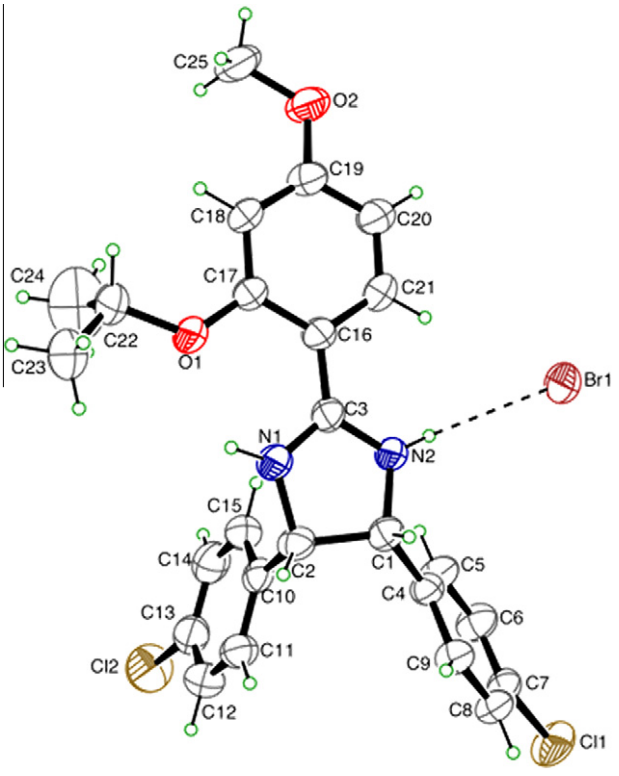
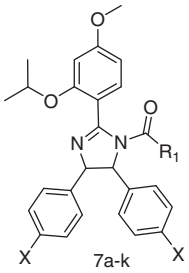


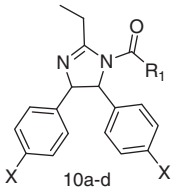
Figure 2. X-ray structure of compound **5** (R_e = 0.044).

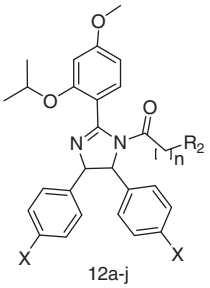
2.2. MDM2–p53 binding inhibition activities

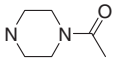
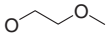
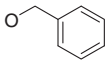
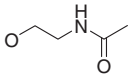
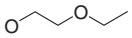
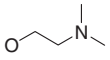
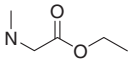
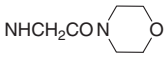
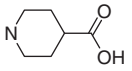
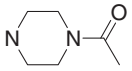
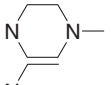
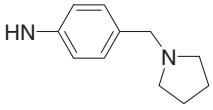
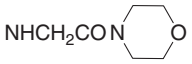
All synthesized compounds were evaluated for their p53–MDM2 binding inhibitory activities by fluorescence-polarization (FP)-based binding assay.[14] Nutlin-1 was used as the positive control. Results are summarized in Table 1.

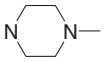
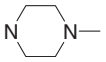
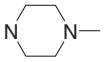
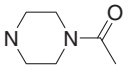
Table 1
p53–MDM2 binding inhibitory activities of imidazoline derivatives (**7a–k**, **10a–d** and **12a–j**)







Compds	X	R_1	K_i[a] (μM)
Nutlin-1	Cl		1.24
7a	Cl		2.3
7b	Cl		32.37
7c	Cl		0.6
7d	Cl		1.38
7e	Cl		0.57
7f	Cl		1.59
7g	Cl		3.32
7h	Cl		0.48
7i	Cl		4.07
7j	Cl		8.77
7k	CF_3		NA[b]
10a	Cl		NA
10b	Cl		NA
10c	Cl		NA
10d	Cl		NA

Compds	X	n	R_2	K_i[a] (μM)
Nutlin-1	Cl	—	—	1.24
12a	H	1		NA
12b	H	1		NA
12c	Cl	1		2.64
12d	Cl	1		25.77
12e	Cl	1		82.07
12f	Cl	1		NA
12g	Cl	2		0.9
12h	Cl	2		1.22
12i	Cl	2		1.3
12j	CF_3	1		NA

[a] Values are means of three experiments.
[b] NA, no activity.

As shown in Table 1, five compounds (**7c, 7e, 7h, 12g**, and **12h**) displayed improved binding inhibitory activities and seven compounds (**7a, 7d, 7f, 7g, 7i, 12c**, and **12i**) showed equivalent similar activities in comparison with that of Nutlin-1. Analogs **10a–d**, in which 2-phenyl group on the imidazoline ring was displaced with ethyl group, did not show significant inhibitory activities to p53–MDM2 binding, which indicated that 2-phenyl group on the imidazoline ring was essential for inhibitory activities. Replacement the para-chlorine atoms on 4,5-diphenyl rings with hydrogen atoms (**12a, b**) or trifluromethyl groups (**7k**, **12j**) caused a loss in potency, suggesting that the presence of chlorine atoms on the 4,5-diphenyl rings played an important role in keeping the binding inhibitory activities. Introduction an ester group to the N-1 side chain (**7a**, **7c–e**) still possessed significant binding inhibitory activities, yet compound **7b** with benzyl formate group on N-1 displayed weak activity (K_i = 32.37 μM). Interestingly, **7d** (K_i = 1.38 μM), **7e** (K_i = 0.57 μM), and **7h** (K_i = 0.48 μM) with longer N-1 side chain exhibited enhanced inhibitory activities than that of **7a** (K_i = 2.30 μM), **7g** (K_i = 3.32 μM), and **7i** (K_i = 4.07 μM). The similar result could also be observed in third series of analogs

Table 2
Anti-proliferative activities of imidazoline derivatives (**7c–h** and **12g–i**) against four human cancer cell lines in vitro

	K_i (μM)	MTT IC_{50}[a,b] (μM)			
		PC3	KB	A549	HCT116
Nutlin-1	1.24	29.2	35.17	2.33	4.58
7c	0.6	33.1	3.6	9.1	5.81
7e	0.57	10.99	8.27	10.85	NA[c]
7h	0.48	14.34	7.39	8.29	10.91
12g	0.90	8.44	5.81	3.19	5.40
12h	1.22	3.32	NT[d]	3.82	3.73
12i	1.30	2.74	NT	3.82	7.57

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are means of three experiments.
[c] NA, no activity.
[d] NT, not tested.

(Table 1), the length of the alkyl linker between the N-1 and heterocycle influenced the binding inhibitory activities obviously. Compounds with a CH_2CH_2 linker (i.e., **12g** and **12i**) showed better activities than molecules with a CH_2 linker (i.e., **12c** and **12d**).

2.3. Cell biology

Potent compounds (**7c**, **7e**, **7h**, **12g**, **12h**, and **12i**) identified in the FP-studies were selected for further evaluation to be certain whether they had effects on intact cells that were consistent with inhibition of MDM2–p53 binding. Four MDM2 amplified cell lines were used, two wild-type p53 cell lines (A549 and HCT116), one p53 null cell line (PC3) and one p53 mutated cell line (KB), Nutlin-1 was used as the positive control. The results are summarized in Table 2. As positive control, Nutlin-1 showed a very good selective profile on wild-type p53 expression cell lines. Although tested six compounds did not show significant improvement on growth inhibitory activities than that of Nutlin-1 on wild-type p53 cell lines (A549 and HCT116), interestingly, as for PC3 or KB cell lines, most of tested compounds exhibited 2–10-folds improvement on growth inhibitory activities than that of Nultin-1. We also noted that, though **12g–i** exhibited weaker p53–MDM2 binding inhibitory activities than that of **7c**, **7e** or **7h**, they exhibited better anti-proliferation activities. A very recent work was reported that Nutlin-3 induced DNA damage response p53-independently,[15] therefore there might be a possibility that besides p53–MDM2 binding inhibitory, compounds **12g–i** could take part into other oncogene pathways to exhibit an enhanced cell growth inhibitory activities.

Moreover, flow cytometric analysis was performed to determine whether target compounds could lead to cell cycle arrest (Fig. 3). A549 cells were treated with **7c** (10 μM) for 72 h, and Nultin-1 (10 μM) was used as positive control. It was shown that **7c** caused an increase in the percentage of cells blocked in the G_2/M phase of cell cycle, with a simultaneous decrease of cells in G_0/G_1 and S phase, which was almost twice effect than that of Nultin-1.

2.4. Molecular docking studies

To address the mechanism behind the different binding inhibitory effects of different N-1 side chain in target compounds, molecular docking study was conducted using CDOCKER of Discovery Studio 2.1 (Accelrys), which uses a CHARMm-based Molecular Dynamics docking algorithm, to study the binding modes between

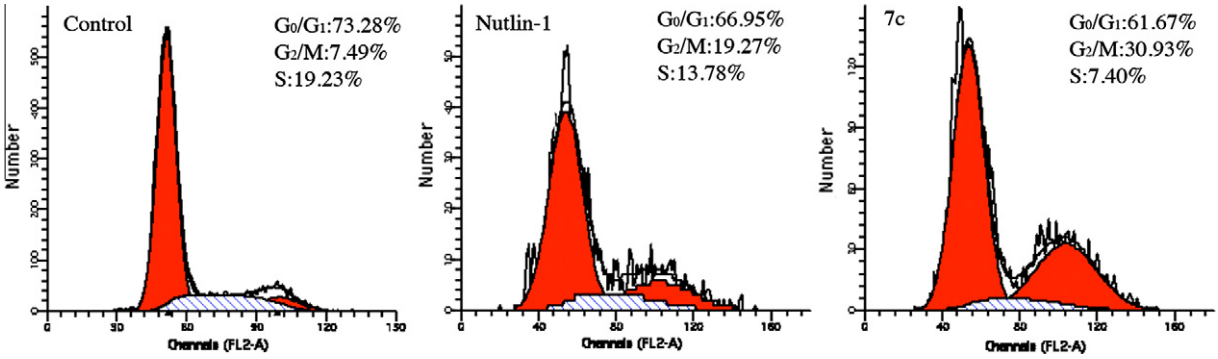


Figure 3. Nutlin-1 and **7c** induced cell cycle arrest in cells with wild-type p53 (A549 cells). After 72 h treatment, **7c** showed almost twice effect, which caused a simultaneous decrease of cells in G_0/G_1 and S phase, than that of Nultin-**1** at a dose of 10 μM.

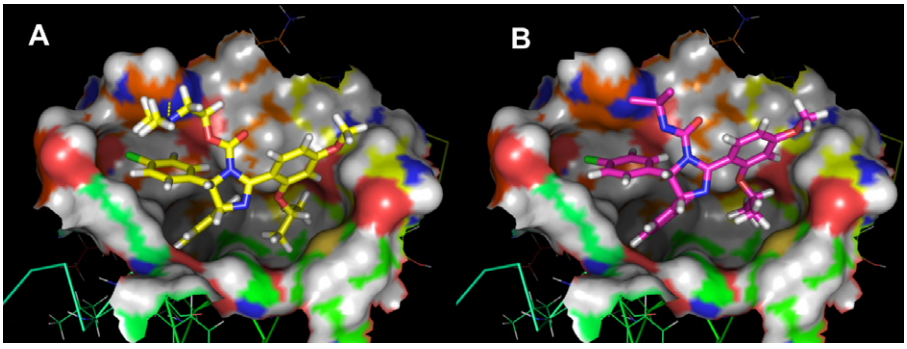


Figure 4. Compound **7e** and **7g** occupying three hydrophobic binding pockets of MDM2 (1RV1), **7e** forms H-bond with His-96 (A). In contrast, **7g** was found no H-bond in the binding mode (B).

molecules (e.g., **7e** and **7g**) and published MDM2 crystal structure (1RV1).[8] The result showed that although both molecules could occupy three hydrophobic binding pockets of MDM2, the CDOCK ENERAGE of **7e** (−**21.223** kcal/mol) was much lower than that of **7g** (−**14.319** kcal/mol). Besides, nitrogen atom on 2-(dimethylamino)ethanol moiety of **7e** could form hydrogen bond with His-96 (Fig. 4A). In contrast, **7g**, with a shorter N-1 side chain, was not able to approach the His-96 and form hydrogen bonds (Fig. 4B). Thus, the results of molecular docking studies could explain the fact that compound **7e** showed more potent activity than that of compound **7g**.

3. Conclusions

Three new series of imidazoline derivatives (**7a–k, 10a–d** and **12a–j**) were designed synthesized and evaluated their biological activities as p53–MDM2 binding inhibitors. Five of the analogs exhibited better p53–MDM2 binding inhibitory activities and anti-proliferative activities in vitro against four human cancer cell lines than that of Nutlin-1. It was suggested that the p53–MDM2 binding inhibitory activities of these compounds was not only related with the length of the N-1 substituted groups but also depended on different substitution patterns on the imidazoline ring. The introduction of heterocyclic residues to the end of N-1 side chain was favorable for the potency. The obtained SAR information is beneficial for the further development of Nutlin derivatives and analogs as p53–MDM2 binding inhibitors for cancer therapy. Further studies are still under investigation.

4. Experimental sections

4.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 400 M spectrometer with $CDCl_3$ as solvent. Chemical shifts were reported in values (ppm), relative to internal TMS, and *J* values were reported in Hertz.). Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. Reagents and solvents were purchased from common commercial suppliers and were used without further purification.

4.1.1. Synthesis of 2-hydroxy-4-methoxybenzaldehyde (1)

Dimethyl sulfate (0.273 mL, 3 mmol) was added to a mixture of 2,4-dihydroxyl-benzaldehyde (0.414 g, 3 mmol) and dry potassium carbonate (0.414 g, 3 mmol) in dry acetone (10 mL) in a period of 15 min and refluxed for additional 5 h. Then, the mixture was cooled to room temperature, filtered and concentrated under vacuum. The obtained residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 12:1) to give a white solid **1** (55%), mp: 40–42 °C (lit.[16]: 39–42 °C).

4.1.2. Synthesis of 2-isopropoxy-4-methoxybenzaldehyde (2)

Isopropyl bromide (0.94 mL, 10 mmol) was added to a mixture of **1** (0.76 g, 5 mmol) and dry potassium carbonate (2.07 g, 15 mmol) in dry DMF (20 mL) under nitrogen. Then, the mixture was stirred at 40 °C for 5 h. After cooling to room temperature, the reaction mixture was filtered and evaporated under reduced pressure, and purified by silica gel column chromatography (petroleum ether/ethyl acetate = 3:1) to give a yellow liquid **2** (92%).

4.1.3. Synthesis of 1,2-bis (4-chlorophenyl) ethane-1,2-diamine (4)

A mixture of 4-chlorobenzaldehyde (0.29 g, 2.1 mmol) and ammonium acetate (0.14 g, 7.8 mmol) was heated to 120 °C and stirred at this temperature for 3 h. The reaction mixture was cooled to room temperature, filtered, and the filter cake was washed with water, 5% NaOH, and hot alcohol successively to get a white solid **3**, which was used without further purification.

Compound **3** (0.35 g) was suspended in 50% concentrated sulfuric acid (3 mL), heated to 180 °C and stirred at this temperature for 3 h. The mixture was diluted with ice water (20 mL) and extracted with EtOAc (3 × 20 mL). The aqueous layer was basified with concentrated ammonium hydroxide and extracted with ether (3 × 15 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a white solid **4** (46.9%). mp: 143.1–146.8 °C. ^{1}H NMR ($CDCl_3$): 7.30 (m, Ar-H, 8H), 4.00 (s, CH, 2H), 1.38 (s, NH_2 × 2, 4H); MS: m/z = 281 $[M+H]^+$.

4.1.4. Synthesis of 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole (5)

A mixture of 1,2-bis(4-chlorophenyl)ethane-1,2-diamine (**4**, 300 mg, 1.1 mmol) and 2-hydroxy-4-methoxy-benzaldehyde (**2**, 190 mg, 1 mmol) in dry methylene chloride (5 mL) was stirred at 0 °C for 30 min. Then, NBS (290 mg, 16.3 mmol) was added. The resulting solution was allowed to warm up to room temperature and stirred overnight. 10% NaOH was added to the reaction mixture to adjust the pH at 8.0. Then, the mixture was extracted with CH_2Cl_2 (3 × 20 mL) and organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate/triethylamine = 3:1:0.01) to give a white solid **5** (81%). ^{1}H NMR ($CDCl_3$): 8.24 (d, *J* = 8.4, Ar-H, 1H); 7.03 (d, *J* = 8.4, Ar-H, 4H); 6.93 (d, *J* = 8.0, Ar-H, 4H); 6.62 (m, Ar-H, 1H); 6.55 (s, Ar-H, 1H); 5.36 (s, CH–N 2, 2H); 4.73 (m, CH, 1H); 3.87 (s, CH3, 3H) ; 1.37 (d, *J* = 6.4, CH3 2, 6H).

4.1.5. Synthesis of 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole (8)

A mixture of 1,2-bis(4-chlorophenyl)ethane-1,2-diamine (**5**, 300 mg, 1.1 mmol) and propionaldehyde (58 mg, 1 mmol) in dry methylene chloride (5 mL) was stirred at 0 °C for 30 min. Then, NBS (290 mg, 16.3 mmol) was added to the mixture. The resulting solution was allowed to warm up to room temperature and stirred overnight. 10% NaOH was added to the reaction mixture to adjust the pH at 8.0. The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate/triethylamine = 3:1:0.01) to give a white solid (94%). ^{1}H NMR ($CDCl_3$): 7.06 (d, *J* = 8.4, Ar-H, 2H); 6.89 (d, *J* = 8.4, Ar-H, 4H); 5.66 (s, –CH–N 2, 2H); 2.77 (q, *J* = 7.6, –CH2, 2H); 1.31 (t, *J* = 8.0, –CH3, 3H).

4.1.6. Synthesis of imidazoline derivatives (7a–k): general procedures

A cooled (0 °C) mixture of **5** (60 mg, 0.132 mmol) and triethylamine (0.13 mL) in methylene chloride (10 mL) was added to a solution of bis(trichloromethyl)carbonate (65.0 mg, 0.34 mmol) in dry methylene chloride (10 mL) in a period of 15 min. The reaction mixture was stirred at 0 °C for 30 min and solvent was removed to dryness. The residue was dissolved in dry methylene chloride (10 mL), and a solution of correspondingly alcohols or secondary amines in methylene chloride (10 mL) was added. After stirring for 15 min, the reaction was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The obtained residue was purified by silica gel column chromatography, gradient elution (petroleum ether/ethyl acetate/triethylamine = 3:1:0.01–1:1:0.01), to give **7a–k**.

4.1.6.1. 2-Methoxyethyl 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazole-1-carboxylate (7a). White solid (80%); mp 155.5–156.5 °C; ^{1}H NMR ($CDCl_3$): 7.52 (d, J = 8.0, Ar-H, 1H); 7.10 (m, Ar-H, 6H); 6.98 (d, J = 8.4, Ar-H, 2H); 6.49 (m, Ar-H, 2H); 5.65 (m, CH × 2, 2H); 4.6 (m, CH, 1H); 4.09 (m, CH_2, 2H); 3.90 (s, OCH_3, 3H); 3.29 (t, J = 4.8, CH_2, 2H); 3.27 (s, CH_3, 3H); 1.41 (d, J = 6.4, CH_3, 3H); 1.26 (d, J = 6.0, CH_3, 3H). MS (ESI), m/z = 557 $[M+H]^+$.

4.1.6.2. Benzyl 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazole-1-carboxylate (7b). White solid (66%); mp 210–212 °C; ^{1}H NMR ($CDCl_3$): 7.52 (d, J = 8.4, Ar-H, 1H); 7.24 (m, Ar-H, 3H); 7.11 (m, Ar-H, 6H); 6.98 (d, J = 8.4, Ar-H, 2H); 6.85 (d, J = 7.6, Ar-H, 2H); 6.53 (m, Ar-H, 1H); 6.40 (d, J = 2.0, Ar-H, 1H); 5.65 (m, CH × 2, 2H); 4.56 (m, CH, 1H); 3.84 (s, OCH_3, 3H); 1.37 (d, J = 5.6, CH_3, 3H); 1.11 (d, J = 6.0, CH_3, 3H). MS (ESI), m/z = 589 $[M+H]^+$.

4.1.6.3. 2-Acetamidoethyl 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4- methoxyphenyl)-4,5-dihydroimidazole-1-carboxylate (7c). White solid (75%); mp: 80–81 °C; ^{1}H NMR ($CDCl_3$): 7.53 (d, J = 8.4, Ar-H, 1H); 7.10 (m, Ar-H, 6H); 6.98 (d, J = 8.4, Ar-H, 2H); 6.56 (d, J = 7.6, Ar-H, 1H); 6.52(s, Ar-H, 1H); 5.68 (m, CH × 2, 2H); 4.70 (m, CH, 1H); 4.05 (m, CH_2, 1H); 3.86 (m, CH_2, 1H); 3.86 (s, OCH_3, 3H); 3.37 (m, CH_2,1H); 3.15 (m, CH_2, 1H); 1.78 (t, CH_3, 3H); 1.44 (d, J = 6.4, CH_3, 3H) ; 1.30 (d, J = 5.6, CH_3, 3H). MS (ESI), m/z = 584 $[M+H]^+$.

4.1.6.4. 2-Ethoxyethyl 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazole-1-carboxylate (7d). White solid (82%); mp: 157–160 °C; ^{1}H NMR ($CDCl_3$): 7.55 (d, J = 8.4, Ar-H, 1H); 7.11 (m, Ar-H, 6H); 6.99 (d, J = 8.4, Ar-H, 2H); 6.54 (m, Ar-H, 2H); 5.65 (m, CH × 2, 2H); 4.70 (m, CH, 1H); 4.10 (m, CH_2, 2H); 3.86 (s, OCH_3, 3H); 3.34 (t, J = 4.8, CH_2, 2H); 3.27 (m, CH_2, 2H); 1.44 (d, J = 6.4, CH_3, 3H); 1.28 (d, J = 5.6, CH_3, 3H); 1.21 (t, J = 7.2, CH_3, 3H). MS (ESI), m/z = 571 $[M+H]^+$.

4.1.6.5. 2-(Dimethylamino)ethyl 4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazole-1-carboxylate (7e). White solid (58%); mp: 177–179 °C; ^{1}H NMR ($CDCl_3$): 7.52 (d, J = 8.0, Ar-H, 1H); 7.08 (m, Ar-H, 6H); 6.97 (d, J = 8.4, Ar-H, 2H); 6.52 (m, Ar-H, 2H); 5.6 (m, CH × 2, 2H); 3.94 (m, CH_2, 2H); 3.84 (s, OCH_3, 3H); 2.30 (m, CH_2, 2H); 2.06 (s, CH_3 × 2, 6H); 1.44 (d, J = 5.6, CH_3, 3H); 1.28 (d, J = 6.4, CH_3, 3H). MS (ESI), m/z = 570 $[M+H]^+$.

4.1.6.6. Ethyl-2-(4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-*N*-methyl-4,5-dihydro-1*H*-imidazole-1-carboxamido) acetate (7f). White solid (66%); mp: 70–72 °C; ^{1}H NMR ($CDCl_3$): 7.65 (d, J = 8.4, Ar-H, 1H); 7.08 (m, Ar-H, 4H); 6.95 (m, Ar-H, 4H); 6.55 (m, Ar-H, 1H); 6.52 (d, J = 2.4, Ar-H, 1H); 5.52 (t, CH × 2, 2H); 4.67 (m, CH, 1H); 4.06 (m, CH_2, 2H); 4.02 (s, OCH_3, 3H); 3.76 (m, CH_2, 2H); 2.71 (s, N–CH_3, 3H); 1.42 (d, J = 6.0, CH_3, 3H); 1.35 (d, J = 6.4, CH_3, 3H); 1.16 (t, J = 7.6, CH_3, 3H). MS (ESI), m/z = 598 $[M+H]^+$.

4.1.6.7. 4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-*N*,*N*-dimethyl-4,5-dihydroimidazole-1-carboxamide (7g). Yellow oil (70%); ^{1}H NMR ($CDCl_3$): 7.54 (d, J = 8.4, Ar-H, 1H); 7.09 (d, J = 8.8, Ar-H, 2H); 7.04 (d, J = 8.4, Ar-H, 2H); 6.98 (d, J = 8.0, Ar-H, 2H); 6.54 (m, Ar-H, 1H); 6.50 (d, J = 2.4, Ar-H, 1H); 5.62 (m, CH × 2, 2H); 4.64 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 2.61 (s, N–CH_3 × 2, 6H); 1.42 (dd, J = 6.4, 18.0, CH_3 × 2, 6H). MS (ESI), m/z = 526 $[M+H]^+$.

4.1.6.8. 4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-*N*-(2-morpholino-2-oxoethyl)-4,5-dihydroimidazole-1-carboxamide (7h). White solid (70%); mp: 105–113 °C; ^{1}H NMR ($CDCl_3$): 7.60 (d, J = 8.4, Ar-H, 1H); 7.18 (d, J = 8.4, Ar-H, 2H); 7.09 (d, J = 8.4, Ar-H, 2H); 7.04 (d, J = 8.4, Ar-H, 2H); 6.97 (d, J = 8.0, Ar-H, 2H); 6.57 (m, Ar-H, 1H); 6.52 (s, Ar-H, 1H); 5.68 (s, NH, 1H); 5.62 (m, CH × 2, 2H); 4.69 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 3.90 (m, CH_2, 2H); 3.63 (m, CH_2 × 2, 4H); 3.53 (m, CH_2, 2H); 3.27 (m, CH_2 × 2, 4H); 1.44 (d, J = 6.4, CH_3, 3H); 1.25 (d, J = 6.0, CH_3, 3H). MS (ESI), m/z = 625 $[M+H]^+$.

4.1.6.9. (4, 5-Bis (4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)(morpholino)methanone (7i). White solid (82%); mp: 78–82 °C; ^{1}H NMR ($CDCl_3$): 7.52 (d, J = 8.4, Ar-H, 1H); 7.10 (d, J = 8.8, Ar-H, 2H); 7.05 (d, J = 8.8, Ar-H, 2H); 6.96 (d, J = 8.4, Ar-H, 2H); 6.87 (d, J = 8.8, Ar-H, 2H); 6.57 (m, Ar-H, 1H); 5.62 (m, CH × 2, 2H); 4.63 (m, CH, 1H); 3.87 (s, OCH_3, 3H); 3.21 (m, CH_2 × 4, 8H); 1.42 (dd, J = 6.0, 18.0, CH_3 × 2, 6H). MS (ESI), m/z = 568 $[M+H]^+$.

4.1.6.10. (4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperidine-4-carboxamide (7j). White solid (72%); mp: 82–84 °C. ^{1}H NMR ($CDCl_3$): 7.54 (d, J = 8.0, Ar-H, 1H); 7.09 (m, Ar-H, 4H); 6.96 (m, Ar-H, 4H); 6.57 (m, Ar-H, 1H); 6.53 (s, Ar-H, 1H); 5.60 (dd, J = 17.0, 8.0, CH × 2, 2H); 5.29 (m, NH_2, 2H); 4.63 (m, CH, 1H); 3.87 (s, OCH_3, 3H); 3.78 (m, CH_2, 2H); 2.54 (m, CH_2, 2H); 2.12 (m, CH, 1H); 1.42 (d, J = 6.0, CH_3, 3H); 1.36 (d, J = 6.0, CH_3, 3H); 1.28 (m, CH_2 × 2, 4H). MS (ESI), m/z = 610 $[M+H]^+$.

4.1.6.11. 1-(4-(2-(2-Isopropoxy-4-methoxyphenyl)-4,5-bis(4-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)ethanone (7k). White solid (82%); mp: 86–88 °C. ^{1}H NMR ($CDCl_3$): 7.79 (s, Ar-H, 1H); 7.36 (m, Ar-H, 5H); 7.10 (m, Ar-H, 4H); 6.54 (s, Ar-H, 1H); 5.74 (m, CH × 2, 2H); 4.70 (m, CH, 1H); 3.97 (s, OCH_3, 3H); 3.70 (m, CH_2, 2H); 3.49 (m, CH_2, 2H); 3.21 (m, CH_2, 4H); 2.11 (s, CH_3, 3H); 1.44 (d, J = 6.0, CH_3, 3H); 1.39 (d, J = 6.0, CH_3, 3H). MS (ESI), m/z = 678 $[M+H]^+$.

4.1.7. Synthesis of imidazoline derivatives (10a–d): general procedure

A solution of bis(trichloromethyl)carbonate (65.0 mg, 0.34 mmol) in dry methylene chloride (10 mL) was added to a cooled (0 °C) mixture of **5** (60 mg, 0.132 mmol) and triethylamine (0.13 mL) in methylene chloride (10 mL) in a period of 15 min. The reaction mixture was stirred at 0 °C for additional 30 min and solvent was removed to dryness. Then, obtained residue was dissolved in dry methylene chloride (10 mL) and added to a solution of correspondingly alcohols or secondary amines in methylene chloride (10 mL). After stirring for 15 min, water (10 mL) was added, the mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The obtained residue was purified by silica gel column chromatography, gradient elution (petroleum ether/ethyl acetate/triethylamine = 3:1:0.01–1:2:0.01), to give **10a–d**.

4.1.7.1. (4,5-Bis(4-chlorophenyl)-2-ethyl-4,5-dihydroimidazol-1-yl)-(4-methylpiperazin-1-yl)methanone (10a). White solid (80%); mp: 43–50 °C; ^{1}H NMR ($CDCl_3$): 7.03 (t, J = 8.4, Ar-H, 4H); 6.80 (m, Ar-H, 4H); 5.40 (s, CH × 2, 2H); 3.07 (m, CH_2 × 2, 4H); 2.70 (m, CH_2, 2H); 2.32 (m, CH_2 × 2, 4H); 2.29 (s, CH_3, 3H); 1.39 (m, 3H, CH_3). MS (ESI), m/z = 445 $[M+H]^+$.

4.1.7.2. 4,5-Bis(4-chlorophenyl)-*N*,*N*,2-triethyl-4,5-dihydroimidazole-1-carboxamide (10b). White solid (79%); mp: 42–45 °C; ^{1}H NMR ($CDCl_3$): 7.06 (d, J = 8.0, Ar-H, 2H); 7.03 (d, J = 8.4, Ar-H, 2H); 6.83 (d, J = 8.4, Ar-H, 2H); 6.78 (d, J = 8.4, Ar-H, 2H); 5.40 (s, CH × 2, 2H); 3.40 (m, CH_2 × 2, 4H); 2.70 (m, CH_2, 2H); 1.34 (m, CH_3, 3H); 0.99 (m, CH_3 × 2, 6H). MS (ESI), m/z = 418 $[M+H]^+$.

4.1.7.3. 4,5-Bis(4-chlorophenyl)-2-ethyl-*N*-(4-(pyrrolidin-1-yl)phenyl)-4,5-dihydroimidazole-1-carboxamide (10c). Oily stuff (46%); ^{1}H NMR($CDCl_3$) : 7.17(d, J = 8.0, Ar-H, 1H); 7.04 (m, Ar-H, 5H); 6.80 (m, Ar-H, 6H); 5.60 (m, CH × 2, 2H); 2.50 (m, CH_2, 2H); 1.94 (m, CH_2 × 2, 4H); 1.42 (t, J = 7.2, CH_3, 3H); 1.25 (m, CH_2 × 2, 4H); MS (ESI), m/z = 507 $[M+H]^+$.

4.1.7.4. 4,5-Bis(4-chlorophenyl)-2-ethyl-*N*-(2-morpholino-2-oxoethyl)-4,5-dihydroimidazole-1-carboxamide (10d). White solid (55%); mp: 175–181 °C; ^{1}H NMR ($CDCl_3$): 7.10 (d, J = 8.4, Ar-H, 2H); 7.05 (d, J = 8.8, Ar-H, 2H); 6.89 (d, J = 8.4, Ar-H, 2H); 6.82 (d, J = 8.0, Ar-H, 2H); 5.57 (m, CH_2, 2H); 5.35 (s, NH, 1H); 3.78 (m, CH_2, 2H); 3.63 (m, CH_2 × 2, 4H); 3.53 (m, CH_2, 2H); 3.32 (m, CH_2, 2H); 3.04 (m, CH_2, 2H); 1.40 (t, J = 7.6, CH_3, 3H). MS (ESI), m/z = 489 $[M+H]^+$.

4.1.8. Synthesis of imidazoline derivatives (12a–j): general procedure

A solution of chloroacetyl chloride (0.36 mmol) or chloropropionyl chloride in acetone (10 mL) was added to a mixture of **5** (0.24 mmol) and anhydrous potassium carbonate in dry acetone (10 mL). The reaction mixture was stirred for additional 30 min at room temperature and solvent was removed to dryness. The obtained residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate/triethylamine = 1:1:0.01) to give intermediate **11**. Then, a solution of secondary amines (1.5 mmol) in acetonitrile (5 mL) was added to the solution of **11** in dry acetone (10 mL) and refluxed for 15 min. After cooling to room temperature, solvent was removed to dryness, and water (5 mL) was added, the resulting mixture was extracted with ethyl estate (3 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The obtained residue was purified by silica gel column chromatography, gradient elution (petroleum ether/ethyl acetate/triethylamine = 3:1:0.01–1:2:0.01), to give **12a–j**.

4.1.8.1. 1-(2-(2-Isopropoxy-4-methoxyphenyl)-4,5-diphenyl-4,5- dihydroimidazol-1-yl)-2-(4-methylpiperazin-1-yl) ethanone (12a). White solid (86%); mp: 191–194 °C; ^{1}H NMR($CDCl_3$): 7.62 (d, J = 8.4, Ar-H, 1H); 7.15 (m, Ar-H, 2H); 7.04 (m, Ar-H, 8H); 6.56 (m, Ar-H, 1H); 6.51 (s, Ar-H, 1H); 5.87 (m, CH × 2, 2H); 4.71 (m, CH, 1H); 3.86 (s, OCH_3, 3H); 2.96 (m, CH_2, 2H); 2.38 (m, CH_2, CH_3, 11H); 1.43 (dd, J = 5.6, 18.0, CH_3 × 2, 6H). MS (ESI), m/z = 527 $[M+H]^+$.

4.1.8.2. 2-(Diethylamino)-1-(2-(2-isopropoxy-4-methoxyphenyl)-4,5-diphenyl-4,5-dihydroimidazol-1-yl)ethanone (12b). Oily stuff (82%); ^{1}H NMR ($CDCl_3$): 7.56 (d, J = 8.0, Ar-H, 1H); 7.15 (m, Ar-H, 2H); 7.03 (m, Ar-H, 8H); 6.53 (s, Ar-H, 1H); 6.51 (2, Ar-H, 1H); 5.88 (m, CH × 2, 2H); 4.71 (m, CH, 1H); 3.84 (s, OCH_3, 3H); 3.20 (m, CH_2, 2H); 2.59 (m, CH_2 × 2, 4H); 1.43 (dd, J = 5.6, 18.0, CH_3 × 2, 6H); 0.90 (m, CH_3 × 2, 6H). MS (ESI), m/z = 500 $[M+H]^+$.

4.1.8.3. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-2-morpholinoethanone (12c). White solid (84%); mp: 177–179 °C; ^{1}H NMR ($CDCl_3$): 7.57 (s, Ar-H, 1H); 7.07 (m, Ar-H, 6H); 6.96 (d, J = 8.4, Ar-H, 2H); 6.54 (m, Ar-H, 1H); 6.50 (d, J = 2.4, Ar-H, 1H); 5.60 (m, CH × 2, 2H); 4.68 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 3.60 (m, CH_2 × 2, 4H); 2.93 (s, CH_2, 2H); 2.31 (m, CH_2, 4H); 1.43 (dd, J = 5.6, 18.0, CH_3 × 2, 6H); MS (ESI), m/z = 582 $[M+H]^+$.

4.1.8.4. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-2-(4-methylpiperazin-1-yl) ethanone (12d). Oily stuff (82%); ^{1}H NMR ($CDCl_3$): 7.57 (d, J = 8.4, Ar-H, 1H); 7.07 (m, Ar-H, 6H); 6.98 (d, J = 8.4, Ar-H, 2H); 6.56 (m, Ar-H, 1H); 6.50 (d, J = 1.6, Ar-H, 1H); 5.60 (m, CH × 2, 2H); 4.68 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 2.94 (m, CH_2, 2H); 2.24 (m, CH_2, CH_3, 11H); 1.43 (dd, J = 5.6, 18.0, CH_3 × 2, 6H). MS (ESI), m/z = 595 $[M+H]^+$.

4.1.8.5. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-2-(piperidin-1-yl)ethanone (12e). White solid (65%); mp: 190–192 °C; ^{1}H NMR ($CDCl_3$): 7.59 (d, J = 9.0, Ar-H, 1H); 7.09 (m, Ar-H, 6H); 6.97 (d, J = 8.4, Ar-H, 2H); 6.55 (m, Ar-H, 1H); 6.50 (d, J = 1.5, Ar-H, 1H); 5.60 (m, CH × 2, 2H); 4.69 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 2.90 (m, CH_2, 2H); 2.27 (m, CH_2, 2H); 1.50 (m, CH_2 × 2, 4H); 1.43 (d, J = 6.0, CH_3 × 2, 6H); 1.36 (m, CH_2, 2H). MS (ESI), m/z = 580 $[M+H]^+$.

4.1.8.6. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-2-(diethylamino)ethanone (12f). White solid (70%); mp: 183–186 °C; ^{1}H NMR ($CDCl_3$): 7.54 (d, J = 8.4, Ar-H, 1H); 7.07 (m, Ar-H, 6H); 6.95 (d, J = 8.0, Ar-H, 2H); 6.56 (d, J = 8.8, Ar-H, 1H); 6.51 (s, Ar-H, 1H); 5.60 (m, CH × 2, 2H); 4.69 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 2.59 (s, CH_2, 2H); 2.40 (m, CH_2, CH_3, 11H); 1.41 (d, J = 5.6, CH_3, 3H); 1.29 (d, J = 6.0, CH_3, 3H); 1.26 (s, CH_2, 2H). MS (ESI), m/z = 568 $[M+H]^+$.

4.1.8.7. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-3-(4-methylpiperazin-1-yl) propan-1-one (12g). Oily stuff (76%); ^{1}H NMR ($CDCl_3$): 7.54 (d, J = 8.4, Ar-H, 1H); 7.07 (m, Ar-H, 6H); 6.95 (d, J = 8.0, Ar-H, 2H); 6.56 (d, J = 8.8, Ar-H, 1H); 6.51 (s, Ar-H, 1H); 5.60 (m, CH × 2, 2H); 4.69 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 2.59 (s, CH_2, 2H); 2.40 (m, CH_2, CH_3, 11H); 1.41 (d, J = 5.6, CH_3, 3H); 1.29 (d, J = 6.0, CH_3, 3H); 1.26 (s, CH_2, 2H). MS (ESI), m/z = 609 $[M+H]^+$.

4.1.8.8. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-3-(diethylamino)propan-1-one (12h). Oily stuff (68%); ^{1}H NMR ($CDCl_3$): 7.54 (d, Ar-H, 1H); 7.07 (m, Ar-H, 6H); 6.96 (d, Ar-H, 2H); 6.56 (d, Ar-H, 1H); 6.52 (s, Ar-H, 1H); 5.71 (dd, CH × 2, 2H); 4.70 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 3.37 (t, CH_2, 2H); 2.84 (t, CH_2, 2H); 2.41 (m, CH_2 × 2, 4H); 1.44 (d, CH_3, 3H); 1.29 (d, CH_3, 3H); 0.96 (t, CH_3 × 2, 6H). MS (ESI), m/z = 582 $[M+H]^+$.

4.1.8.9. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-3-morpholinopropan-1-one (12i). Oily stuff (70%); ^{1}H NMR ($CDCl_3$): 7.54 (d, J = 9.0, Ar-H, 1H); 7.10 (m, Ar-H, 6H); 6.96 (m, Ar-H, 2H); 6.55 (m, Ar-H, 1H); 6.52 (d, J = 1.5, Ar-H, 1H); 5.67 (m, CH × 2, 2H); 4.72 (m, CH, 1H); 3.85 (s, OCH_3, 3H); 3.61 (m, CH_2, 4H); 2.55 (m, CH_2, 2H); 2.34 (m, CH_2, 2H); 2.24 (m, CH_2, 4H); 1.44 (d, J = 6.0, CH_3, 3H), 1.30 (d, J = 6.0, CH_3, 3H). MS (ESI), m/z = 596 $[M+H]^+$.

4.1.8.10. 1-(4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl)-3-morpholinopropan-1-one (12j). Oily stuff (46%); ^{1}H NMR ($CDCl_3$): 7.79 (s, Ar-H, 1H); 7.36 (m, Ar-H, 5H); 7.10 (m, Ar-H, 4H); 6.54 (s, Ar-H, 1H); 5.74 (m, CH × 2, 2H); 4.70 (m, CH, 1H); 3.99 (s, OCH3, 3H); 3.87 (s, CH_2, 2H); 2.38 (m, CH_2, CH_3, 11H); 1.43 (dd, J = 5.6, 18.0, CH_3 × 2, 6H). MS (ESI), m/z = 623 $[M+H]^+$.

4.2. Biological evaluation

4.2.1. MDM2 protein expression and purification

MDM2 (1-118) plasmid was provided by Dr. Shaomeng Wang's group, and transformed into *Escherichia coli* BL-21 (DE3). Cultures were grown at 37 °C in TB medium, and induced by 0.4 mM IPTG at an OD_{600} of 0.6 at 18 °C for 20 h. Cells were lysed in 50 mM Tries, pH 7.5 buffer containing 500 mM NaCl and 10% glycerol. MDM2 (1-118) was purified from the soluble fraction using Ni-NTA resin, and desalted in PBS buffer pH 7.5, 150 mM NaCl and 10% glycerol. The protein was purified to >95% as judged SDS–PAGE.

4.2.2. Fluorescence polarization competitive binding assay

Measurements were made with an EnVision Multilabel Plate Reader using a 480 nM excitation filter and a 535 nM emission filter. Assays were performed in Corning 384-well black plates. Nutlin-1 was used as a positive control, while DMSO was used as negative controls. Assays were performed in duplicate and repeated at least twice on separate days. Competition experiments were carried out in a total volume of 20 μL 40 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 1 mM DTT, 4% DMSO. Probe peptide was present at a final concentration of 1 nM, and MDM2 was present at a final concentration of 10 μM. Plates were allowed to incubate at room temperature for 1 h prior to measurement.

4.2.3. Cell viability assay

A549, HCT116, KB and PC3 cells were planted in 96-well plates (4×10^3/well) for 24 h, and subsequently treated with different concentrations of compounds **7c**, **7e**, **7h**, **12g**, and **12h** for 72 h. Viable cells were determined using MTT assay. MTT solution (5.0 mg/mL in RPIM-1640, Sigma, St. Louis, MO) was added (10.0 μL/well), and plates were incubated for a further 4 h at 37 °C. The purple formazan crystals were dissolved in 100.00 μL DMSO. After the crystal dissolved, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 nm. The concentration of drug inhibit for 50% of cells (IC_{50}) was calculated using the software of dose–effect analysis with microcomputers.

4.2.4. Flow cytometric analysis

For flow cytometric analysis of DNA content, A549 cells in exponential growth were treated with **7c** (10 μM) for 72 h. The cells treated with **7c** were collected and washed twice with PBS, and then fixed with 75% alcohol overnight. Then, the cells were washed with PBS and resuspended in 100.0 μL of PBS 200.0 mg/mL RNase was added for 30 min to eliminate the interference of RNA and then 20.0 mL/L propidium iodide (PI; Sigma) was added for 30 min. Then, the cells were washed, and the DNA content was detected by FACSCalibur (Becton Dickinson, Lincoln Park, New Jersey, USA).

4.3. Molecular docking

Molecular docking was performed using CDOCKER, a CHARMm-based molecular dynamics docking algorithm on Discovery Studio 2.1 (Accelrys). The MDM2 structure cocrystallized with an imidazoline compound was obtained from the PDB data bank (PDB code: 1RV1).[8] A protein clean process and a CHARMm-force field were sequentially applied. The area around imidazoline compounds was chosen as the active site, with a radius set as 9 A°. After removing the ligand from the structure of the complex, a binding sphere in the three axis directions was constructed around the active site. All default parameters were used in the docking process. CHARMm-based molecular dynamics (1000 steps) were used to generate random conformations. Final energy minimization was set as the full potential mode. The final binding conformation of **7e** and **7g** was determined on the basis of CDOCKING ENERAGE. The most stable binding mode among the top 10 of docking poses of each compound was presented in Figure 4, respectively.

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