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# Searching for dual inhibitors of the MDM2-p53 and MDMX-p53 protein-protein interaction by a scaffold-hopping approach

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ABSTRACT

Two libraries of substituted benzimidazoles were designed using a 'scaffold-hopping' approach based on reported MDM2-p53 inhibitors. Substituents were chosen following library enumeration and docking into an MDM2 X-ray structure. Benzimidazole libraries were prepared using an efficient solution-phase approach, and screened for inhibition of the MDM2-p53 and MDMX-p53 protein-protein interactions. Key examples showed inhibitory activity against both targets.

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INTRODUCTION

The design of small molecule inhibitors of protein-protein interactions has received considerable interest in recent years.(1-4) In particular, the interaction of p53 with the regulatory proteins MDM2 and MDMX have provided a focus for efforts because of their importance in a number of cancers, and the prospect that inhibition of these interactions may provide new therapies. Overexpression of the regulatory proteins MDM2 and MDMX has been demonstrated to silence the tumor suppressor function of p53. MDM2 amplification occurs in around 11% of all tumors, and is most common in hepatocellular carcinoma (44%), osteosarcomas (20%), and soft tissue sarcomas (31%). Similarly, MDMX (MDM4) occurs in approximately 10-25% of tumors, e.g. brain (11%), breast (5-40%), and sarcomas (17%).(5-8)

Expression of MDM2 is p53-dependent, and forms a regulatory feedback loop with p53 by binding to the p53 transactivation domain. Acting as an E3-ligase, MDM2 promotes proteasomal degradation of p53.(9-12) MDMX expression is not p53-dependent and MDMX levels remain constant. The protein inhibits p53 transcriptional activity, but does not act as an E3 ligase independently of MDM2.(13) Thus, MDM2 and MDMX both regulate p53, but their functions are different and non-redundant.(14)

The X-ray crystal structure of MDM2 bound to a p53-derived peptide reveals the $\alpha$-helical peptide bound into a deep groove on the surface of the protein, and key interactions are formed with three hydrophobic residues of p53 (Figure 1).(15) Inhibitors of MDM2 with diverse chemotypes have been reported.(16, 17) X-ray crystal structures of a range of potent MDM2-p53 inhibitors have been published, such as Nutlin-3 (**1**), spirooxindoles, e.g. MI-63 (**2**) and pyrrolidones (**3**).(18-21) Inhibitors show cellular activity and in vivo antitumor activity.(18, 22) Recently, potent MDM2-p53 inhibitors have entered clinical trials, including RG7112,(23, 24) RG7388(25) and MI-773(26) (Chart 1).

The p53 transactivation loop binds in a cleft on the surface of MDMX similar to that identified for MDM2 but with a closed conformation for Tyr99.(27, 28). Steric hindrance of Met53 in the Leu23 pocket for MDMX may be responsible for the lack of potency against MDMX for MDM2-p53 inhibitors such as the Nutlins and spirooxindoles.(29) A high degree of flexibility has been measured in computational studies of MDMX, and significant conformational differences, owing to the induced fit of ligands are observed in crystal structures.(30-32) Reports of small-molecule MDMX-p53 inhibitors have not been as numerous as for MDM2.(21) The selective MDMX inhibitor

5-oxo-pyrazolylidene SJ-172550 (**4**) was identified in an MDMX high-throughput assay.(33) The 3-imidazolylindole **5** is a mixed MDM2- and MDMX-p53 inhibitor with modest potency against MDMX.(29) Recently, indolylhydantoins, e.g. (**6**) have been reported as MDM2-p53 and MDMX-p53 inhibitors.(34)

In this paper, we describe the application of a scaffold-hopping approach to design new inhibitor chemotypes for MDM2 and MDMX(35, 36) based on heterocycle replacement, and derived from two MDM2-p53 inhibitors with significantly different core structures. In particular, starting from *cis*-imidazoline (**1**),(18) and a class of oxindoles (**7**),(37, 38) benzimidazole derivatives were designed and evaluated through docking studies. The benzimidazole heterocycle was chosen as a 'privileged structure' in medicinal chemistry that is readily synthetically accessible.(39) The synthesis of small libraries yielded mixed MDM2-p53 and MDMX-p53 inhibitors with promising potency.

## MATERIALS AND METHODS

**MDM2-p53 ELISA assay.** Compounds were assayed for MDM2-p53 inhibitory activity using ELISA assays in a 96-well format using the published method.(40)

**MDMX-p53 ELISA assay.** MDMX-p53 inhibitory activity was determined using an analogous method incorporating a pCMV-XL5-MDMX cDNA construct (OriGene Technologies) for the *in vitro* coupled T7 transcription and rabbit reticulocyte lysate translation of MDMX, and a rabbit anti-MDMX antigen affinity-purified polyclonal antibody (Bethyl Laboratories Inc, via UK supplier Cambridge Bioscience, UK, Cat No. A300-287 A).(41)

**Docking experiments**. Docking calculations were performed with the GOLD software.(42) Ligands were docked within the p53 binding site of MDM2, using the crystal structure of human MDM2 in complex with the small molecule inhibitor Nutlin-2 (PDB code: 1RV1).(18) A single protein chain was selected from the unit cell. The binding site was defined by hydrophobic fitting points calculated on the target for a 8Å radius around the co-crystallized ligand. GoldScore was used as a fitness function, while default genetic algorithm parameter settings were applied. 30 poses were generated for each ligand, early termination being allowed when the top 10 solutions were within 1.5Å R.M.S.D.

**Chemical Synthesis.**

**General Procedure A – Synthesis of compounds (14).**

A mixture of the appropriate aldehyde (1.2 eq.), sodium dithionite (86% purity; 1.98 g, 9.78 mmol, 3 eq.) and **13** (1.16 g, 3.26 mmol, 1.0 eq.) in methanol (12 mL) and water (3 mL) was heated in a microwave reactor for 10 minutes at 100 $^{o}$C. The sample was diluted with ethyl acetate (20 mL) and washed with water (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), and evaporated. Chromatography (silica; ethyl acetate, petrol) gave compound **14**.

**General Procedure B.** Compound **14** was dissolved in DCM (2 mL) and TFA (3 mL) and the mixture stirred at rt for 1 hour, then evaporated. The residue was diluted with DCM (20 mL) and neutralised with sodium carbonate (sat.; 20 mL). The organic layer was dried ($MgSO_4$) to give **15** which was used without further purification.

**General procedure C.** Either an aliquot of a solution of **15** in dry DCM (1 mL, 0.049 M), or weighed **15** in dry DCM (1 mL) was treated with the named isocyanate (1.5 eq.). The mixture was quenched with $NH_2$-silica and filtered through a plug of $NH_2$-silica. Evaporation gave **8** without further purification.

**(*S*)-Ethyl 3-(3-(5-chloro-2-(3-methoxybenzyl)-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxamido)benzoate 8**{*3,7*}**.**

**General procedure C: 15**{*3*} (1 mL, 49.3 µmol) and ethyl 3-isothiocyanatobenzoate (12 µl, 76 µmol). **8**{*3,7*} (26 mg, 96%) as a white solid; HPLC purity (as area %) > 98; $^{1}$H NMR (300MHz, $CDCl_3$) 1.38 (1H, t, *J* 7.2, $CH_2CH_3$), 1.54 (1H, qt, *J* 3.6 and 13.1), 1.72 (1H, app.dd), 1.87 (1H, app.d), 2.20 (1H, qd, *J* 4.0 and 12.7), 2.80 (1H, td, *J* 2.2 and 13.1), 3.38(1H, app.t), 3.63 (3H, s, $OCH_3$), 3.77 (1H, app. dd), 4.21 (1H, app.d), 4.30 (2H, s, $CH_2$), 4.36 (2H, q, *J* 7.2), 6.54 (1H, br.d), 6.65-6.69 (1H, m, Ar), 6.77 (2H, d, *J* 8.3, Ar), 7.17 (1H, app.t, Ar), 7.20 (1H, m, Ar), 7.32-7.39 (2H, m, Ar), 7.67-7.81 (4H, m, Ar); $^{13}$C NMR (100MHz, $CDCl_3$) 14.3, 24.8, 28.6, 35.0, 44.1, 47.2, 53.5, 55.2, 61.1, 112.2, 112.5, 114.3, 119.8, 120.5, 121.0, 122.7, 124.4, 124.7, 127.7, 128.9, 129.9, 131.0, 132.1, 137.5, 138.9, 144.0, 154.2, 154.7, 160.0, 166.3.

**(*S*)-Ethyl 3-(3-(5-chloro-2-(4-methoxybenzyl)-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxamido)benzoate 8**{*4,7*}**.**

**General procedure C: 15**{*4*} (1 mL, 49 µmol) and 3-isocyanatobenzoate (12 µl, 76 µmol). **8**{*4,7*} (23 mg, 85%), white solid; HPLC purity (as area %) > 98; $^{1}$H NMR (400MHz, $CDCl_3$) 1.33 (3H, t, *J* 4.1, $CH_2CH_3$), 1.48 (1H, qt, *J* 4.1 and 13.3), 1.62 (1H, app.d), 1.82 (1H, app.d), 2.17 (1H, qd, *J* 4.1 and 12.8),

2.78 (1H, td, *J* 2.3 and 13.3), 3.36 (1H, app.t), 3.55 (3H, s, OC$H_3$), 3.82 (1H, dd, *J* 4.1 and 12.8), 4.15 (1H, d, *J* 16.0), 4.17 (1H, app.d), 4.23 (1H, d, *J* 16.0), 4.25-4.33 (1H, m), 4.30 (2H, q, *J* 7.3, C$H_2$CH$_3$), 6.70-6.73 (3H, app.d), 7.04 (2H, d, *J* 8.7, Ar), 7.15 (1H, dd, *J* 1.8 and 8.7), 7.28-7.33 (2H, m, Ar), 7.59-7.62 (1H, m, Ar), 7.63-7.70 (2H, m, Ar), 7.81 (1H, app.d, Ar); $^{13}$C NMR (100MHz, $CDCl_3$) 14.3, 24.9, 28.6, 34.1, 44.2, 47.2, 53.4, 55.0, 61.0, 112.2, 114.2, 119.7, 121.1, 124.4, 124.8, 127.6, 127.7, 128.9, 129.4, 131.0, 132.2, 139.0, 144.0, 154.7, 154.9, 158.7, 166.3; MS (ESI+) m/z = 547.3 $[M+H]^+$.

**(*S*)-*N*-Benzyl-3-(2-benzyl-5-chloro-1H-benzo[d]imidazol-1-yl)piperidine-1-carbothioamide 16.**

**General procedure C: 15**{*1*} (16 mg, 49.2 µmol) and benzyl isothiocyanate (7.2 µl, 54.1 µmol). **16** (15 mg, 64%) as a white solid; HPLC purity (as area %) > 93; $^1$H NMR (500 MHz, $CDCl_3$) 1.43 (1H, qt, *J* 4.1 and 13.3), 1.55 (1H, app.d), 1.77-1.80 (1H, m), 2.25 (1H, qd, *J* 12.8 and 4.4), 3.03 (1H, td, *J* 13.4 and 2.4), 3.50 (1H, app.t), 4.35-4.46 (4H, m), 4.67-4.71 (1H,m), 4.74 (1H, dd, *J* 14.6 and 4.6), 4.97 (1H, dd, *J* 14.6 and 5.4), 5.65 (1H, app.t, N*H*), 7.15-7.22 (6H, m, Ar), 7.30-7.36 (6H, m, Ar), 7.73 (1H, d, *J* 2.0, Ar); $^{13}$C NMR (125 MHz, $CDCl_3$) 24.5, 28.4, 35.0, 48.0, 50.4, 51.3, 52.7, 112.0, 119.9, 122.7, 127.2, 127.7, 127.8, 127.9, 128.4, 128.8, 128.9, 132.1, 136.2, 137.8, 144.1, 154.5, 183.2. LC-MS (ESI+) m/z = 475.3 $[M+H]^+$.

**(*S*)- 2-Benzyl-1-(1-(benzylsulfonyl)piperidin-3-yl)-5-chloro-1H-benzo[d]imidazole 17.**

Phenylmethanesulfonyl chloride (12 mg, 60.9 µmol, 1.1 eq) was added to a solution of **15** (18 mg, 55.4 µmol, 1eq) and triethylamine (11.6 µl, 83.1 µmol, 1.5 eq) in DCM (1mL). The mixture was stirred at RT for 1h, then quenched with water (3mL). The organic layer was separated, dried ($MgSO_4$), and evaporated. Chromatography (5% DCM, methanol) gave **17** (25 mg, 94%) as a white solid; HPLC purity (as area %) > 96; $^1$H NMR (500 MHz, $CDCl_3$) 1.34-1.41 (2H, m), 1.64-1.68 (1H, m), 1.94 (1H, qd, *J* 12.6 and 4.2), 2.54 (1H, td, *J* 12.6 and 2.6), 3.01 (1H, app.t), 3.54 (1H, dd, *J* 12.3 and 4.6), 3.61-3.64 (1H, m), 4.13 (1H, d, *J* 14.1), 4.17 (1H, d, *J* 14.1), 4.19 (1H, d, *J* 15.9), 4.24 (1H; tt, *J* 12.0 and 4.1), 4.40 (1H, d, *J* 15.9), 7.11 (1H, d, *J* 8.7, Ar), 7.14 (1H, dd, *J* 8.7 and 1.8, Ar), 7.18-7.23 (3H, m, Ar), 7.27-7.30 (3H, m, Ar), 7.32-7.37 (4H, m, Ar), 7.72 (1H, d, *J* 1.8, Ar); LC-MS (ESI+) m/z = 480.3 $[M+H]^+$.

**General Procedure E: Benzimidazole Intermediate Synthesis (20)**

A mixture **18** (0.3g, 1.41 mmol), sodium dithionite (0.67g, 3.84 mmol) and **19** (1.0 eq.) in methanol (16 mL) and water (4 mL) was heated by microwave for 10 mins at 100 $^o$C, then diluted with ethyl acetate (20 mL), washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$) and evaporatated. Recrystallisation from ethyl acetate, petrol gave **20**.

**General Procedure F: Boc deprotection (21)**

A mixture of **20** (0.40 g, 0.94 mmol ), TFA (0.6 mL) and DCM (2 mL) was stirred at rt for 4.5 h, then concentrated and diluted with methanol (4 mL). $K_2CO_3$ (0.2 g) was added and the suspension was stirred at rt, then filtered, and concentrated. Chromatography (5% DCM, MeOH) gave **21**. Intermediates **21**{*2*},**21**{*3*}, **21**{*4*} were used directly in the subsequent step without purification.

**General Procedure G: Benzimidazole Synthesis (9)**

**21** (0.01 g, 0.028 mmol) was added to a solution of the appropriate isocyanate (1.2 eq.) in DCM (2 mL) and the mixture was stirred at rt for 1 h, then concentrated. Chromatography (NH-silica; 2% MeOH, DCM) gave **9** as glassy solids.

***N*-(4-Acetylphenyl)-4-(6-chloro-1-(3-chlorobenzyl)-1*H*-benzo[*d*]imidazole-2-yl)piperidine-1-carboxamide 9**{*2,2*}

**General Procedure G: 21**{*2*} (0.011 g, 0.029mmol), 4-acetylphenylisocyanate (0.005 g, 0.032 mmol). **9**{*2,2*} 81%; HPLC purity (as area %) > 99; $^1$H NMR (400 MHz ,$CDCl_3$) 1.88 (m, 2H, C$H_2$CH), 2.09 (m, 2H, C$H_2$NCO), 2.49, (s, 3H, COC$H_3$) 2.99 (m, 2H, C$H_2$NCO), 3.07(m, 1H, $CH_2$C*H*), 4.17 (m, 2H, C$H_2CH_2$NCO), 5.31 (s, 2H, C$H_2$Ar), 6.49 (s, 1H, N-H), 6.85 (m, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.14 (d, 2H, *J* 8.37 Hz, Ar-H) 7.17 (d, 1H, *J* 1.82 Hz, Ar-H), 7.39 (d, 3H, *J* 7.91 Hz Ar-H), 7.38 (d, 2H, *J* 8.85 Hz, Ar-H), 7.81 (d, 1H, *J* 8.56 Hz, Ar-H); LC-MS (ESI+) *m/z* = 519 $[M+H]^+$.

**4-(6-Chloro-1-(3-chlorobenzyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*-(3,4,5-trimethoxyphenyl)piperidine-1-carboxamide 9**{*2,3*}

**General Procedure G: 21**{*2*} (0.05 g, 0.14 mmol), 3,4,5-trimethoxyphenylisocyanate (0.035 g, 0.16 mmol). **9**{*2,3*} 73 %; HPLC purity (as area %) > 92; $^1$H NMR (400 MHz ,$CDCl_3$) 1.82 (m, br, 2H, C$H_2CH_2$-NH), 2.10 (m, 2H, C$H_2CH_2$-NH), 2.99 (m, 3H, C$H_2$-$CH_2$-NH, CH-$CH_2$), 3.73 (s, 3H, Ar-OMe), 3.77 (s, 6H, Ar-$(OMe)_2$), 4.12 (m, 2H, C$H_2$-N), 5.27 (s, 2H, C$H_2$-N), 6.31 (s, 1H, N-*H*), 6.59 (s, 2H, Ar-*H*), 6.72 (d,1H, *J* 7.21, Ar-H), 7.01 (s, 1H, Ar-H), 7.13 (m, 3H, Ar-H), 7.62 (d, 1H, 8.33 Hz, Ar-H); LC-MS (ESI+) *m/z* 569 $[M+H]^+$;

**Ethyl-4-(4-(6-chloro-1-(3-chlorobenzyl)-1*H*-benzo[*d*]imidazole-2-yl)piperidine-1-carboxamido) benzoate 9**{*2,8*}

**General Procedure G: 21**{*2*} (0.011 g, 0.029 mmol), ethyl 4-isocyanobenzoate (0.006 g, 0.032 mmol). **9**{*2,8*} 80%; HPLC purity (as area %) > 98; $^1$H NMR (400 MHz ,$CDCl_3$) 1.40 (t, 3H, 7.11 Hz, $OCH_2CH_3$), 1.89 (m, 2H, C$H_2$CH), 2.05 (m, 2H, C$H_2$NCO), 3.04 (m, 2H, C$H_2$NCO), 3.08 (m, 1H, $CH_2$C*H*), 4.19 (m, 2H,

C*H*$_2$CH$_2$NCO), 4.37 (q, 2H, *J* 7.12 Hz OC*H*$_2$CH$_3$), 5.35 (s, 2H, C*H*$_2$Ar), 6.58 (s, 1H, N-H), 6.85 (m, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.21 (d, 1H, *J* 1.82 Hz, Ar-H), 7.38 (m, 3H, Ar-H), 7.43 (d, 2H, *J* 8.83 Hz, Ar-H), 7.69 (d, 1H, *J* 8.56 Hz, Ar-H), 7.97 (d, 2H, *J* 8.77 Hz, Ar-H; LC-MS (ESI+) *m/z* = 549 $[M+H]^+$.

**_N_-(4-Acetylphenyl)-4-(6-chloro-1-(4-methoxybenzyl)-1_H_-benzo[_d_]imidazol-2-yl)piperidine-1-carboxamide 9**{*4,2*}

**General Procedure G: 21**{*4*} (0.097 g, 0.27 mmol), 4-acetylphenylisocyanate (0.074 g, 0.46 mmol). Chromatography (50% EtOAc, petrol), **9**{*4,2*} 55 %; HPLC purity (as area %) > 96; $^1$H NMR (400 MHz ,$CDCl_3$) 1.82 (m, 2H, C*H*$_2$CH$_2$-NH), 2.05 (m, 2H, C*H*$_2$CH$_2$-NH), 2.48(s, 3H, Ar-COC*H*$_3$) 2.94 (m, 3H, C*H*$_2$-CH$_2$-NH and CH-CH$_2$), 3.71 (s, 3H, Ar-OMe), 4.12 (m, br 2H, C*H*$_2$-N), 5.22 (s, 2H, C*H*$_2$-N), 6.62 (s, 1H, N-*H*), 6.79 (d, 2H, *J* 8.7, Ar-H), 6.84 (d, 2H, *J* 8.7, Ar-H), 7.18 (m, 2H, Ar-H), 7.39 (d, 2H, *J* 8.8, Ar-H), 7.6 (dd, 1H, *J* 8.1, 1.04, Ar-*H*), 7.89 (d, 2H, *J* 8.8, Ar-H).; LC-MS (ESI+) *m/z* 517 $[M+H]^+$.

RESULTS

*Design*

The search for MDM2–p53 inhibitors has revealed several classes of small molecules able to bind MDM2 at the p53 binding site and restore p53 activity.(21) Reflecting the hydrophobic nature of the MDM2-p53 interaction, all of these compounds are characterized by a central scaffold that directs hydrophobic substituents towards the three MDM2 sub-pockets, thus mimicking the key amino acid side-chains of p53.

In an attempt to identify novel chemotypes acting as an anchor point for hydrophobic substituents, our attention was focused on compounds **1** and **7**, taken as representative of two potent and structurally different classes of MDM2 inhibitors. The scaffold-hopping approach initially matched the two nitrogens of the Nutlin imidazoline ring **1** with the two nitrogens in the benzimidazole scaffold (Figure 2, red). Overlay of the Nutlin chlorophenyl rings with the benzimidazole gave two possible orientations of the chloro group in the scaffold (**8** and **9**). Positions 1 and 2 of the benzimidazole ring were used to append the hydrophobic piperidinyl amide substituents judged to be necessary for activity, resulting in two different substitution patterns, represented by general structures **8** and **9** (Figure 2).

Docking experiments showed two separate binding modes for the enantiomers of **8**{*1,6*}. In the case of the (*R*)-enantiomer the chlorobenzimidazole occupies the Phe19 pocket and the aryl urea fills the Leu26 pocket, whereas for the (*S*)-enantiomer the positions are reversed (Figures 3a and 3b, respectively). The docked pose for **9**{*1,6*} shows the arylurea occupying the Leu26 pocket and the

benzyl group positioned within the Phe19 pocket (Figure 3c). Interestingly, the Trp23 subpocket is unoccupied for all of the binding modes generated. This pocket is known to be important from the published X-ray structures of small-molecule MDM2-p53 inhibitors. The other subpockets (Phe19 and Leu26) were occupied by the hydrophobic moieties of the ligands, thus rendering both libraries **8** and **9** worthy of exploration. For the alternative benzimidazoles **8**, the (*S*)-enantiomers appeared to be more promising than (*R*)-enantiomers and therefore were prioritized for synthesis.

*Synthesis*

The rapid synthesis of benzimidazole libraries has been described recently using a number of approaches, including solid-phase synthesis,(43, 44) and solution-phase methods.(45-48) In this case, the required $N^1$-substitution was introduced *via* an $S_NAr$ reaction with an *o*-fluoronitrobenzene, followed by *in-situ* nitro-reduction, using sodium dithionite under microwave heating, and cyclisation with the desired aldehyde to give the the appropriately substituted benzimidazoles **8** and **20**.(49)

The synthesis of benzimidazoles **8** bearing substituted aryl groups required the use of suitably substituted arylacetaldehydes (**10**). These were prepared from the respective benzaldehydes using a Wittig reaction giving the methyl enol ethers (**11**), which were subjected to acidic hydrolysis (Scheme 1). The $S_NAr$ reaction of (*S*)-**12** and 4-chloro-1-fluoro-2-nitrobenzene gave nitroaniline (**13**) in good yield. Reductive cyclisation of **13** with sodium dithionite in the presence of arylacetaldehydes **10**, under microwave heating, provided benzimidazoles **14**{1-4} cleanly and in good yields. Deprotection with TFA gave piperidines **15**{*1-4*} which were reacted in parallel with a series of isocyanates to give the final (*S*)-benzimidazoles **8**{*1-4,1-10*} (Scheme 2). Single examples of the thiourea **16** and sulfonamide **17** derivatives were also prepared (Scheme 3).

The synthesis of 2-(piperidin-4-yl)-benzimidazoles **9** required the Boc-protected isonipecotic aldehyde (**18**), prepared according to a literature procedure (Scheme 4).(50)

Reaction of 4-chloro-1-fluoro-2-nitrobenzene with the required benzylamine under microwave heating gave nitroanilines (**19**). Reductive cyclisation of **19** with sodium dithionite in the presence of aldehydes **14**, under microwave heating gave the benzimidazoles **20**{1-4} in good yields. Deprotection with TFA gave piperidines **21**{*1-4*} which were reacted in parallel with a series of isocyanates giving the final benzimidazoles **9**{*1-4,1-10*} (Scheme 5).

*Biological evaluation*

We have previously demonstrated that the MDM2 and MDMX ELISA assays show good sensitivity over a wide range of $IC_{50}$ values. In particular, compounds with low potency and poor predicted solubility have given reliable results.(40, 41) Compounds from libraries **8** and **9** were assayed against MDM2 and MDMX in parallel. The results are displayed in Tables 1 and 2. Overall, both series displayed limited MDM2 inhibition with only **8**{*4,7*}, and **9**{2,8} showing sub-100 μM activity (MDM2 LE = 0.14 and 0.15, respectively). In contrast, 27/40 examples of series **8** were sub-100 μM inhibitors of MDMX, and two examples showed sub-50 μM activity, i.e. **8**{*3,4*} and **8**{*4,7*} (MDMX LE = 0.15). Series **9** also had a greater number of sub-100 μM MDMX inhibitors (10/30), and four examples showed sub-50 μM activity, i.e. **9**{*2,2*}, **9**{*2,3*},**9**{*2,8*} and **9**{*4,2*} (MDMX LE = 0.15 - 0.16). Interestingly, the unsubstituted thiourea derivative **16** and the sulfonamide **17** showed improved MDMX inhibitory activity when compared with the equivalent urea **15**{*1,6*}.

## DISCUSSION

Recently, analysis of the contribution to binding of each portion of Nutlin-3 (**1**) has been reported, by the synthesis and analysis of fragments of the lead compound.(51) Systematic removal of the groups accessing each of the three sub-pockets of MDM2 allowed an assessment of their relative contribution to binding, and showed that removal of the 4-chlorophenyl group accessing the Trp23 pocket resulted in the most significant loss of activity. For example, **22** showed a $K_d$ for MDM2 of 1 mM (LE = 0.10). The activity of libraries **8** and **9** are consistent with the loss of potency associated with leaving the Trp23 sub-pocket vacant as predicted in the docked binding modes of both series. Interestingly, the results for MDMX suggest that the Trp23 sub-pocket may play a somewhat less significant role in the overall binding affinity, as libraries **8** and **9** showed improved activity.

## CONCLUSIONS

Libraries of substituted benzimidazoles based on scaffold-hopping from structures **1** and **7** have been docked into MDM2 and evaluated. The efficient synthesis of two libraries (**8** and **9**) has been achieved by microwave-assisted reductive cyclisation. Both libraries were assayed for inhibition of the MDM2-p53 and MDMX-p53 protein-protein interaction. Members of each library showed sub-100 μM activity for MDM2 or MDMX and several mixed MDM2-MDMX inhibitors were identified with modest potency.

SUPPORTING INFORMATION

Synthetic and analytical details for compounds **8, 13, 14, 15, 16, 19, 20, 21.**

ACKNOWLEDGEMENTS

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TABLE CAPTIONS

Table 1. MDM2-p53 and MDMX-p53 inhibitory activity of compound libraries **13** and **8**.

Table 2. MDM2-p53 and MDMX-p53 inhibitory activity of compound libraries **20** and **9**.

FIGURE CAPTIONS

Chart 1: MDM2-p53 clinical trials candidates with disclosed structures.

Figure 1: Structure of the p53-MDM2 complex (1RV1) (MDM2 white, p53 peptide yellow).(15)

Figure 2: Schematic for scaffold hopping and docking approach.

Figure 3: Putative binding modes for compounds: a) **8** (*R*)-enantiomers; b) **8** (*S*)-enantiomers; c) **9**.

SCHEME CAPTIONS

Scheme 1: Synthesis of substituted phenylacetaldehydes **10**.[a]

[a] Reagents and Conditions: a) $MeOCH_2PPh_3Br$, KO*t*-Bu, THF, rt; b) $HCO_2H$, DCM, rt.

Scheme 2: Synthesis of benzimidazole library **8**.[a]

[a] Reagents and Conditions: a) DMF, $Na_2CO_3$, 70 $^oC$; b) $ArCH_2CHO$, **13**, $Na_2S_2O_4$, MeOH, $H_2O$, MW, 100 $^oC$; c)i) TFA, DCM, rt; ii) $K_2CO_3$, MeOH, rt; d) R'NCO, DCM, rt.

Scheme 3: Synthesis of benzimidazoles **16** and **17**.[a]

[a] Reagents and Conditions: a) R'NCS, DCM, rt; b) $PhCH_2SO_2Cl$, $Et_3N$, DCM, rt.

Scheme 4: Synthesis of piperidine **18**.[a]

[a] Reagents and Conditions: a) $Boc_2O$, NaOH, dioxane, rt; b) $BH_3$-THF, THF, rt; c) PCC, NaOAc, 4Å molecular sieves, DCM, rt.

Scheme 5: Synthesis of benzimidazole library **9**.[a]

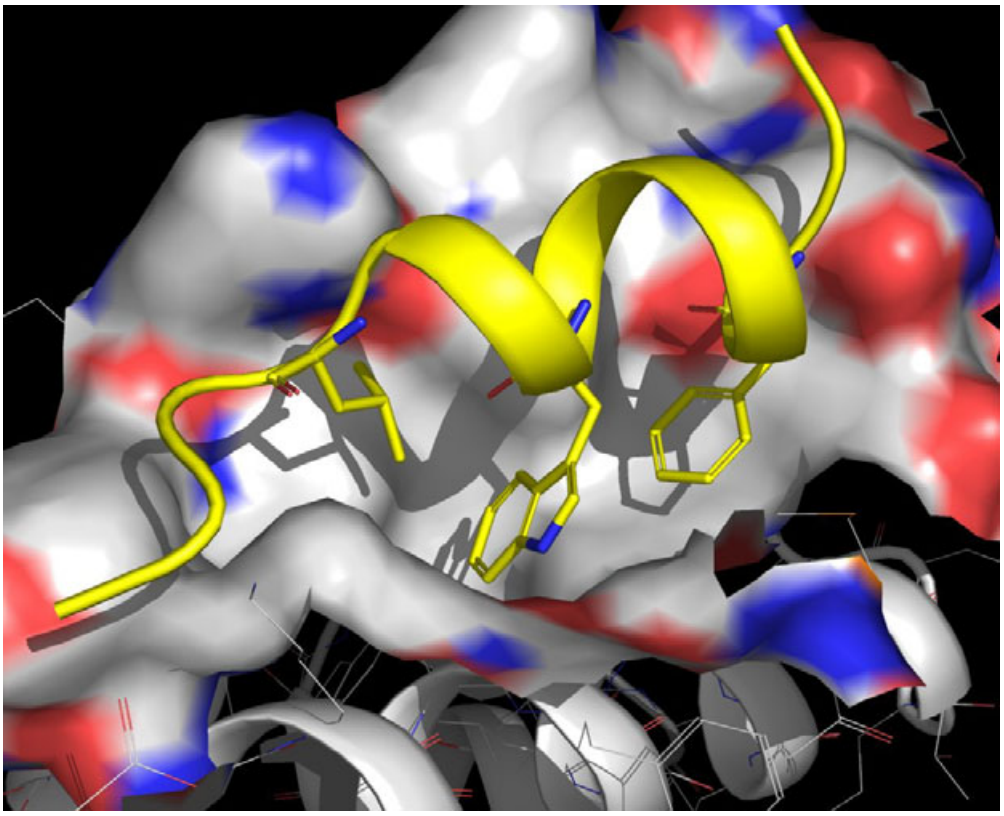
[a] Reagents and Conditions: a) $ArNH_2$, $K_2CO_3$, EtOH, rt; b) **19**, $Na_2S_2O_4$, MeOH, $H_2O$, MW, 100 $^oC$; c) i)TFA, DCM, rt; ii) $K_2CO_3$, rt; d) R'NCO, DCM, rt.

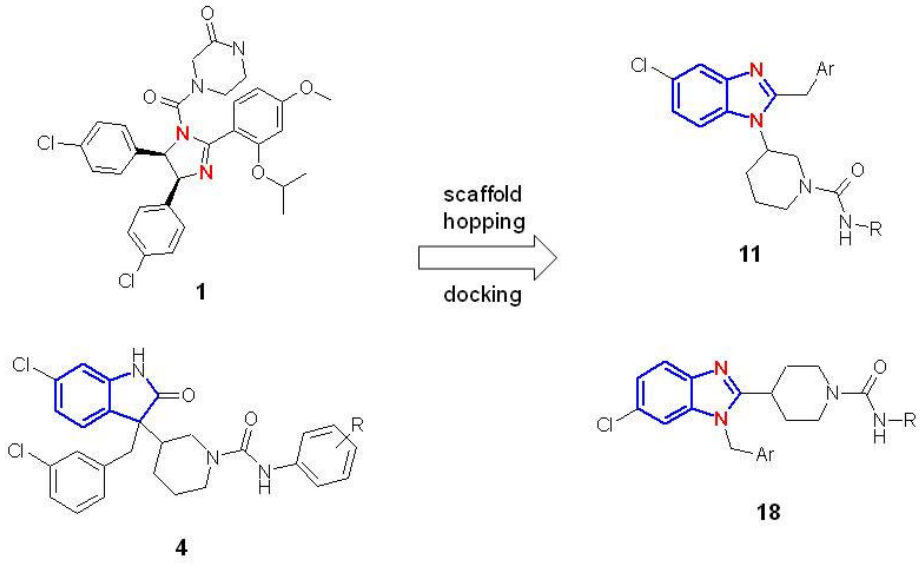
Table 1.

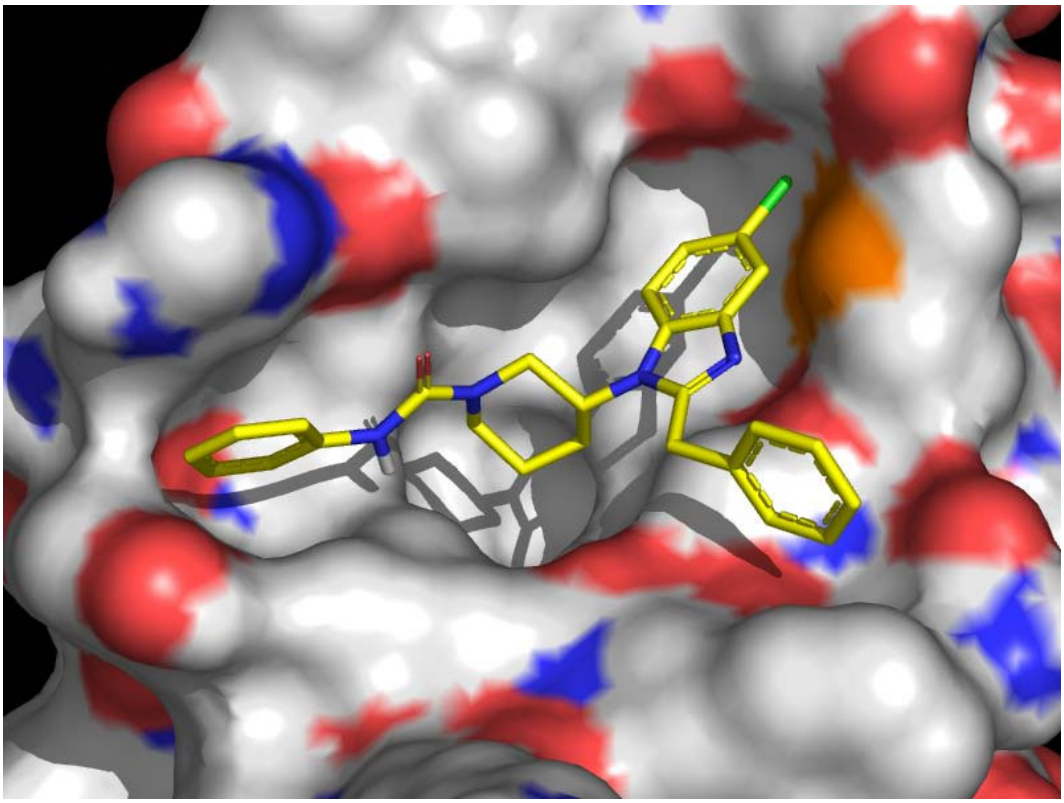
| Compound | MDM2 $IC_{50}$ (μM) | MDMX $IC_{50}$ (μM) | Compound | MDM2 $IC_{50}$ (μM) | MDMX $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **13**{*1*} | >500 | 248 | **8**{*3,1*} | 170 | 64 ± 20 |
| **13**{*2*} | 290 ± 38 | 104 ± 23 | **8**{*3,2*} | - | - |
| **13**{*3*} | >500 | >500 | **8**{3,3} | 121 ± 40 | 92 ± 18 |
| **13**{*4*} | 211 | 130 | **8**{*3,4*} | 120 ± 37 | 43 ± 15 |
| **8**{*1,1*} | 183 ± 20 | 70 ± 19 | **8**{*3,5*} | 213 ± 158 | 74 ± 12 |
| **8**{1,2} | 208 ± 33 | 105 ± 54 | **8**{*3,6*} | 191 | 187 |
| **8**{*1,3*} | 202 ± 24 | 54 ± 14 | **8**{*3,7*} | 146 ± 63 | 77 ± 20 |
| **8**{*1,4*} | 176 | 117 | **8**{*3,8*} | >500 | 116 |
| **8**{*1,5*} | 202 ± 51 | 92 ± 51 | **8**{*3,9*} | 257 ± 146 | 131 ± 48 |
| **8**{*1,6*} | 218 | 100 | **8**{*3,10*} | 177 | 100 |
| **8**{*1,7*} | 203 ± 80 | 61 ± 30 | **8**{*4,1*} | - | - |
| **8**{*1,8*} | 196 ± 61 | 69 ± 5 | **8**{*4,2*} | 201 ± 96 | 95 ± 13 |
| **8**{*1,9*} | >500 | 156 | **8**{*4,3*} | 115 | 207 |
| **8**{*1,10*} | 257 | 169 | **8**{*4,4*} | 166 ± 55 | 78 ± 16 |
| **8**{*2,1*} | 151 | 86 | **8**{*4,5*} | 188 ± 102 | 173 ± 121 |
| **8**{*2,2*} | 134 ± 86 | 81 ± 33 | **8**{*4,6*} | 188 ± 33 | 109 ± 39 |
| **8**{*2,3*} | 148 ± 61 | 60 ± 10 | **8**{*4,7*} | 72 ± 20 | 43 ± 24 |
| **8**{*2,4*} | 201 ± 128 | 154 ± 79 | **8**{*4,8*} | 202 ± 157 | 76 ± 24 |
| **8**{*2,5*} | 114 ± 38 | 80 ± 13 | **8**{*4,9*} | 198 ± 75 | 99 ± 26 |
| **8**{*2,6*} | 125 ± 35 | 83 ± 29 | **8**{*4,10*} | 131 ± 45 | 64 ± 13 |
| **8**{*2,7*} | 154 ± 81 | 57 ± 14 | **16** | 144 ± 44 | 49 ± 14 |
| **8**{*2,8*} | 153 ± 112 | 50 ± 6 | **17** | 212 ± 1 | 59 ± 36 |
| **8**{*2,9*} | 165 ± 30 | 73 ± 14 | | | |
| **8**{*2,10*} | 103 ± 36 | 63 ± 35 | | | |

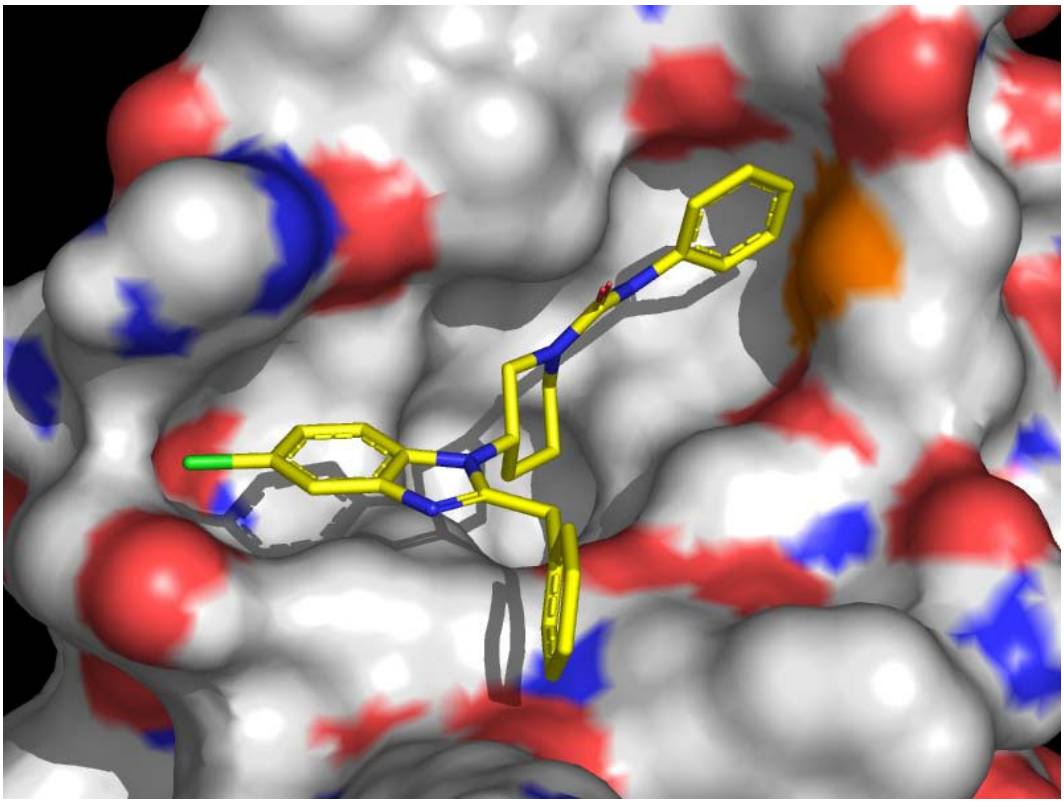
Table 2.

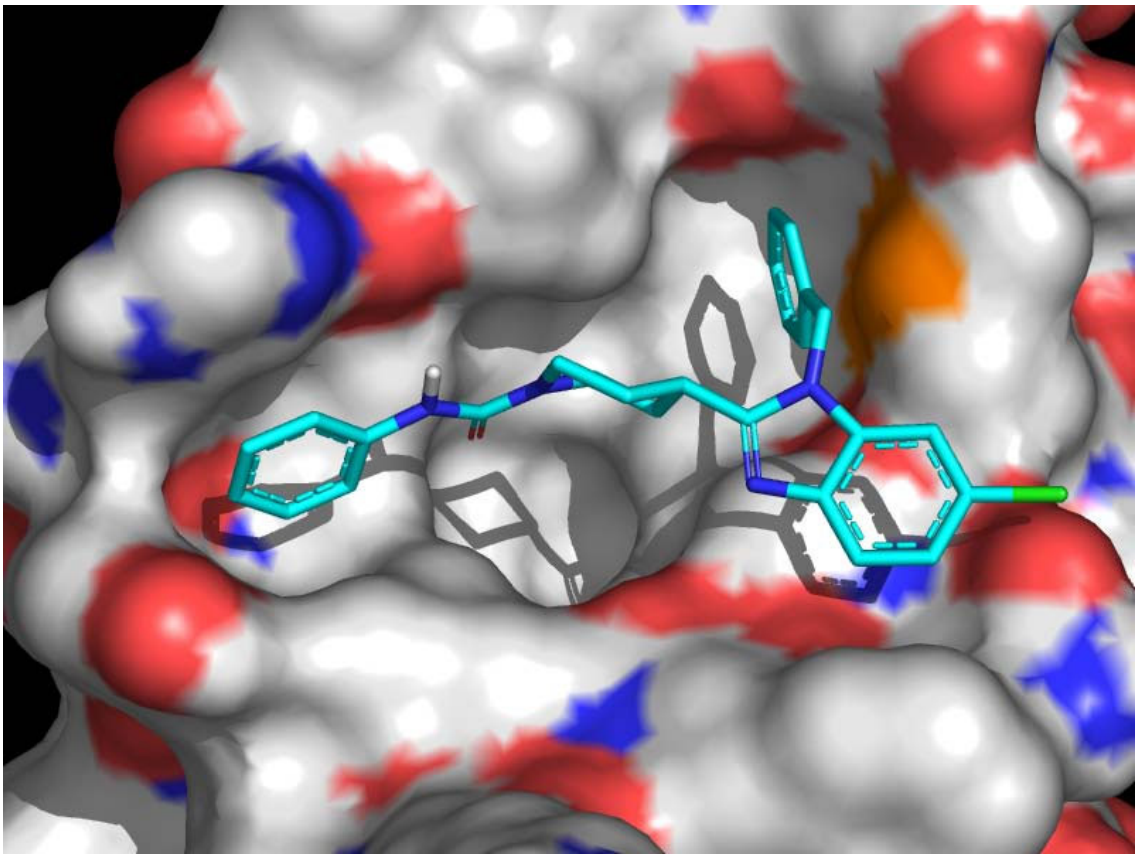
| Compound | MDM2 $IC_{50}$ (μM) | MDMX $IC_{50}$ (μM) | Compound | MDM2 $IC_{50}$ (μM) | MDMX $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **20**{*1*} | 253 | >500 | **9**{*2,9*} | - | - |
| **20**{*2*} | 248 | 217 | **9**{*2,10*} | 212 | 101 |
| **20**{*3*} | >500 | >500 | **9**{*3,1*} | - | - |
| **20**{*4*} | >500 | >500 | **9**{*3,2*} | 201 | 103 |
| **21**{*1*} | 282 | >500 | **9**{*3,3*} | 182 ± 21 | 81 ± 20 |
| **9**{*1,1*} | - | - | **9**{*3,4*} | 291 ± 117 | 252 ± 136 |
| **9**{*1,2*} | 307 ± 132 | 154 ± 70 | **9**{*3,5*} | 183 | >500 |
| **9**{*1,3*} | 256 ± 51 | 94 ± 37 | **9**{*3,6*} | 269 ± 39 | 200 ± 100 |
| **9**{*1,4*} | 141 ± 22 | 98 ± 62 | **9**{*3,7*} | >500 | >500 |
| **9**{*1,5*} | >500 | 151 | **9**{*3,8*} | 149 ± 61 | 94 ± 38 |
| **9**{*1,6*} | >500 | 231 | **9**{*3,9*} | 218 | 123 |
| **9**{*1,7*} | >500 | 194 | **9**{*3,10*} | 218 | 201 |
| **9**{*1,8*} | 151 | 194 | **9**{*4,1*} | >500 | >500 |
| **9**{*1,9*} | >500 | >500 | **9**{*4,2*} | 314 ± 65 | 49 ± 22 |
| **9**{*1,10*} | 204 ± 69 | 111 ± 63 | **9**{*4,3*} | >500 | 145 |
| **9**{*2,1*} | - | - | **9**{*4,4*} | - | - |
| **9**{*2,2*} | 130 ± 65 | 46 ± 11 | **9**{*4,5*} | - | - |
| **9**{*2,3*} | 243 | 45 | **9**{*4,6*} | >500 | 245 |
| **9**{*2,4*} | 156 ± 40 | 113 ± 74 | **9**{*4,7*} | - | - |
| **9{***2,5*} | - | - | **9**{*4,8*} | 180 ± 15 | 73 ± 16 |
| **9**{*2,6*} | - | - | **9**{*4,9*} | - | - |
| **9**{*2,7*} | 161 ± 68 | 68 ± 29 | **9**{*4,10*} | >500 | 184 |
| **9{***2,8*} | 88 ± 43 | 31 ± 10 | | | |

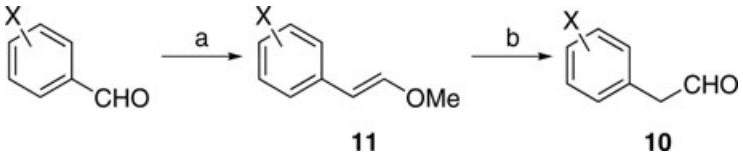




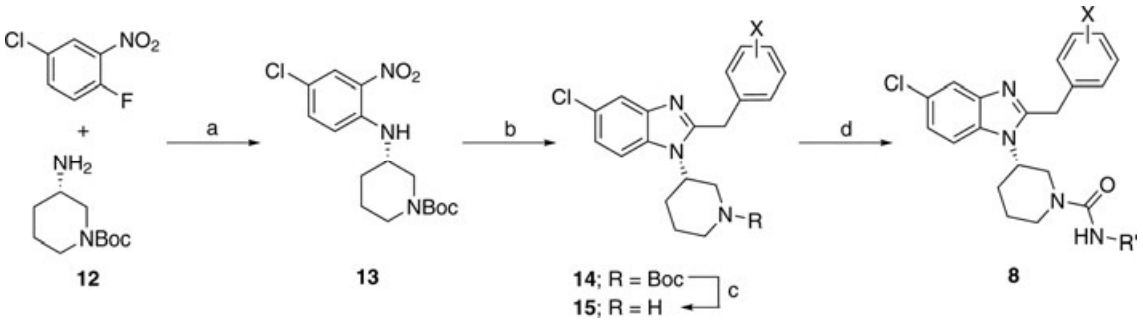




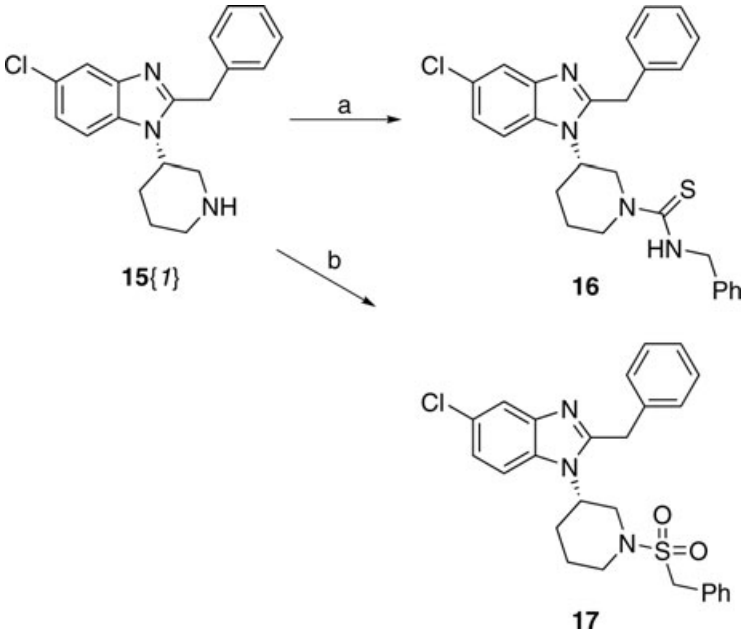




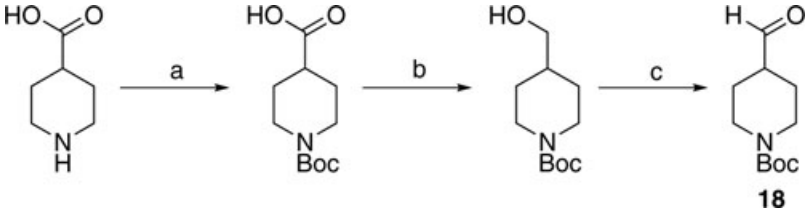

**11** **10**




**12** **13** **14**; R = Boc **15**; R = H c **8**




15{1} 16 17

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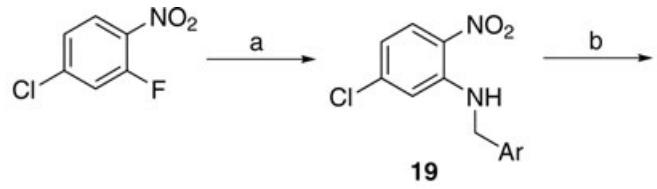


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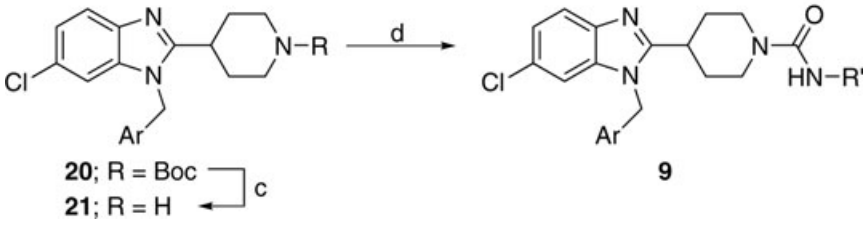


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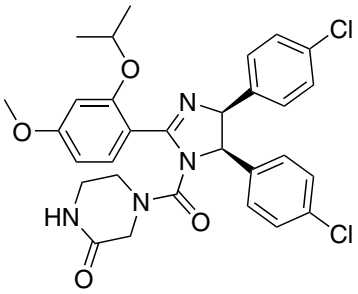
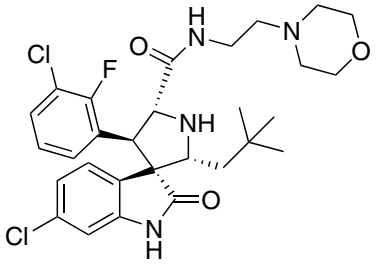
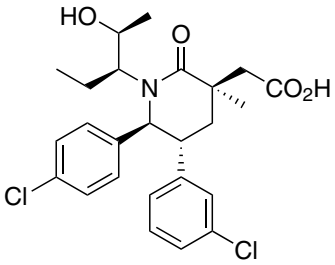


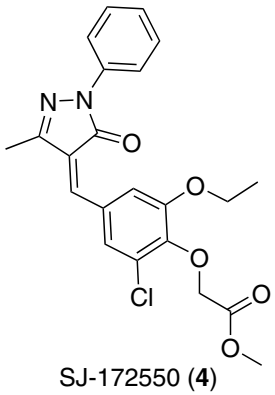
Nutlin-3 (**1**)



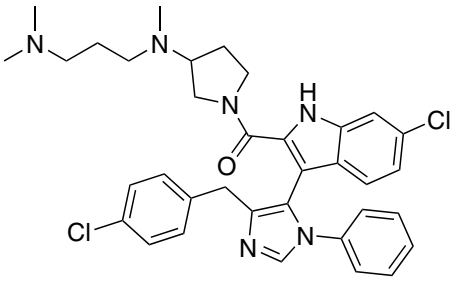
MI-63 (**2**)



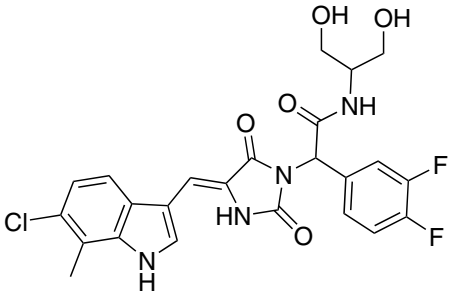
(**3**)



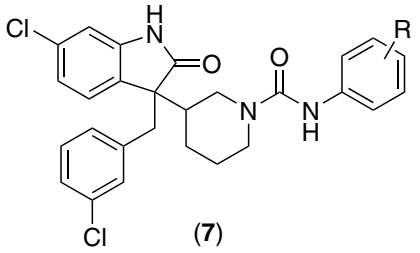
SJ-172550 (**4**)



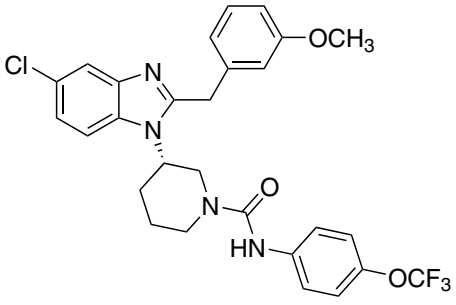
(**5**)



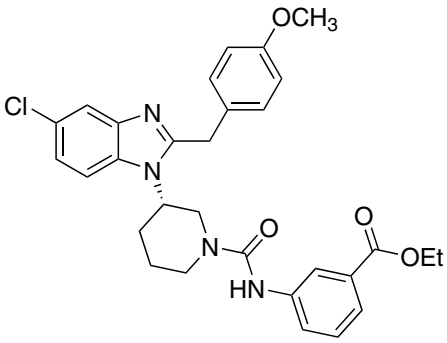
RO-5963 (**6**)



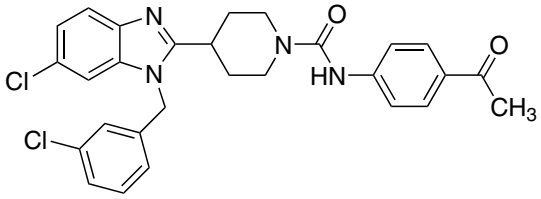
(**7**)



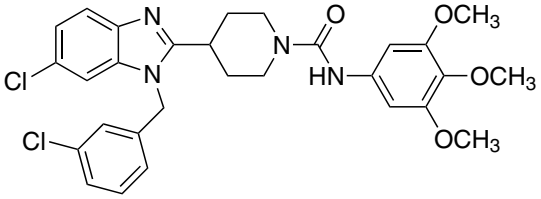
**8**{*3,4*}



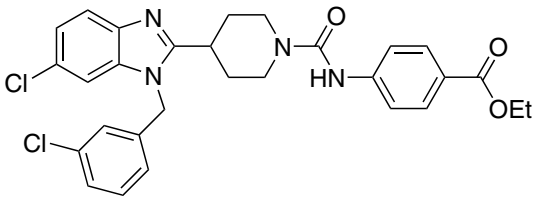

**8**{*4,7*}



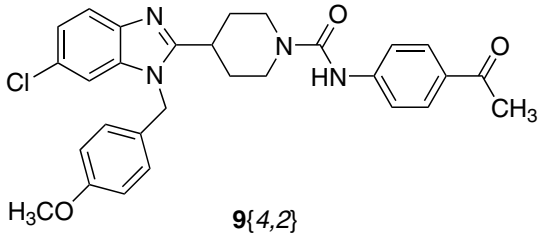

**9**{*2,2*}



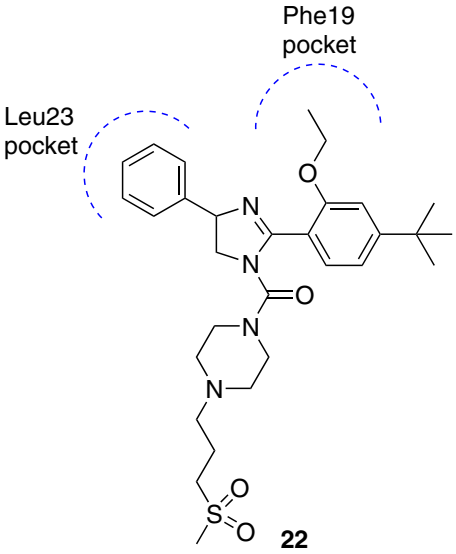

**9**{*2,3*}



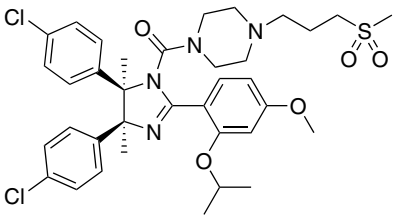

**9**{*2,8*}



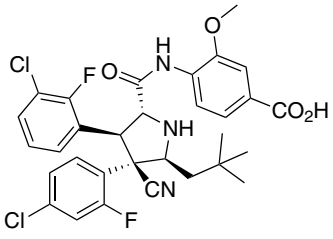
**9**{*4,2*}



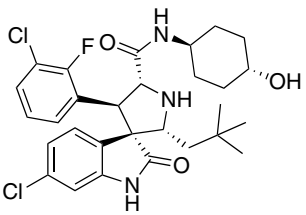

**22**



RG7112

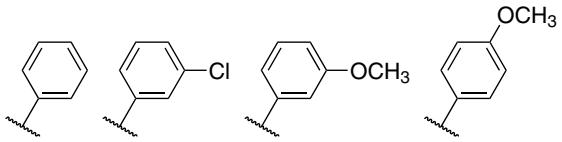


RG7388



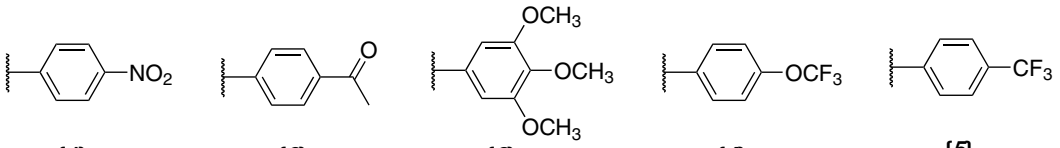
MI-773

Ar =

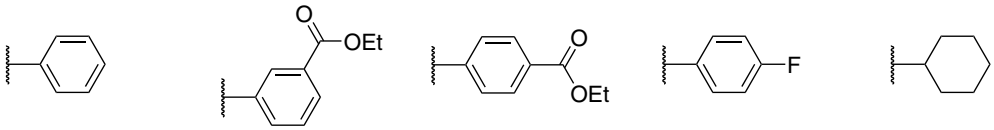



{1} {2} {3} {4}

R' =




{1} {2} {3} {4} {5}




{6} {7} {8} {9} {10}