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Improvement of the synthesis and pharmacokinetic properties of chromenotriazolopyrimidine MDM2-p53 protein-protein inhibitors

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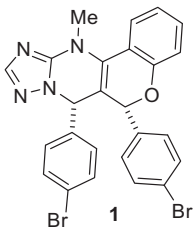
ABSTRACT

Human murine double minute 2 (MDM2) is a negative regulator of p53, which plays an important role in cell cycle and apoptosis. We report several optimizations to the synthesis of the chromenotriazolopyrimidine series of MDM2-p53 protein-protein interaction inhibitors. Additionally, the in vitro and in vivo stability, pharmacokinetic properties and solubility were improved through N-substitution.

The tumor suppressor p53 is responsible for the transcription of several genes controlling cell cycle arrest and apoptosis and is known to play an important role in regulating tumor formation and proliferation.[1] The oncoprotein MDM2 directly blocks p53 activity and promotes the degradation of p53.[2] MDM2 is often over expressed in cancer and its expression has been correlated with accelerated disease progression.[3] Inhibition of the MDM2-p53 protein-protein interaction, and the resulting p53 activation, has been proposed as a novel oncology therapeutic strategy.[4] The chromenotriazolopyrimidine MDM2 inhibitor **1** demonstrated p53 dependent inhibition of HCT116 cancer cell proliferation (Fig. 1).[5] Although exhibiting moderate cellular antiproliferative activity, **1** displayed poor pharmacokinetic (PK) properties. In rat in vivo experiments,[6] **1** exhibited moderate-to-high clearance (2.4 L/h/kg) and an oral bioavailability of only 23%. In addition, **1** suffered from low solubility in PBS (<1 μg/mL).[7] Herein we report our efforts to improve the PK properties within this series of MDM2 inhibitors.

With the aim of rapidly exploring the SAR and improving the physicochemical and PK properties of **1**, a more robust and scalable synthetic route to this series of inhibitors was required. The original synthesis provided **1** in an overall yield of 1%.[5] We were able to make several improvements to the synthesis (Scheme 1). For example, in the initial condensation reaction, adjustments to the workup procedure improved the yield of enone **2** from 65% to 90% yield. By slowly adding the reaction mixture to a rapidly stirring solution of ice water, we were able to obtain a free flowing off-white powder of enone **2**. Employing the finely powdered enone **2** in the subsequent melt reaction with aminotriazole resulted in an improvement of the yield for phenol **3** from 41% to 86%. The previous procedure for the condensation of **3** employed 1.3 equiv of bromobenzaldehyde at 160 °C and led to a 1:4 ratio of **4**/**5** in 20% yield. Since the desired diastereomer **4** was the minor product in this transformation, we sought to further optimize this reaction. It was found that decreasing the reaction temperature to 140 °C and increasing the equivalents of bromobenzaldehyde to 3.0



HTRF IC_{50} = 1.4 uM
SJSA-1 p21 IC_{50} = 32 uM
PK (rat)
MRT (hr) 2.4
Vdss (L/kg) 9.9
CL (L/hr/kg) 2.4
F (%) 23
solubility (μg/mL)
PBS <1

Figure 1. Potency and PK properties of **1**.

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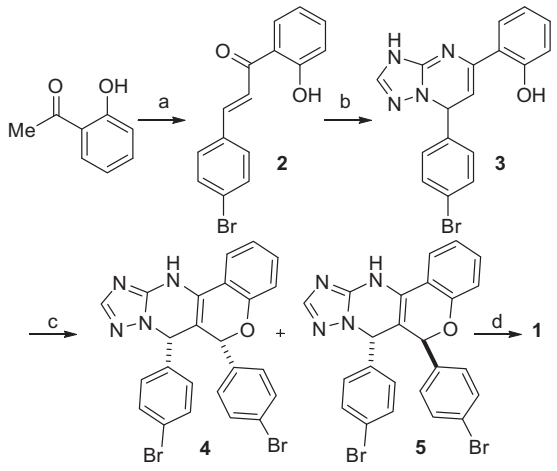


Scheme 1. Optimized synthesis of **1**. Reagents and conditions: (a) 4-bromobenzaldehyde (1.0 equiv), KOH (3.6 equiv), EtOH, rt, 16 h, 90%; (b) 1,2,4-triazol-5-amine (1.0 equiv), neat, 160 °C, 30 min, 86%; (c) 4-bromobenzaldehyde (3.0 equiv), neat, 140 °C, 10 min, 75%; (d) MeI (2.0 equiv), Cs_2CO_3 (1.9 equiv), rt, 30 min, 57%.

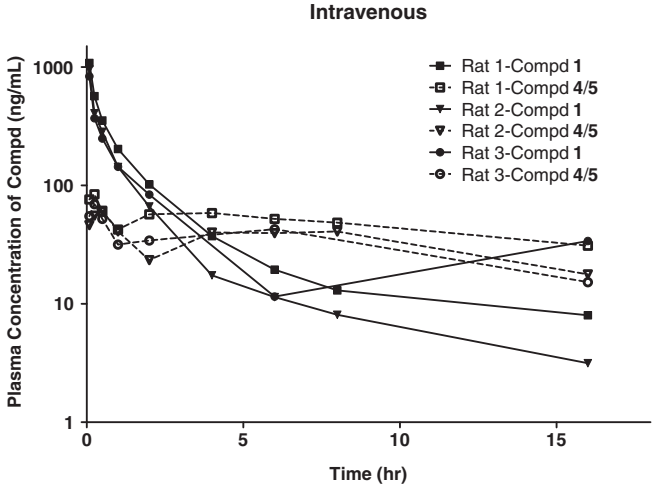


Figure 2. In vivo conversion of **1** to **4**/**5** in rats following an intravenous dose of **1**.[6]

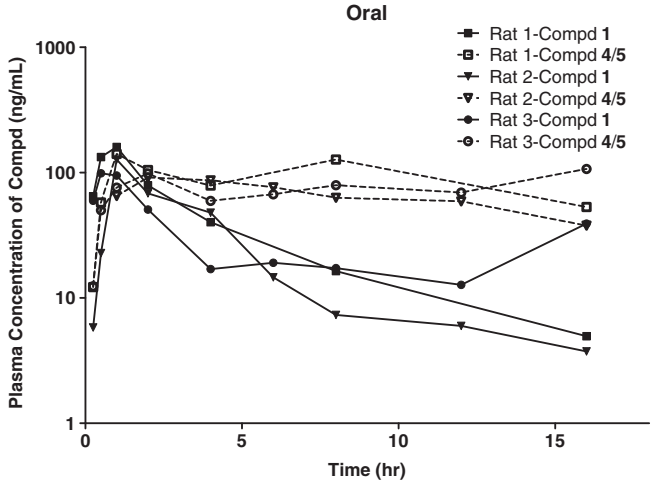
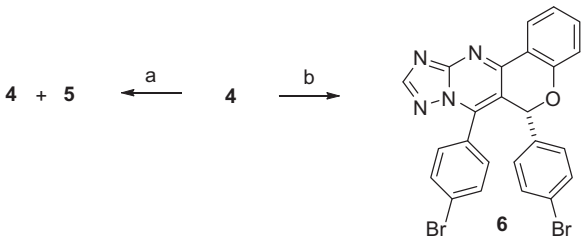


Figure 3. In vivo conversion of **1** to **4**/**5** in rats following an oral dose of **1**.[6]



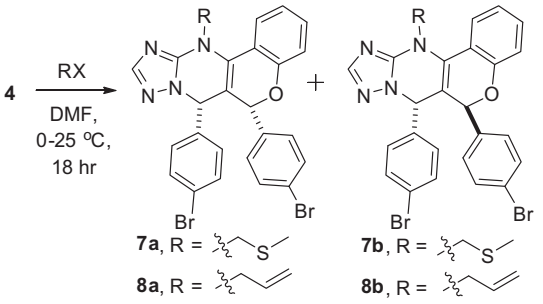
Scheme 2. Chemical instability of **4**. Reagents and conditions: (a) DMSO, rt, 12 h; (b) dioxane, 45 °C, 5 min.

resulted in a 10:1 ratio of **4**/**5** in 75% yield. Utilizing the optimized conditions, we were able to obtain the desired chromenotriazolopyrimidine core **4** in 53% overall yield. After the requisite N-alkylation, chiral separation provided the active enantiomer **1**.[8]

In order to evaluate the metabolic stability of **1**, it was incubated with rat liver microsomes (RLM) and after 30 min <5% of **1** remained.[9] Furthermore, the plasma concentration of **1** in vivo rapidly declined with time (Fig. 2).[6] Interestingly, after intravenous administration of **1** in rats, measureable concentrations of **4**/**5** were found in the plasma. Following an oral dose of **1**, quantities of **4**/**5** were also observed in the rat plasma (Fig. 3). We hypothesized that oxidative N-demethylation and tautomeric isomerization was responsible for the rapid biotransformation of **1** to **4**/**5** in vitro and in vivo.

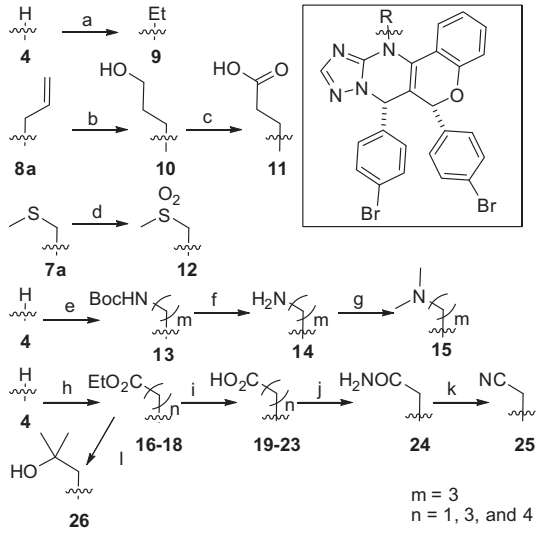
In order to test our N-demethylation hypothesis, we sought to improve the metabolic stability of **1** by installing *N*-alkyl substituents that would block the metabolic soft-spot.[10] The X-ray co-crystal structure of **4** bound to MDM2 revealed the N–H makes no

Table 1
N-alkylation conditions



Entry	Base	Equiv of base	RX	Equiv of RX	Ratio **a**:**b**,[a] yield of **a**[b]
1	Pyridine	1.0	Cl⁄⁄S	1.5	NR
2	2,6-Lutidine	1.0	Cl⁄⁄S	1.5	NR
3	DMAP	1.0	Cl⁄⁄S	1.5	NR
4	Imidazole	1.0	Cl⁄⁄S	1.5	NR
5	Morpholine	1.0	Cl⁄⁄S	1.5	NR
6	TMG	1.0	Cl⁄⁄S	1.5	1:1.3
7	Cs_2CO_3	1.5	Cl⁄⁄S	1.25	1:1
8	DBU	1.5	Cl⁄⁄S	1.25	1:1.3
9	DBU	0.9	Cl⁄⁄S	1.25	1.9:1, 20%
10	iPr_2NEt	2.0	Allylbr	2.0	1:2.8
11	DBU	1.0	Allylbr	2.0	2.7:1, 35%

DMF was degassed with Ar (g) for 10 min before use.
[a] Ratio of **a**:**b** determined by LCMS.
[b] Isolated yield.



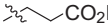
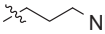
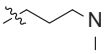
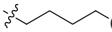
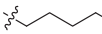
Scheme 3. Preparation of *N*-alkyl compounds **9**–**26**. Reagents and conditions: (a) iodoethane (2.0 equiv), Cs_2CO_3 (2.0 equiv), DMF, 25 °C, 1 h, 38%; (b) 9-BBN (6.0 equiv), THF, 25 °C, 2 h; then 3 N NaOH/30% H_2O_2 (1:1), 30 min, 80%; (c) KBr (1.0 equiv), TEMPO (1.1 equiv), NaOCl (1.3 equiv), acetone/5% aq $NaHCO_3$ (4:1), 0 °C, 30 min, 2%; (d) oxone, alumina, H_2O, $CHCl_3$, 50 °C, 10 h, 38%; (e) 3-(Boc-amino)propyl bromide (1.0 equiv), Cs_2CO_3 (1.0 equiv), DMF, 18 h, 8%; (f) TFA (15 equiv), DCM, 25 °C, 30 min, 90%; (g) formaldehyde (2.0 equiv), $NaBH_3CN$ (4.0 equiv), MeOH, 4 Å sieves, 25 °C, 8 h, 47%; (h) *n* = 1 ethyl iodoacetate (1.2 equiv) , Cs_2CO_3 (1.0 equiv), DMF, 0–25 °C, 30 min, 67%; *n* = 3 ethylbromobutyrate (2.0 equiv), DBU (1.0 equiv), DMF, 0–25 °C, 30 min, 67%; *n* = 4 ethylbromovalerate (1.2 equiv), DBU (1.0 equiv), DMF, 0–25 °C, 30 min, 31%; (i) *n* = 1 LiOH (26 equiv), THF/MeOH (1:1), 25 °C, 1 h, 42%; *n* = 3 LiOH (5 equiv), THF/MeOH (1:1), 25 °C, 24 h, 40%; *n* = 4 LiOH (10 equiv), THF/MeOH (1:1), 25 °C, 2 h, 35%; (j) $SOCl_2$ (1.3 equiv), THF, reflux, 30 min; then NH_3OH (25 equiv), DCM, 25 °C, 1 h, 65%; (k) $POCl_3$ (1.0 equiv), DMF, 100 °C, 1 h, 54%; (l) MeMgBr (2.5 equiv), THF, 0–25 °C, 18 h, 50%.

contacts with the protein.[5] Thus, we predicted that N-substitution would project into solvent and would not have a detrimental impact on potency of this series. A reliable method for N-alkylation of **4** was required in order to prepare several *N*-alkyl analogs. Unfortunately, **4** readily epimerizes to a 1:1 mixture of **4**/**5** upon standing at room temperature in DMSO (Scheme 2).[11] Furthermore, **4** undergoes facile oxidation to **6** in the presence of oxophilic solvents (i.e., dioxane, DMA or DMF) and mild temperature (45 °C) within 5 min. Thus, N-alkylation reactions would require both precautions to remove oxygen and short reaction time in order to minimize undesired side-products.

First we explored alkylation of **4** using chlorodimethylsulfide. Weak bases (Table 1, entries 1–5) resulted in no reaction while the use of TMG (tetramethylguandine) provided a 1:1.3 ratio of **7a**:**7b** (Table 1, entry 6).[12] Using 1.5 equiv of DBU or Cs_2CO_3, afforded a 1:1.3 and 1:1 ratio of **7a**:**7b**, respectively (Table 1, entries 7 and 8). Reducing the equivalents of DBU to 0.9 provided a 1.9:1 ratio of **7a**:**7b** with a 20% isolated yield of the desired **7a** (Table 1, entry 9). Alternatively, allylation of **4** with allyl bromide employing iPr$_2$NEt provide a 1:2.8 ratio of **8a**:**8b** (Table 1, entry 10). DBU provided the most favorable result, giving a 2.7:1 ratio of **8a**:**8b** and a 35% isolated yield of the desired **8a** (Table 1, entry 11).

With the optimized N-alkylation reaction conditions, we rapidly prepared several *N*-alkyl analogs as shown in Scheme 3. Alkylation of **4** with iodoethane afforded the desired **9** in 38% yield. *N*-allyl analog **8a** was converted to the alcohol **10** using hydroboration-oxidation conditions. The oxidation of **10** to provide acid **11** proved difficult. Many conditions were explored,[13] but only TEMPO oxidation gave the acid **11**. Sulfide **7a** could be readily oxidized to the desired sulfone **12** in 38% yield. N-alkylation of **4** with 3-(Boc-amino)propyl bromide provided **13**, which was converted to **14**. Amine **14** was further transformed into dimethylamine **15**. N-alkylation of **4** with several bromoesters afforded **16**–**18** which were hydrolyzed to provide acids **19**–**23**. Acid **19** was further converted into amide **24** and nitrile **25**. Ester **16** was also used to prepare tertiary alcohol **26**.

Table 2
Potencies and RLM stability of *N*-alkyl analogs

Compd	R=	HTRF[a] IC_{50} (μM)	SJSA-1 p21[b] IC_{50} (μM)	RLM[c] % remaining
1[d]	Me	1.4 ± 0.78	32	<5
8		2.4 ± 0.06	>50	<5
9	Et	1.8 ± 0.23	>50	35
10		0.43 ± 0.007	19	11
11		0.51 ± 0.02	19	>90
12		0.51 ± 0.12	26	27
13		4.4 ± 0.15	>50	44
14		3.7 ± 0.17	>50	>90
15		7.5 ± 0.007	>25	66
19		0.91 ± 0.06	>50	87
20[d]		0.48 ± 0.15	38	>90
21		0.65 ± 0.04	46	83
22		0.76 ± 0.07	20	73
23[d]		0.35 ± 0.02	12	87
24		0.54 ± 0.001	26	22
25		0.73 ± 0.04	>50	13
26		0.37 ± 0.06	14	36

[a] Values reported as mean of at least two experiments, followed by standard deviation, see Ref. 15.
[b] See Ref. 16.
[c] See Ref. 9.
[d] Compound **1**, **20**, and **23** are single enantiomers, other compounds are racemic mixtures.

The binding and cellular potencies for the *N*-alkyl analogs are shown in Table 2.[14] Several *N*-alkyl groups were tolerated and a few analogs exhibited increased stability in the presence of RLM. Small groups, such as allyl **8a** and ethyl **9**, maintained potency but exhibited high turnover in the presence of microsomes. While, the addition of polarity to the terminal alkyl position enhanced the MDM2 affinity (i.e., **10**, **12**, and **24**–**26**), these compounds lacked improved intrinsic clearance. Propyl carbamate **13** and amines **14**–**15** showed increased stability in the presence of RLM, but these compounds suffered from reduced potency. On the other hand, acids **11**, and **19**–**23** maintained potency and showed improved stability in the presence of RLM.

Since acids **19**[17] and **22**[18] were prepared in reasonable yield, potent in the biochemical assay, and stable to RLM, the racemates were separated using chiral chromoatography.[19] The single enantiomers **20** and **23** were found to be more potent and more metabolically stable than **1**. Also, **20** and **23** exhibited improved solubility

Table 3
PK and solubility properties of **20** and **23**

Compd	In vivo PK[a]				Solubility[b] PBS (μg/mL)
	MRT (iv, h)	V_{dss} (iv, L/kg)	CL (iv, L/h/kg)	*F* (po, %)	
20	0.46	2.6	5.7	14	132
23	4.1	1.7	0.32	54	6.1

[a] Dosed iv 0.5 mg/kg in 100% DMSO; po 2.0 mg/kg in 0.5% methylcellulose/1% Tween 80/98.5% water.
[b] See Ref. 7.

in PBS. We characterized these analogs in rat in vivo PK experiments (Table 3). Acid **20** exhibited high clearance of 5.7 L/h/kg. However, **23** demonstrated low clearance (0.32 L/h/kg) and had modest oral bioavailability (*F* 54%).

In conclusion, by blocking the metabolic soft-spot we were able to substantially improve the PK properties of the chromenotriazolopyrimidine series of MDM2–p53 inhibitors while maintaining both the biochemical and cellular potencies. Further in vivo pharmacodynamic experiments of this series will be reported in due course.

Acknowledgements

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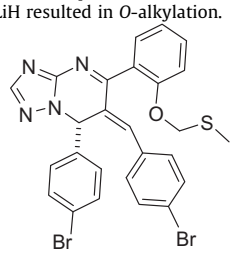
6. Iv 2.0 mg/kg dose in DMSO; po 5.0 mg/kg dose in 2% HPMC, 1% Tween 80, pH 2.2 witsh HCl.
7. PBS = phosphate buffer saline; for experimental details see Tan, H.; Semin, D.; Wacker, M.; Cheetham, J. *J. Assoc. Lab. Autom.* **2005**, *10*, 364.
8. For chiral separation procedure see Ref. 5.
9. Compound (1 μM) was incubated with liver microsomes (0.25 mg/mL) and NADPH (1 mM) in PBS for 30 min.
10. For example of incorporation of water-solubilizing moieties to improve properties of MDM2 inhibitors see: Leonard, K.; Marugan, J. J.; Raboisson, P.; Calvo, R.; Gushue, J. M.; Koblish, H. K.; Lattanze, J.; Zhao, S.; Cummings, M. D.; Player, M. R.; Maroney, A. C.; Lu, T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3463.
11. For hypothesized mechanism of epimerization see Ref. 5.
12. Stronger bases such as LiH resulted in *O*-alkylation.



13. Both *m*-CPBA and Dess-Martin periodinate failed to provide any acid **11**.
14. All biologically active compounds were characterized by ^{1}H NMR, and their purity was determined to be >95% by reverse phase HPLC; ^{1}H NMR (Bruker either 400 or 500 MHz), HPLC (A: 0.1% TFA in H_2O, B: 0.1% TFA in CH_3CN, 10–100% B in 15 min.
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16. mRNA p21 levels in SJSA-1 cells with 10% FBS were measured using Taqman; see Ref. 5 for experimental details; the standard deviation for benchmark compound ±1.0, (n = 34).
17. ^{1}H NMR of **19** (400 MHz, MeOH) δ ppm 4.79 (s, 1H) 4.84 (s, 1H) 5.62 (s, 1H) 6.06 (s, 1H) 6.75–6.84 (m, 3H) 6.96–7.03 (m, 2H) 7.06–7.12 (m, 3H) 7.12–7.18 (m, 2H) 7.20–7.28 (m, 1H) 7.51 (dd, J = 7.83, 1.57 Hz, 1H) 7.70 (s, 1H).
18. ^{1}H NMR of **21** (400 MHz, chloroform-*d*) δ ppm 1.56–1.67 (m, 4H) 2.37 (t, J = 6.65 Hz, 2H) 4.21 (t, J = 7.53 Hz, 2H) 5.50 (s, 1H) 5.84 (s, 1H) 6.79 (dq, J = 8.90, 4.47 Hz, 4H) 6.98 (dd, J = 8.22, 1.17 Hz, 1H) 7.13–7.20 (m, 3H) 7.22–7.26 (m, 2H) 7.34 (ddd, J = 8.22, 7.34, 1.47 Hz, 1H) 7.48 (dd, J = 7.83, 1.57 Hz, 1H) 8.03 (br s, 1H).
19. For **20**: OD-H column with SFC [24 g/min MeOH/0.2% diethylamine + 46 g/min CO_2]; for **23**: separated at ester **18** 250 × 30 mm OD-H column with SFC [20 g/min MeOH/0.2% diethylamine + 60 g/min CO_2].