Targeted Synthesis of Complex Spiro[3H-indole-3,2′-pyrrolidin]-2(1H)-ones by Intramolecular Cyclization of Azomethine Ylides: Highly Potent MDM2–p53 Inhibitors

Andreas Gollner[+],* Harald Weinstabl[+],* Julian E. Fuchs, Dorothea Rudolph, Geraldine Garavel, Karin S. Hofbauer, Jale Karolyi-Oezguer, Gerhard Gmaschitz, Wolfgang Hela, Nina Kerres, Elisabeth Grondal, Patrick Werni, Juergen Ramharter, Joachim Broeker, and Darryl B. McConnell[a]

Mouse double minute 2 (MDM2) is a main and direct inhibitor of the crucial tumor suppressor p53. Reports from initial clinical trials showed that blocking this interaction with a small-molecule inhibitor can have great value in the treatment of cancer for patients with p53 wild-type tumors; however, it also revealed dose-limiting hematological toxicities and drug-induced resistance as main issues. To overcome the former, an inhibitor with superior potency and pharmacokinetic properties to ultimately achieve full efficacy with less-frequent dosing schedules is required. Toward this aim, we optimized our recently reported spiro-oxindole inhibitors by focusing on the crucial interaction with the amino acid side chain of His96$_{MDM2}$. The designed molecules required the targeted synthesis of structurally complex spiro[indole-3,2′-pyrrolo[2,3-c]pyrrole]-2,4′-diones for which we developed an unprecedented intramolecular azomethine ylide cycloaddition and investigated the results by computational methods. One of the new compounds showed superior cellular potency over previously reported BI-0252. This finding is a significant step toward an inhibitor suitable to potentially mitigate hematological on-target adverse effects.

The transcription factor tumor protein p53 (TP53), frequently referred to as the "guardian of the genome", is a pivotal tumor suppressor protein and mainstay of the body's cellular anticancer defense system.[1] TP53 is activated following cellular stress and regulates multiple downstream target genes implicated in cell-cycle control, apoptosis, senescence and DNA repair.[2] The TP53 gene is mutated in about 50% of all human cancers whereas the other 50% have tumors with TP53 wild-type status.[3] However, the function of TP53 is frequently attenuated in these TP53 wild-type cancers by other mechanisms, including overexpression of its key negative regulator HDM2, which is the human homologue of mouse double minute 2 (MDM2). Stabilization and activation of TP53 by the inhibition of TP53 binding to its negative regulator MDM2 has been explored as a novel approach to cancer therapy in patients with TP53 wild-type tumors.[4] These research efforts have yielded several MDM2–p53 protein–protein interaction (PPI) inhibitors, which have been or are currently still being evaluated in early clinical development.[5]

High-grade thrombocytopenia was reported for several MDM2–p53 inhibitors as a dose-limiting toxicity (DLT) in the clinic, in particular when testing continuous dose schedules.[5a, 6] To clinically manage thrombocytopenia, a next generation of MDM2–p53 inhibitors with the potency and pharmacokinetic properties to allow less-frequent dose schedules,[7] is needed. Our recently reported MDM2–p53 inhibitor BI-0252 (1) resulted in tumor regressions in all animals of a mouse SJSA-1 xenograft study with a single, but high oral dose of 100 mg kg^{-1}. To deliver a compound suitable to test less-frequent dose schedules in the clinic, we strived for further improvements in potency and pharmacokinetic properties of our MDM2–p53 inhibitors to decrease the required human dose on a less-frequent dose schedule. Herein we report the targeted syntheses of structurally complex and highly potent MDM2–p53 inhibitors with modified spiro-oxindole core structures, which were made accessible by employing unprecedented 1,3-dipolar cycloaddition chemistry.
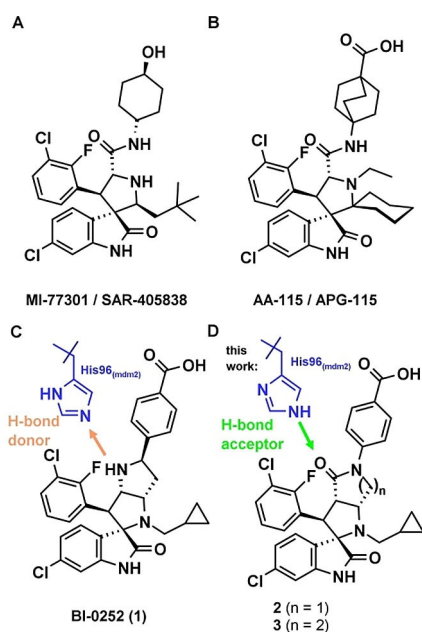
BI-0252 (1) is as a chemically stable and orally active inhibitor of the MDM2–p53 interaction which bears a spiro[3H-indole-3,2′-pyrrolidin]-2(1H)-one core structure.[8] In contrast to the pioneering spiro[3H-indole-3,3′-pyrrolidin]-2(1H)-ones initially reported by Wang et al. (Scheme 1 A)[9] and later by other groups,[10] which can undergo epimerization to four diastereomers via a retro-Mannich/Mannich reaction in solution,[11] Additional spiro-oxindole MDM2 inhibitors include clinical candidate DS-3032b[12] and others.[13] The new class of spiro[3H-indole-3,2′-pyrrolidin]-2(1H)-ones is not prone to this epimeri-

[a] Dr. A. Gollner,[+] Dr. H. Weinstabl,[+] Dr. J. E. Fuchs, Dr. D. Rudolph,
Dr. G. Garavel, K. S. Hofbauer, J. Karolyi-Oezguer, G. Gmaschitz, W. Hela,
Dr. N. Kerres, E. Grondal, P. Werni, Dr. J. Ramharter, Dr. J. Broeker,
Dr. D. B. McConnell
Boehringer Ingelheim RCV GmbH & Co. KG, Dr. Boehringer-Gasse 5-11A,
1121 Vienna (Austria)
E-mail: andreas.gollner@boehringer-ingelheim.com
          harald.weinstabl@boehringer-ingelheim.com

[+] These authors contributed equally to this work.

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
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Scheme 1. A) Clinical spiro[3*H*-indole-3,3′-pyrrolidin]-2(1*H*)-one inhibitor MI-77301/SAR-405838. B) Chemically stable clinical spiro-oxindole inhibitor AA-115/APG-115. C) Spiro[3*H*-indole-3,2′-pyrrolidin]-2(1*H*)-one BI-0252 (**1**). D) Novel spiro[indole-3,2′-pyrrolo[2,3-*c*]pyrrole]-2,4′-diones.
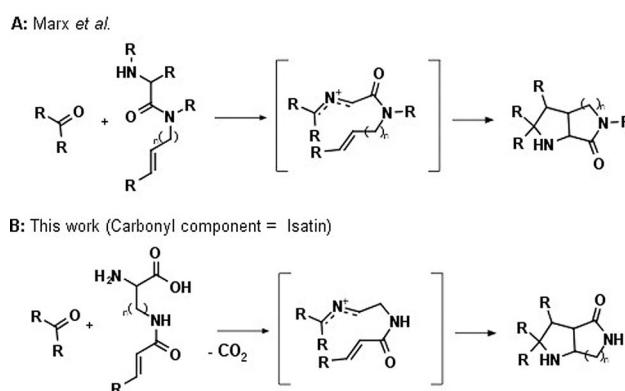
zation.[8] The problem of epimerization was also recently addressed by Aguilar et al. leading to chemically stable inhibitors and the clinical candidate AA-115/APG-115, Scheme 1 B.[14]

The X-ray co-crystal structure of **1** in MDM2 (PDB ID: 5LAZ) revealed a hydrogen bond between the basic secondary nitrogen of **1** and the side chain of His96 of the MDM2 protein as being important for the binding of **1** to MDM2 (Scheme 1 C, Figure 2 B).[8] In contrast many other MDM2–p53 inhibitors address His96 with a carbonyl oxygen functioning as hydrogen bond acceptor.[11b] Striving for further potency optimization we aimed at evaluating the influence of replacing the secondary amine (hydrogen bond donor) by a carbonyl group oxygen (hydrogen bond acceptor) in our lead series (Scheme 1 D). To test this hypothesis we designed the five-membered lactam analogue **2** (NH to C=O) and the six-membered lactam analogue **3** as close analogues of **1**.

1,3-Dipolar cycloadditions of azomethine ylides are a versatile tool for the generation of highly substituted pyrrolidines with dense stereochemistry.[15] We took advantage of this powerful method in our earlier study to generate the core structure of **1** by reacting 6-chloroisatin, 1-(2-fluoro-3-chlorophenyl)-2-nitroethene and L-homoserine in a three-component reaction.[8] For the synthesis of the new core structures the use of an intermolecular cycloaddition seemed less attractive, as published reports suggest that the outcome of such reactions using substituted styrene analogues as dipolarophiles with electron-withdrawing groups other than a nitro group would favor an undesired regioisomer.[16] We therefore envisioned an intramolecular cycloaddition which should favor the desired regioisomer.

Intramolecular cycloadditions of azomethine ylides have been used successfully to generate complex fused pyrrolidine,
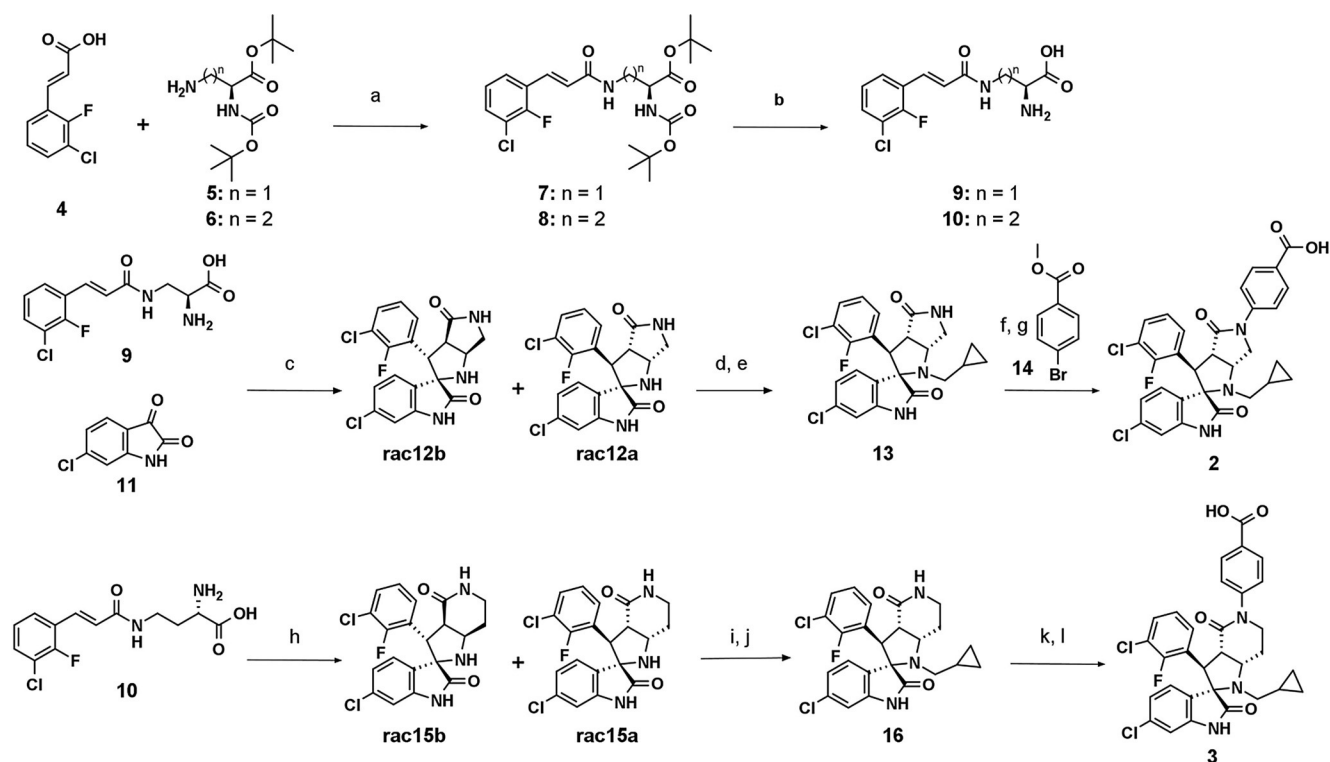
dihydropyrrole or pyrrole ring systems and are particularly valuable for the synthesis of natural products.[17] Marx et al. reported the usefulness of this method to generate novel polycyclic lactams for the design of screening libraries (Scheme 2 A)

Scheme 2. A) Application of intramolecular cyclization with azomethine ylides to access octahydropyrrolo[2,3-*c*]pyrrol-6-ones by Marx et al.[18] B) This work: novel approach to access complex octahydropyrrolo[2,3-*c*]pyrrol-4-ones (*n* = 1, 2).

and therein describes the reaction of isatin with 2-(methylamino)-N-phenyl-N-(prop-2-en-1-yl)acetamide which yielded a octahydro-1′*H*-spiro[indole-3,2′-pyrrolo[2,3-*c*]pyrrole]-2,6′-dione compound with undetermined stereochemistry in moderate yield.[18] To access the desired unprecedented octahydro-1′*H*-spiro[indole-3,2′-pyrrolo[2,3-*c*]pyrrole]2,4′-dione core structure we planned to modify the amine components to 2-amino-3-(prop-2-enamido)propanoic acids and 2-amino-3-(prop-2-enamido)butanoic acids (Scheme 2 B), which should readily generate the azomethine ylide after imine formation with an isatin analogue followed by subsequent decarboxylation. The ylide was expected to react with the remaining double bond to yield the desired product.

We started the synthesis by preparing the amino acid cyclization precursors **9** and **10** (Scheme 3) from commercially available 3-amino-N-(tert-butoxycarbonyl)-L-alanine tert-butyl ester (**5**) and (*S*)-2-tert-butoxycarbonylamino-4-aminobutyric acid tert-butyl ester (**6**) by amide coupling with (2*E*)-3-(3-chloro-2-fluorophenyl)prop-2-enoic acid (**4**). Both reactions proceeded in close to quantitative yields to give compounds **7** and **8**. To remove the Boc and tert-butyl protecting groups, compounds **7** and **8** were treated with trifluoroacetic acid in CH_2Cl_2 at RT, and after completion of the reactions amino acids **9** and **10**, respectively, were precipitated from water at pH 6–7 in excellent yields. With the cyclization precursors **9** and **10** now in hand, we performed the first decarboxylative cycloaddition by heating **9** with one equivalent of 6-chlorisatin (**11**) in methanol at 100 °C for 30 min in a microwave reactor.[19] We were able to isolate an inseparable mixture of the diastereomers *rac*-**12a** and *rac*-**12b** in 23 % yield and a diastereomeric ratio of 1:3. After reductive amination of this mixture with cyclopropylcarboxaldehyde we were able to isolate *rac*-**13** in quantitative yield based on the content of *rac*-**12a** which was separated by chiral SFC to obtain enantiomerically pure **13**.

Scheme 3. Synthesis of **2** and **3**: a) HATU (1.2 equiv), DIPEA (3.0 equiv), DMF, 20 min (for **7**), 1 h (for **8**), RT, (the crude products of **7** and **8** were used directly for the next step); b) TFA, 70 °C, 1 h, 85 % (for **9**, over two steps), 89 % (for **10**, over two steps); c) **11** (1.0 equiv), MeOH, 100 °C, microwave, 30 min, 23 % d.r. = 3:1 for *rac*-**12 b** and *rac*-**12 a** (the mixture of diastereomers was used directly for the next step); d) cyclopropanecarbaldeyde (5 equiv), AcOH, RT, 20 min, then NaBH(OAc)$_3$ (2 equiv), RT, 2 h, 100 % (based on content *rac*-**12 a**); e) chiral SFC separation to obtain **13**; f) **14** (1.5 equiv), Cs$_2$CO$_3$ (2 equiv), Xantphos (0.2 equiv), Pd(TFA)$_2$ (0.1 equiv), dioxane, 120 °C, microwave, 1 h, 45 %; g) NaOH (9.7 equiv), dioxane, 80 °C, 20 h, 86 %; h) **11** (1.0 equiv), MeOH, 100 °C, microwave, 30 min, 61 % d.r. = 1:1.7 for *rac*-**15 b** and *rac*-**15 a**; i) cyclopropanecarbaldeyde (5 equiv), AcOH, RT, 20 min, then NaBH(OAc)$_3$ (2 equiv), RT, 1 h, 86 %; j) chiral SFC separation of *rac*-**16**; k) **14** (1.5 equiv), Cs$_2$CO$_3$ (2 equiv), Xantphos (0.2 equiv), Pd(TFA)$_2$ (0.1 equiv), dioxane, 120 °C, microwave, 1 h, 44 %; l) NaOH (9.7 equiv), dioxane, 80 °C, 20 h, 91 %.

Buchwald coupling with methyl 4-bromobenzoate (**14**) and subsequent saponification delivered target compound **2**. Despite the low yield and unfavorable selectivity in the cycloaddition step we were able to obtain the complex polycyclic structure of **2** in only six synthetic steps to obtain sufficient material for biological testing.

For the synthesis of the six-membered lactam analogue **3** we reacted the cyclization precursor **10** with one equivalent of **11** under the same conditions and isolated the two diastereomers *rac*-**15 a** and *rac*-**15** in a yield of 61 % favoring the desired compound *rac*-**15 a** (d.r. = 1.7:1). Reductive amination of *rac*-**15 a** with cyclopropylcarboxaldehyde and subsequent chiral SFC separation gave compound **16**. Buchwald coupling of lactam **16** with methyl 4-bromobenzoate and saponification delivered compound **3** in good overall yields (Scheme 3).

To test whether the higher yield for the ylide intermediate **10** ($n = 2$) versus **9** ($n = 1$) is due to pre-organization we explored their conformational ensembles using a fine-grained systematic conformational search in MOE 2016.0802 (Figure 1). We enumerated accessible conformations in an energy window of 10 kcal mol^{-1} with a minimum pair-wise RMSD of 0.1 Å using the default AMBER10:EHT force field along with a dielectric constant of 32.7 resembling methanol. Resulting conformations were subsequently energy minimized on B3LYP-D3/

6-31G* level in implicit methanol solvation using Gaussian 09.[20] Resulting conformations were analyzed with respect to their compactness using the radius of gyration r_{gyr} as well as with respect to their RMSD to modelled intermediate conformations leading to desired and undesired reaction products.
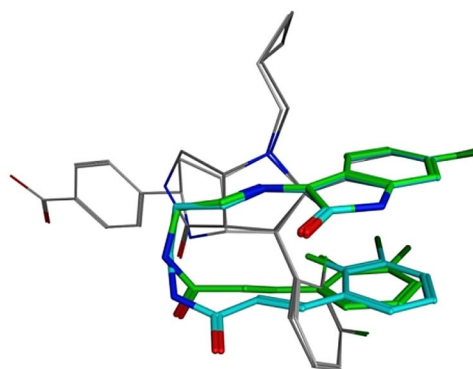

Figure 1. Superposition of **1** (white) and **13** (grey) extracted from crystal structures of MDM2 ligand complexes (PDB IDs: 5LAZ and 6I3S) with low-energy conformation for linker length $n = 1$ (green) and the lowest-energy conformation for $n = 2$ (cyan). We find a preorganization of the reaction educts in solution allowing an intramolecular cycloaddition starting from low-energy conformations.

For both linker lengths we found a large set of collapsed conformations within the respective ensemble that are pre-organized for an intramolecular cycloaddition. For $n=1$ we found 60 of 175 total conformations (34%) with $r_{gyr}<4$ Å which typically indicates stacking interactions between both π-systems in the ylide intermediate. For the molecule with linker length $n=2$, we similarly found 57 of 148 conformations with $r_{gyr}<4$ Å (39%). For compounds with $n=1$ collapsed conformations do not directly correspond to the minimum energy conformations but are very close with a strain energy of only $+0.7$ kcal mol^{-1}. Elongating the linkage to $n=2$ focusses the conformational ensemble specifically around the collapsed form. The lowest non-collapsed conformation is strongly unfavorable and only found at $+6.5$ kcal mol^{-1} in the latter case. These findings are consistent with the higher reaction yields obtained for educts with linker length $n=2$. In addition to the formation of pre-organized collapsed conformations of the ylide intermediates, we found a focusing of the conformations around structures leading to specific diastereomeric reaction products. Amongst all conformations with $r_{gyr}<4$ Å precursor conformations locking the lactam 5-ring in the undesired direction are found more often (60%) than in the desired conformation (40%). For the larger intermediates leading to the lactam 6-ring product we found only 51% of conformations pre-organized to form the undesired diastereomer versus 49% for the desired one. Both, dominance of the precursor to the undesired product for the 5-ring lactam as well as an almost equal distribution of precursor conformations for the 6-ring lactam are in line with the observed syntheses yields.

To evaluate the influence of replacing the secondary amine (hydrogen bond donor) by a carbonyl group (hydrogen bond acceptor) we measured biochemical (IC$_{50}$ (MDM2–p53)) and also the cellular potency in the p53 wild-type osteosarcoma SJSA-1 cell line proliferation assay (Table 1). Like **1**, compounds **2** and **3** showed low nanomolar potency in the MDM2–p53 assay (4 nM) which is at the assay-wall of this assay. We observed a significant improvement in the cellular potency when comparing compound **1** (471 nM) and the new five-membered lactam compound **2** (161 nM) while the six-membered lactam analogue **3** showed potency similar to **1**. This showed that for our

spiro-oxindole core structure compounds addressing His96$_{MDM2}$ with a carbonyl oxygen (hydrogen bond acceptor) are favored in terms of potency. To assess off-target selectivity we tested compounds **2** and **3** against the p53 mutant cell line SK-OV-3 which showed no effect on the growth of the p53 mutant SK-OV-3 cell line up to a concentration of 25 μM. This indicates that compounds **2** and **3** are selective MDM2–p53 PPI inhibitors similar to the data reported for **1**.

An X-ray crystal structure of compound **13** in MDM2 (Figure 1A) showed that the lactam carbonyl indeed forms a hydrogen bond interaction with the side chain of His96$_{MDM2}$. The overlay of the structure with the X-ray crystal structure of compound **1** (PDB ID: 5LAZ) shows a very similar binding mode with compound **13** slightly shifted to enable hydrogen bond formation with His96$_{MDM2}$ (Figure 2).
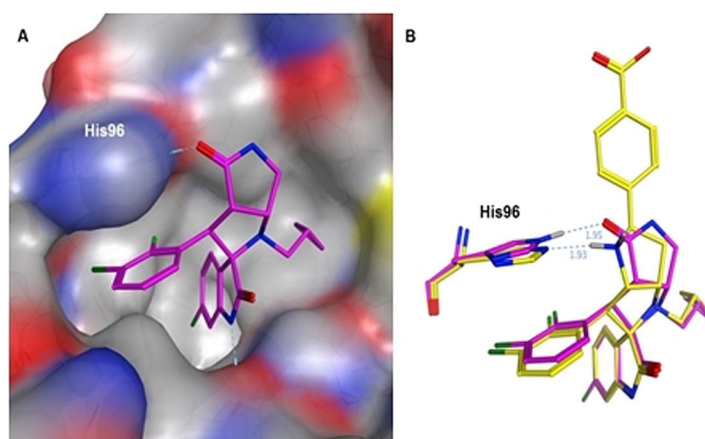

Figure 2. A) X-ray co-crystal structure of **13** (magenta) in MDM2. (The racemic compound *rac*-**13** was used for crystallization, only the eutomer **13** was found in the co-crystal structures (PDB ID: 6I3S). B) Overlay of X-ray co-crystal structure of **13** (magenta) in MDM2 with X-ray co-crystal structure of compound **1** (yellow) as observed in PDB ID 5LAZ.

In conclusion, we have developed an unprecedented intramolecular cyclization of azomethine ylides which enabled access to octahydropyrrolo[2,3-c]pyrrol-4-ones and octahydropyrrolo[2,3-c]pyridine-4-ones and allowed the targeted synthesis of structurally complex and highly functionalized spiro-oxindoles **2** and **3** in highly efficient 6-steps sequences. We investigated the pre-organization of the ylide intermediates of the cycloaddition reaction to rationalize the outcome of the experiments by computational methods which could facilitate future synthesis planning of intramolecular cycloadditions. The compounds were prepared to investigate the effect of introducing a hydrogen bond acceptor (lactam carbonyl oxygen) to our spiro-oxindole MDM2–p53 PPI inhibitors to address the side-chain of His96$_{MDM2}$ in comparison with the earlier compound BI-0252 (**1**) which carried a hydrogen bond donor at an equivalent position. Compound **2** displayed a threefold improvement in potency in the p53 wild-type osteosarcoma SJSA-1 cell line proliferation assay relative to **1**. This finding guided our optimization efforts toward hydrogen bond acceptors at this position and was an important milestone toward a MDM2–p53 PPI in-

Table 1. Enzymatic and cellular potency on SJSA-1 (p53 WT) and SK-OV-3 (p53 mutant) cell lines.

| Compd | IC$_{50}$ [nM][a] | | |
	MDM2–p53[b]	SJSA-1 CTG[c]	SK-OV-3 Alamar
1	4	471	>25 000
13	90	1272	>25 000
2	4	161	>25 000
3	4	547	>25 000

[a] Values are expressed as the mean of at least two measurements. [b] α-Assay. [c] CellTiter-Glo.

hibitor suitable to test less frequent dose schedules with the intention to manage thrombocytopenia in the clinic. In vivo profiling of **2** and additional compounds will be reported in due course.

Acknowledgements

Conflict of interest

Keywords: antitumor agents · azomethine ylides · cycloaddition · protein–protein interactions · spiro compounds

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