Structural correction of 3-Thiomorpholin-8-oxo-8*H*-acenaphtho[1,2-b]pyrrole-9-carbonitrile (**S1**) Based Molecules as dual Bcl-2/Mcl-1 inhibitors: *Re. J. Med. Chem: 2011, 54, 1101*

Ting Song, [†] Qingbin Chen, [‡] Xiangqian Li, [†] Gaobo Chai, [‡] and Zhichao Zhang, [*, †]

[†] School of Chemistry, Dalian University of Technology, Dalian 116012, People's Republic of China,

[‡] School of Life Science and Technology, Dalian University of Technology, Dalian 116024, People's Republic of China.

[*] To whom correspondence should be addressed. Phone: 86-411-84986032. Fax: 86-411-84986032. E-mail: zczhang@dlut.edu.cn.

Abstract

A structure-activity relationship (SAR) study of a novel series of Bcl-2/Mcl-1 dual inhibitors, **S1** and its derivatives was reported in *J. Med. Chem*, *2011, 54, 1101-1105*. Because the core structure of **S1** has recently been corrected based on the two-dimensional (2D) NMR and X-ray diffraction, we corrected **S1** and its derivatives. The reanalysis of the structure-activity relationship and the corrected binding mode of these inhibitors within the BH3-binding groove were reported.

Results

The precursor of **S1** was firstly synthesized by Qian's research group in 2005, which was assigned to have the backbone structure of 8-oxo-8*H*-acenaphtho[1,2-b]pyrrol-9-carbonitril (left, in Scheme 1).[1] The rigidity and strong ICT (intramolecular charge transfer) nature provide some of the derivatives good spectroscopic properties or DNA intercalating abilities.[2-4] However, **S1**'s non-DNA interaction but potent anticancer activity inspired us to explore its alternative anticancer targets. It was identified as a Bcl-2 inhibitor by us in 2007.[5] Until now, eight papers regarding pharmacological studies of **S1** in multiple human cancers including leukemia, breast cancer, lung cancer, and ovarian cancer were published.[6-13] These studies identified **S1** as an authentic BH3 mimetic acting completely through Bcl-2 signaling pathway. Consistent with these cell-based studies, the sub-μM binding affinities of **S1** toward Bcl-2, Mcl-1, and Bcl-xL were determined by isothermal titration calorimetry (ITC), fluorescence polarization assays (FPAs) and enzyme-linked immunosorbent assay (ELISA).[6, 14] A combination of SAR studies with molecular docking revealed the functional basis of **S1** that it binds into the BH3 grooves of Bcl-2 and Mcl-1 proteins.

This work was published in *J. Med. Chem*, *2011, 54, 1101-1105*. Further, this binding mode has been identified by ^{1}H-^{15}N HSQC spectrum on Mcl-1/**S1** complexes.[14]

Recently, Qian and Wang reassigned the backbone structure of **S1** by two-dimensional (2D) NMR and X-ray diffraction (right, in Scheme 1).[15, 16] The detailed synthesis methods and process are completely the same with our synthesis in this journal. The UV-Vis, MS and 1D NMR data are accordingly the same. Therefore, we confirmed that the compound **S1** we used in all the previous experiments, including *in vitro* biochemical assays and cell-based assays, actually has the backbone structure of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile.

In previous JMC report, we developed a series of more potent Bcl-2/Mcl-1 inhibitors **6a-i** from S_NAr^H products of **S1** backbone. The structures should also be revised here. The structural correction study has testified that the C_6 of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile is the most electrophilic carbon thus more favorable for S_NAr^H reaction through the crystal structure of 6-((4-methoxyphenyl)thio)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile.[15] The synthesis procedures of this compound are the same as we used to synthesize compounds **6a-i** in previous JMC. Accordingly, the structures of **6a-i** should be corrected as shown in Scheme 1.

Since some wrong structures were used in the three-dimensional docking and SAR study in the previous JMC, we reanalyzed the binding mode based on the corrected structures. The docking result of corrected structure of **S1** in complex with Mcl-1 showed a similar position and orientation with the previous one. The hydrogen bond is still able to form between the carbonyl group and the arginine 263 (R263) (Figure 1a), and the 6-position (numbered as 3-position in original report) group occupies p2 pocket. In addition, corrected **6f** was chosen to reanalyze SAR. Consistent with the previous report, the *N*, *N*-dimethylamino group could partly mimic the isobutyl group of L62 in the Bim BH3 peptide to occupy p2 pocket. Taken together, it seems that the differences in the backbone have little influence on SAR study, providing an explanation on why the previous docking gained supports from experimental data. Notably, the corrected structures provided a more comfortable position for hydrogen bond formation due to the shorter backbone (Figure 1a and b).

Conclusions

We corrected the structures of **S1** and its derivatives based on the recently reassigned backbone structure, and reanalyze the docking and SAR study of them as Bcl-2/Mcl-1 inhibitors. Based on

these corrected structures, the binding mode of **S1** and 6-substituted **S1** derivatives was determined. The carbonyl substitution of **S1** binds near R263 of Mcl-1 through a hydrogen bond, whereas the 6-position substituent extends into p2 pocket. This study provides a solid foundation for the further application of **S1** series compounds on both anticancer study and novel inhibitors development, as long as the structures could be determined with the combination of different 2D NMR techniques rather than the only MS and 1D NMR data.

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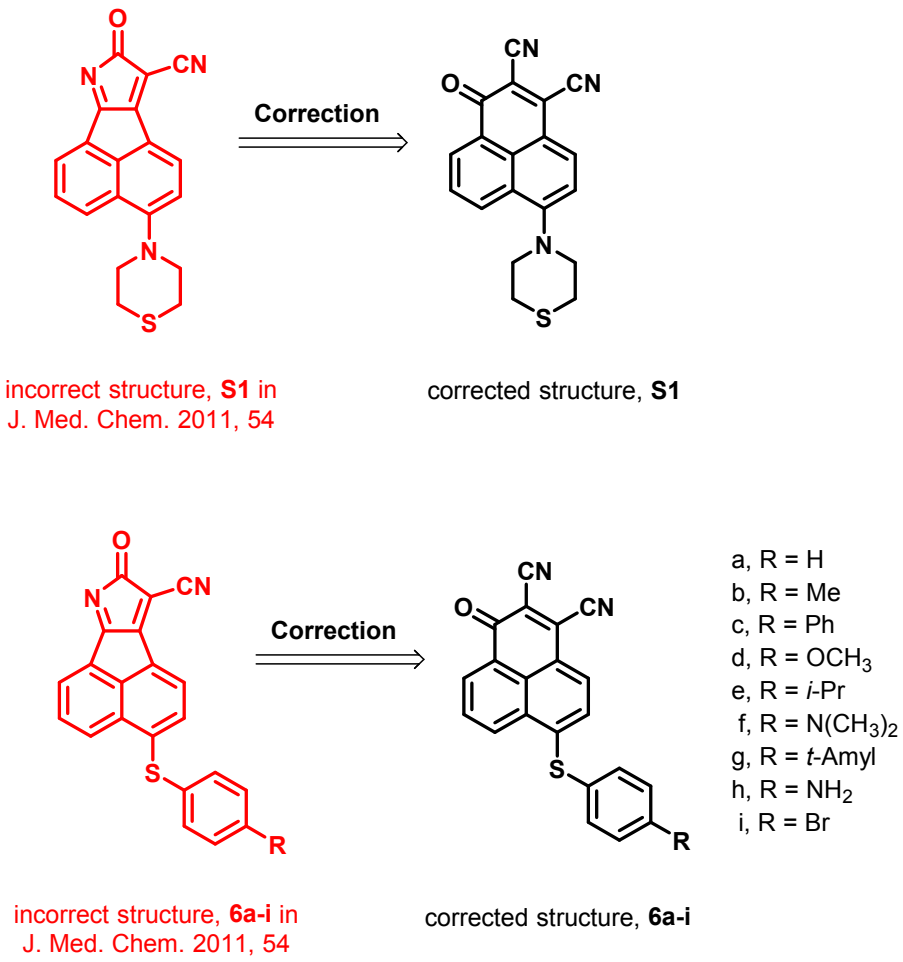

Scheme 1. Comparison of the corrected structures **S1**, **6a-i** with the mistakenly assigned structures in literature.

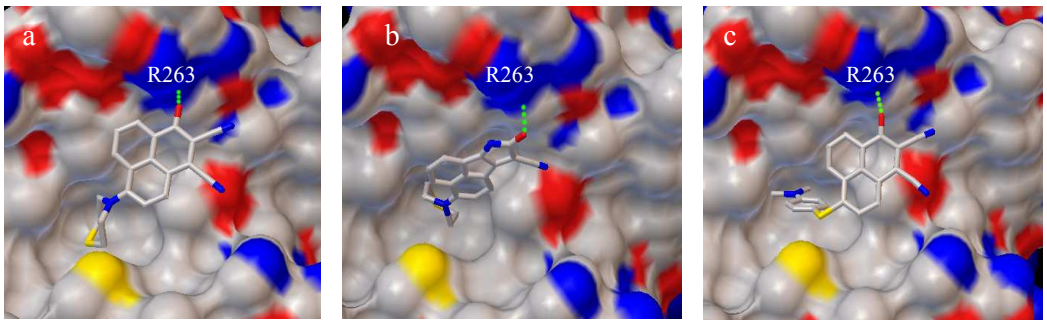

Figure 1. Binding model of the corrected structure of **S1** (a) and the mistakenly assigned one (b) in complex with Mcl-1. (c) Binding model of corrected structure of **6f** in complex with Mcl-1. The carbon, oxygen, nitrogen, and sulfur atoms of Mcl-1 and indicated compounds are shown in gray, red, blue, and yellow, respectively. Hydrogen bonds are depicted as green dashed lines.