Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2023, **33**, 67–69

Mendeleev Communications

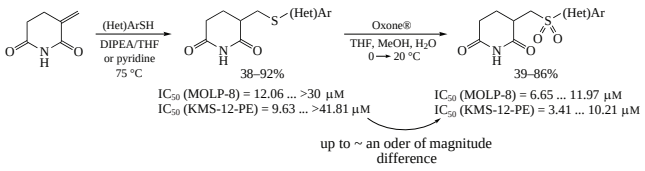
Synthesis of novel glutarimide derivatives *via* the Michael addition of (hetero)aromatic thiols: pronounced effect of sulfur oxidation on cytotoxicity towards multiple myeloma cell lines

Maria Adamchik,[a] Andrey Bubyrev,[a] Daniil Zhukovsky,[a,†] Petr Zhmurov,[a,‡] Alexander Bunev[b] and Mikhail Krasavin*[a,c]

[a] *St. Petersburg State University, 199034 St. Petersburg, Russian Federation. E-mail: m.krasavin@spbu.ru*
[b] *Medicinal Chemistry Center, Togliatti State University, 445020 Togliatti, Russian Federation*
[c] *Immanuel Kant Baltic Federal University, 236016 Kaliningrad, Russian Federation*

DOI: 10.1016/j.mencom.2023.01.021

2-Methylidenepiperidine-2,6-dione was employed as a substrate in the thio-Michael addition of a series of (hetero)-aromatic thiols. Oxidation of the resulting (hetero)arylthio derivatives with Oxone® gave the corresponding sulfones. Testing of the latter against multiple myeloma cell lines MOLP-8 and KMS-12-PE alongside with selected precursor sulfides confirmed the earlier observed significantly higher cytotoxicity of sulfones.



Keywords: 2-aminoglutarimide, thio-Michael reaction, Oxone® oxidation, sulfones, Cereblon, stronger binding, hydrogen bonding, cytotoxicity, multiple myeloma.

The immunomodulatory imide drugs (IMiDs) thalidomide, lenalidomide and pomalidomide are clinically used for the treatment of multiple myeloma [Figure 1(*a*)].[1] They exert their effect through the interaction with Cereblon (CRBN), a substrate receptor subunit of the E3 ubiquitin ligase complex CRL4.[2] Binding of IMiDs to CRBN leads to stronger binding of transcription factors IKZF1/3 to CRBN, their ubiquitination and, ultimately, proteasomal degradation. The end-point of IMiD action is the growth inhibition of multiple myeloma cells.[3] Binding of IMiDs to CRBN is also central to the functioning of the so-called proteolysis targeting chimeras (PROTACs), heterobifunctional small molecules consisting of ligands of a protein of interest (POI) and an E3 ubiquitin ligase (CRBN) connected by a linker.[4] PROTACs act catalytically by bringing the POI and CRBN together in a ternary complex. This leads to the ubiquitination and ultimate proteolytic degradation of the POI by the proteasome.[5] Such highly specific, targeted protein degradation is a fundamentally novel platform for pharmacological intervention [see Figure 1(*b*)].[6] Yet, thalidomide and its derivatives are not devoid of potential teratogenic effects, which hampers their use in the PROTAC design and promotes the extensive search for alternative CRBN ligands.[7–9] While the glutarimide portion of IMiDs is essential for CRBN binding, the search for CRBN ligands is focused on 2-substituted glutarimides,[10] starting from readily available glutarimide building blocks.[11] Recently, we developed[12] a series of simple glutarimide derivatives **1** bearing an (arylthio)methyl group in position 2 of glutarimide cycle. The series displayed specific binding to CRBN as confirmed by their affinity to the thalidomide-binding domain of human CRBN using the recently developed microscale thermophoresis assay.[13] Derivatives were also investigated for their antiproliferative effects against multiple myeloma cell lines and displayed moderate ($IC_{50} \sim 10^{-5}$ M) to weak ($IC_{50} \sim 10^{-4}$ M) cell growth inhibition. However, when the sulfur atom in an exemplary compound **1** was oxidized to obtain the corresponding sulfone **2**, the growth inhibition increased markedly ($IC_{50} \sim 10^{-6}$ M). Seeking to identify the basis for such an improvement of the anti-myeloma effect observed on sulfur atom oxidation, we obtained and solved a crystal structure of sulfone **2** (Ar = Ph) bound to CRBN, the target through which multiple myeloma cell growth inhibition is exerted. The X-ray structure revealed that the sulfone moiety was engaged in additional hydrogen bond acceptor interaction with W99 and N50 residues [see Figure 1(*c*)], which was deemed the reason for the stronger effect of sulfone **2** on multiple myeloma cells, compared to the relative sulfide **1**. Encouraged by this finding, we set off to prepare and investigate *in vitro* anti-myeloma efficacy of a wider range of sulfones **2** bearing aromatic and heteroaromatic moieties and compare them with selected precursor sulfides **1** to establish if the stronger antiproliferative activity of the former compared to that of the latter is a sustained phenomenon. Herein, we present the results of this investigation.

3-Methylidenepiperidine-2,6-dione **3** which served as a starting material for the preparation of all glutarimide analogues investigated in this work was prepared on multigram scale as described in the literature.[14] Thio-Michael addition of various thiophenols was performed in THF at 75 °C in the presence of DIPEA to afford compounds **1a**–**h** in good to excellent yields (Scheme 1). Sulfur oxidation with Oxone® gave the corresponding sulfones **2a**–**h** in excellent yields.

† Current address: Research & Development Department, BratskChemSyntez LLC, PharmaSyntez Company, 5A/1 Kommunalnaya St., 665717 Bratsk, Russian Federation.
‡ Current address: School of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH Scotland, United Kingdom.

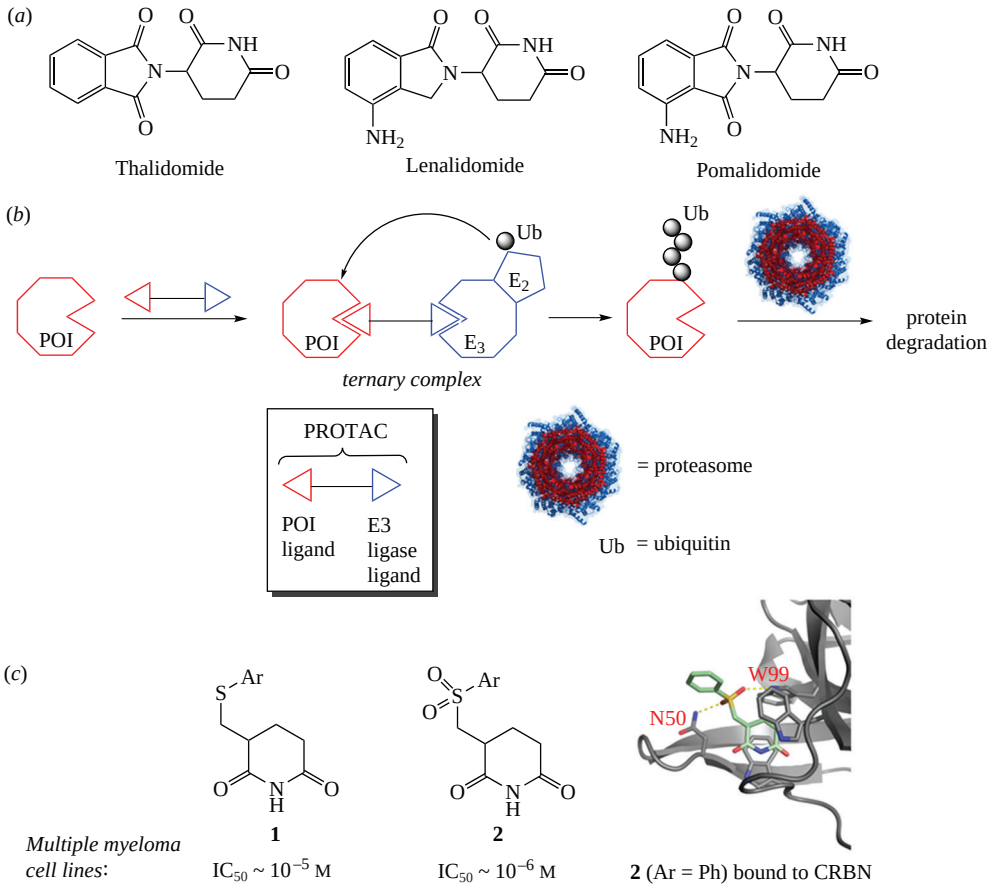
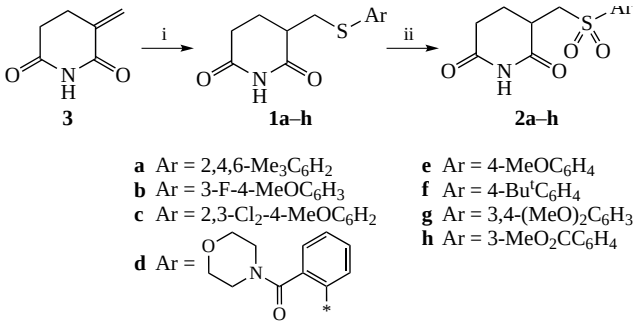
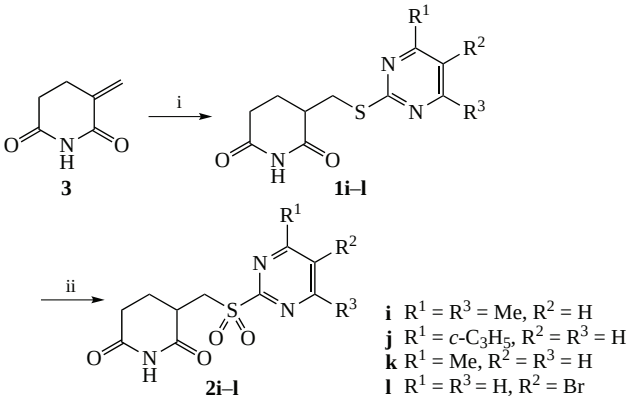


Figure 1 (*a*) Structures of the clinically used IMiDs; (*b*) the principle of PROTAC functioning; (*c*) glutarimide derivatives investigated earlier[12] and in this work.



Scheme 1 *Reagents and conditions*: i, ArSH, DIPEA, THF, 75 °C (66–92%); ii, Oxone®, THF, MeOH, H_2O, 0 °C to room temperature (45–86%).



Scheme 2 *Reagents and conditions*: i, HetArSH, pyridine, 75 °C (38–44%); ii, Oxone®, THF, MeOH, H_2O, 0 °C to room temperature (35–47%).

The same procedure applied to electron-deficient heterocyclic thiols (pyrimidine-2-thiols) was found ineffective and furnished unacceptably low yields. However, the thio-Michael addition reaction performed in pyridine as the reaction medium gave the target compounds **1i–l** in moderate yields. Sulfur oxidation with Oxone® furnished sulfones **2i–l** in excellent yields (Scheme 2).

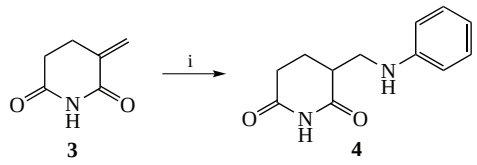
As described previously,[12,15] we selected MOLP-8[16] and KMS-12-PE[17] multiple myeloma cell lines to evaluate the compounds' antiproliferative properties (Table 1).

The data presented in Table 1 confirmed the earlier observed enhancement of antiproliferative activity through sulfur oxidation (**1** → **2**) as in nearly all cases sulfones **2** displayed more potent multiple myeloma cell growth inhibition compared to sulfides **1**. This difference, however, appears to be sensitive to substitution type in the (hetero)aromatic ring. Specifically, placing hydrogen bond accepting groups such as carboxamide (**1d**/**2d**) or, to a lesser extent, carboxylic ester (**1h**/**2h**) (which can, presumably, interfere with the hydrogen bonding of the sulfone linkage with CRBN, *vide supra*) leads to a significantly smaller (and negligible in case of pair **1d**/**2d**) difference in the antiproliferative properties of sulfides and sulfones.

The obvious influence of the nature of the linker between the aromatic moiety and the glutarimide portion in the compounds investigated on their antiproliferative properties made us to hypothesize that replacing a hydrogen bond accepting linker such as sulfide and, especially, sulfone with hydrogen bond donating group such as N-H may have a pronounced effect on the cancer cell growth inhibition profile. To test this hypothesis, we synthesized anilino-substituted compound **4** (Scheme 3) and tested its antiproliferative properties against multiple myeloma cell lines. Indeed, the compound turned out to be virtually

Table 1 Antiproliferative activity of compounds **1a–l** and **2a–l** against MOLP-8 and KMS-12-PE multiple myeloma cell lines.

Compound	IC_{50}/μM[a]		Compound	IC_{50}/μM[a]	
	MOLP-8	KMS-12-PE		MOLP-8	KMS-12-PE
1a	21.02±0.97	23.05±3.60	**2a**	7.25±0.41	6.46±0.66
1b	>30	35.47±7.66	**2b**	8.93±0.61	6.13±0.50
1c	20.68±5.11	16.28±1.72	**2c**	7.79±0.39	4.96±0.78
1d	20.68±3.53	33.51±10.02	**2d**	9.31±0.87	7.07±0.93
1e	23.25±5.81	36.65±7.61	**2e**	7.89±0.33	5.97±1.26
1f	n.d.	n.d.	**2f**	7.64±0.58	8.90±0.98
1g	12.06±1.79	17.27±2.35	**2g**	11.97±2.12	10.21±0.63
1h	>27	37.46±8.79	**2h**	7.39±1.51	4.52±0.94
1i	n.d.	n.d.	**2i**	6.65±1.34	5.49±0.66
1j	>30	25.73±2.59	**2j**	6.95±1.71	5.47±0.30
1k	22.48±4.85	41.81±8.17	**2k**	11.71±0.99	5.45±0.23
1l	19.46±4.66	9.63±2.39	**2l**	7.34±1.5	3.41±0.74



Scheme 3 *Reagents and conditions*: i, $PhNH_2$, DIPEA, THF, 75 °C (50%).

inactive against either MOLP-8 (IC_{50} > 60 μM) or KMS-12-PE cells (IC_{50} > 100 μM).

In conclusion, we have demonstrated that the earlier described (hetero)arylthiomethyl-substituted glutarimides possess significantly weaker antiproliferative properties towards multiple myeloma cell lines compared to their sulfone counterparts. This difference was observed for a wider range of aromatic groups and is thought to have to do with the hydrogen bond accepting properties of the sulfone linkage. Placing hydrogen bond accepting groups in the aromatic portion was shown to nearly level out the difference in the antiproliferative activity of sulfones and sulfides. Replacing the hydrogen bond accepting sulfide or sulfone linkage with an N-H group produced glutarimides completely devoid of activity towards the same cancer cell lines. These findings enhance our knowledge base for the design of glutarimide ligands of E3 ubiquitin ligase Cereblon.

This research was supported by the Russian Science Foundation (grant no. 22-13-00005). We thank the Research Center for Magnetic Resonance, the Center for Chemical Analysis and Materials Research, and Research Resource Center for Molecular and Cell Technologies of Saint Petersburg State University Research Park for obtaining the analytical data.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.01.021.

References

1 H. Quach, D. Ritchie, A. K. Stewart, P. Neeson, S. Harrison, M. J. Smyth and H. M. Prince, *Leukemia*, 2010, **24**, 22.
2 E. S. Fischer, K. Böhm, J. R. Lydeard, H. Yang, M. B. Stadler, S. Cavadini, J. Nagel, F. Serluca, V. Acker, G. M. Lingaraju, R. B. Tichkule, M. Schebesta, W. C. Forrester, M. Schirle, U. Hassiepen, J. Ottl, M. Hild, R. E. J. Beckwith, J. W. Harper, J. L. Jenkins and N. H. Thomä, *Nature*, 2014, **512**, 49.
3 J. Krönke, N. D. Udeshi, A. Narla, P. Grauman, S. N. Hurst, M. McConkey, T. Svinkina, D. Heckl, E. Comer, X. Li, C. Ciarlo, E. Hartman, N. Munshi, M. Schenone, S. L. Schreiber, S. A. Carr and B. L. Ebert, *Science*, 2014, **343**, 301.
4 B. Coll-Martinez, A. Delgado and B. Crosas, *Molecules*, 2020, **25**, 5956.
5 M. Scheepstra, K. F. W. Hekking, L. van Hijfte and R. H. A. Folmer, *Comput. Struct. Biotechnol*., 2019, **17**, 160.
6 M. Békés, D. R. Langley and C. M. Crews, *Nat. Rev. Drug Discov*., 2022, **21**, 181.
7 M. Krasavin, A. Bubyrev, A. Kazantsev, C. Heim, S. Maiwald, D. Zhukovsky, D. Dar'in, M. D. Hartmann and A. Bunev, *J. Enzyme Inhib. Med. Chem*., 2022, **37**, 527.
8 A. Kazantsev and M. Krasavin, *Exp. Opin. Ther. Pat.*, 2022, **32**, 171.
9 A. Bricelj, C. Steinbach, R. Kuchta, M. Gütschow and I. Sosič, *Front. Chem.*, 2021, **9**, 707317.
10 L. J. Alcock, Y. Chang, J. A. Jarusiewicz, M. Actis, S. Nithianantham, A. Mayasundari, J. Min, D. Maxwell, J. Hunt, B. Smart, J. J. Yang, G. Nishiguchi, M. Fischer, C. G. Mullighan and Z. Rankovic, *ACS Med. Chem. Lett.*, 2022, **13**, 475.
11 I. Boichenko, K. Bär, S. Deiss, C. Heim, R. Albrecht, A. N. Lupas, B. Hernandez Alvarez and M. D. Hartmann, *ACS Omega*, 2018, **3**, 11163.
12 M. Krasavin, M. Adamchik, A. Bubyrev, C. Heim, S. Maiwald, D. Zhukovsky, P. Zhmurov, A. Bunev and M. D. Hartmann, *Eur. J. Med. Chem.*, 2023, **246**, 114990.
13 S. Maiwald, C. Heim, B. Hernandez Alvarez and M. D. Hartmann, *ACS Med. Chem. Lett.*, 2021, **12**, 74.
14 M. J. Wanner and G. J. Koomen, *Synthesis*, 1988, 325.
15 M. Krasavin, A. Bubyrev, A. Kazantsev, C. Heim, S. Maiwald, D. Zhukovsky, D. Dar'in, M. D. Hartmann and A. Bunev, *J. Enzyme Inhib. Med. Chem.*, 2022, **37**, 527.
16 Y. Matsuo, H. G. Drexler, A. Harashima, A. Okochi, A. Hasegawa, K. Kojima and K. Orita, *Leuk. Res.*, 2004, **28**, 869.
17 T. Ohtsuki, Y. Yawata, H. Wada, T. Sugihara, M. Mori and M. Namba, *Br. J. Haemotol.*, 1989, **73**, 199.

Received: 24th June 2022; Com. 22/6940