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# Novel 2-amino-substituted (thio)morpholine-3,5-diones: synthesis and cytotoxicity studies

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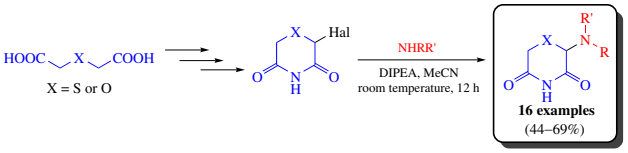
**The present study explores the synthesis of new potential Cereblon ligands, 2-amino derivatives of (thio)morpholine-3,5-diones. The resulting compounds demonstrate notable cytotoxic effects in tests conducted *in vitro* on myeloma cell lines, highlighting the potential of these compounds as molecular glues.**




*Keywords*: thiomorpholine-3,5-dione, morpholine-3,5-dione, amines, halogenation, nucleophilic substitution reaction, cytotoxicity studies, immunomodulatory drugs, Cereblon.

The 3-aminoglutarimide fragment is the basis of many immunomodulatory drugs (IMiDs) with anti-angiogenic and anti-inflammatory properties (Figure 1).[1] The first-in-class IMiD, thalidomide, was approved by the US Food and Drug Administration (FDA) in 2006 for the treatment of multiple myeloma (MM).[2] The ability of IMiDs to bind to CRBN is also the basis for the creation of proteolysis targeting chimeras (PROTACs).[3] This process facilitates ubiquitination and ultimately the proteolytic degradation of the POI by the proteasome.[4,5] This strategy, widely known as targeted protein degradation, shows great promise as a potential substitute for small molecule inhibitors and other methods that decrease atypical protein levels or activity.[5] Finding new potential IMiDs is therefore undoubtedly the most important area of modern medicinal chemistry.

Most of the currently known IMiDs contain glutarimide moiety, which is essential for Cereblon (CRBN) binding. In this study, we have prepared new glutarimide analogues, 2-amino derivatives of thiomorpholine-3,5-dione and morpholine-3,5-dione, as potential CRBN ligands. We also evaluated the cytotoxic properties of the compounds obtained.

The synthesis began with thiodiglycolic **1a** and diglycolic **1b** acids as the starting materials (Scheme 1). Their cyclic imides

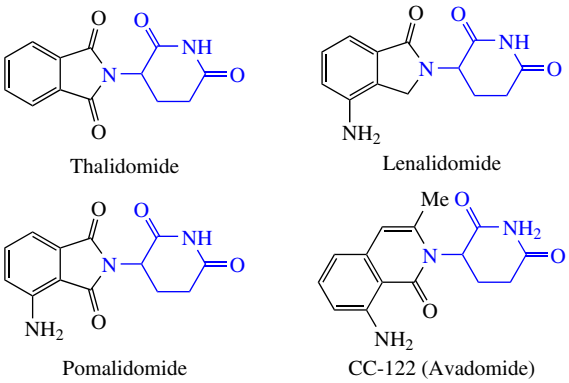
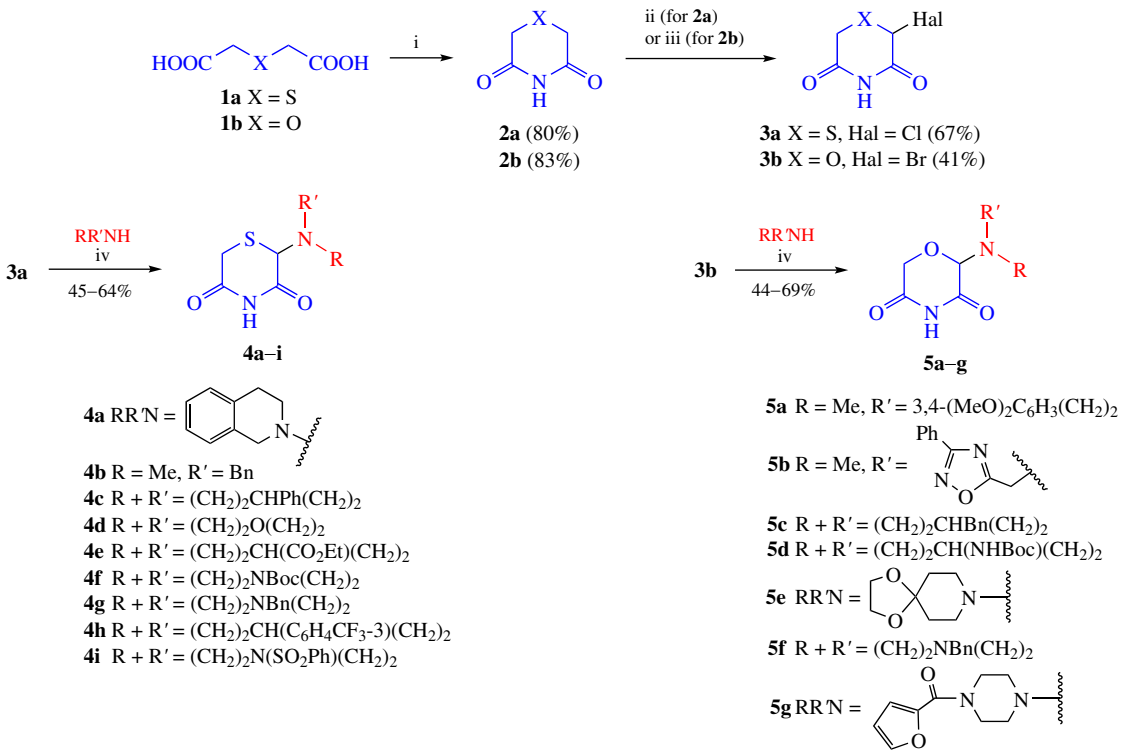



**Figure 1** Structures of some immunomodulatory drugs (IMiDs).

**2a**,**b** were synthesized using the previously described procedure.[6] In the next stage, 2-halo derivatives of **3a**,**b** were obtained by the reaction of **2a**,**b** with halogenating agents. Only one example of chlorination of compound **2a** in low yield by treatment with $PCl_5$ in boiling toluene has been reported.[7] We have proposed carrying out this synthesis under milder conditions, namely, with the use of $SO_2Cl_2$ in $CH_2Cl_2$ at room temperature. Slow addition of $SO_2Cl_2$ to the reaction mixture over 5–6 h provided 67% yield of product **3a**. Unfortunately, oxygen analogue **2b** did not react with $SO_2Cl_2$, even after boiling the reaction mixture for 12 h. Luckily, the synthesis of bromo derivative **3b** could be performed by reacting **2b** with $Br_2$ in a $CHCl_3$ solution at 100 °C under irradiation with a 250 W lamp in a screw vial. Product **3b** was obtained with the yield of 41% after purification. In the final stage, the halogen atom in compound **3a**,**b** was replaced by an amino group. The reactions of **3a**,**b** with secondary amines were carried out in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in MeCN solution at room temperature to afford amino-substituted thiomorpholinediones **4a**–**i** and morpholinediones **5a**–**g** in reasonable yields (see Scheme 1).

The structures of products **4** and **5** were confirmed based on $^1$H and $^{13}$C NMR spectroscopy data and high-resolution mass spectrometry. The $^1$H NMR spectra recorded in $CDCl_3$ contained the characteristic signal for the imide proton as a broad singlet in the region of 7.73–8.90 ppm. The methine CHN proton resonated as a singlet at 4.67–4.98 ppm (for **5a**–**g**) or 4.26–4.53 ppm (for **4a**–**i**); signals for the methylene protons appeared as two doublets ($J$ = 16.9–17.1 Hz) at 4.48–4.59 and 4.20–4.30 ppm (for **5a**–**g**) or at 3.73–3.82 and 3.36–3.54 ppm (for **4a**–**i**). The $^{13}$C NMR spectra recorded in $CDCl_3$ contained two signals for imide carbonyl groups at 168.8–170.0 and 167.0–168.3 ppm; the signal for the methine C*H*N carbon appeared at 90.1–90.9 ppm (for **5a**–**g**) or at 67.5–68.8 ppm (for **4a**–**i**) while the methylene group signals were observed at 64.5–65.2 ppm (for **5a**–**g**) or 28.8–30.0 ppm (for **4a**–**i**).

We conducted *in vitro* assays to test the anti-myeloma activity of all synthesized derivatives **4** and **5**. According to the findings presented in Table 1, all compounds exhibited




**Scheme 1** *Reagents and conditions*: i, $(NH_4)_2CO_3$, 230 °C, 5 h; ii, $SO_2Cl_2$, $CH_2Cl_2$, room temperature, 8 h; iii, $Br_2$, $CHCl_3$, *hv*, 100 °C, 4 h; iv, DIPEA, MeCN, room temperature, 12 h.

**Table 1** Cytotoxicity studies of 2-amino-substituted (thio)morpholin-3,5-diones **4** and **5**.

| Compound | Survival (%) | |
|---|---|---|
| | KMS-12-PE | MOLT-4 |
| **4a** | 7.59 ± 4.02 | 16.03 ± 4.73 |
| **4b** | 7.00 ± 4.42 | 16.37 ± 1.37 |
| **4c** | 8.20 ± 6.4 | 16.57 ± 9.69 |
| **4d** | 11.01 ± 3.27 | 30.77 ± 1.39 |
| **4e** | 9.13 ± 4.37 | 13.13 ± 0.54 |
| **4f** | 15.00 ± 4.35 | 37.06 ± 12.96 |
| **4g** | 8.96 ± 5.55 | 26.70 ± 3.31 |
| **4h** | 11.02 ± 3.62 | 18.34 ± 2.31 |
| **4i** | 8.58 ± 4.52 | 20.14 ± 2.86 |
| **5a** | 12.62 ± 4.39 | 16.58 ± 3.32 |
| **5b** | 15.68 ± 6.72 | 22.97 ± 1.23 |
| **5c** | 18.70 ± 3.28 | 38.87 ± 4.88 |
| **5d** | 10.29 ± 9.93 | 20.06 ± 0.81 |
| **5e** | 19.31 ± 5.01 | 28.88 ± 2.43 |
| **5f** | 17.28 ± 4.56 | 24.27 ± 3.31 |
| **5g** | 14.69 ± 4.91 | 27.42 ± 1.89 |
| Pomalidomide | 3.43 ± 0.87 | 18.34 ± 2.44 |

noticeable cytotoxicity when tested at a concentration of 30 μM during a 72-hour treatment of KMS-12-PE and MOLT-4 cell lines. It is noteworthy that the thiomorpholine derivatives **4a–i** demon-strated higher toxicity. This observation highlights the need for a more in-depth investigation into the nature of this activity, specifically exploring the potential of the synthesized compounds as molecular glues for the transcription factors IKZF1/3.

In conclusion, we have developed a convenient method for preparing 2-amino derivatives of (thio)morpholine-3,5-diones involving the stage of halogenation of cyclic imides and the nucleophilic substitution reaction to replace the halogen atom by the amino group. This allowed us to obtain target compounds in 44–69% yields. All of the synthesized compounds showed significant cytotoxicity against the myeloma cell lines KMS-12-PE and MOLT-4. This study demonstrates the potential for these compounds to be used as molecular glue for the IKZF1/3 transcription factors. These factors play a crucial role in the proliferation and survival of myeloma cells, making this investigation even more significant.

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In commemoration of the 300th anniversary of St. Petersburg State University's founding.

*Online Supplementary Materials*

Supplementary data associated with this article (experimental procedures, analytical data and copies of $^1H$ and $^{13}C$ NMR spectra) can be found in the online version at doi: 10.1016/j.mencom.2024.02.032.

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