Synthesis of a Sulfonimidamide-Based Analog of Tasisulam and Its Biological Evaluation in the Melanoma Cell Lines SKMel23 and A375

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Keywords

A375 cell line · Antiproliferative effect · LY573636 · SKMel23 cell line · Sulfonimidamide · Tasisulam · Melanoma · Chemotherapy

Abstract

Tasisulam is a promising antitumor agent with complex pharmacology, which is used as an antiproliferative agent in patients with metastatic melanoma and other solid tumors. Phase 2 melanoma studies showed promising results but had to be stopped because of insufficient tasisulam clearance leading to toxic side effects. To reduce the negative effects of tasisulam, we synthesized a novel sulfonimidamide-based analog to evaluate its antiproliferative effects in comparison to the original compound by performing a cell proliferation assay in melanoma cell lines SKMel23 and A375. The results revealed that the analog had inhibitory effects on the proliferation comparable to tasisulam in both investigated cell lines. These results could contribute to a reduced toxicity of tasisulam and lead to further clinical trials in metastatic melanoma.

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Anne-Dorothee Steinkamp and Laurenz Schmitt contributed equally to this work.

Introduction

Although comprising only approximately 4% of all skin cancer cases, melanoma is still responsible for almost 80% of skin cancer deaths [1, 2]. The combination of targeted therapy with BRAF and MEK inhibitors has become the standard of care in patients with BRAF (V600E) mutant melanoma and can be used in about one half of patients but they mostly develop resistance a few months after initiation [3–5]. Approximately 19–58% of patients with metastasis of melanoma benefit from a systemic immunotherapy, depending on mono- or combination therapy [6, 7]. Ipilimumab targets the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) receptor whereas nivolumab and pembrolizumab target programmed cell death protein 1 (PD-1) receptors, which has been proven to be superior to ipilimumab alone. The combination of ipilimumab and nivolumab has yielded higher response rates, greater tumor shrinkage, and longer progression-free survival than monotherapy alone [7–9]. However, novel effective therapies for patients with metastatic melanoma are required but have remained scarce. Especially in chemotherapy new developments and insights are missing. A phase 2 study of tasisulam achieved partial response, with an objective response rate (RR) of 11.8% in 8 of 68 patients with

Fig. 1. Overview of structures related to sulfonimidamides.

second-line metastatic melanoma [10]. Tasisulam is an acyl sulfonamide with a unique antitumor dual mechanism of action, involving antiangiogenesis and mitotic inhibition [11]. An *in vitro*, antitumor screening analysis conducted by the National Cancer Institute (NCI COMPARE analysis) demonstrated a broad range of activity for tasisulam in 60 tumor cell lines including leukemia, melanoma, non-small-cell lung cancer, colon, ovarian, renal, and breast cancers [12]. Subsequently, tasisulam was evaluated as a single agent in several tumor types [10, 13–16]. In this study we focused on the antiproliferative effect of tasisulam and a newly synthesized sulfonimidamide-based analog, which both were studied in melanoma cell lines *in vitro*.

It is already known that isosteric replacements in bioactive effector molecules are of interest in medicinal chemistry [17]. Along these lines, we focused on the formal conversion of a sulfone into a sulfoximine through substitution of a sulfonyl group by a sulfoximido group (Fig. 1) [18]. As a result, various compounds with good cyclooxygenase-2 inhibition potency were obtained [19–21]. In addition, we studied sulfoximine-based analogs of zolimidine [22], dapsone [23], and S0859 [24]. The most advanced examples stem from industry, where Bayer-Pharma [25] and AstraZeneca [26] have promoted kinase inhibitors with sulfoximido groups in their respective anticancer programs to clinical trials [27]. Switching from a sulfone to a sulfoximine allows retaining the drug effectiveness while improving the overall bioactivity profile by positively affecting solubility [28] and ADME properties [29].

Sulfonamides are important compounds in medicinal chemistry [30–40], and, in particular, N-acylated derivatives have revealed interesting antitumor activities in combination with attractive pharmacokinetics [41, 42]. As surrogates for sulfonamides, the corresponding sulfonimidamides have lately been promoted (Fig. 1). For example, Sehgelmeble et al. [43] found significant potencies when profiling sulfonimidamide-based analogs of sulfonamidic γ -secretase inhibitors. Furthermore, N-acylated sulfonimidamides were shown to have bioisosteric properties to carboxylic acid [44–49].

Although the first sulfonimidamide syntheses date back to the 1960s [50–55], the preparative chemistry related to such compounds has remained rather underdeveloped mainly due to the difficulties in accessing appropriately substituted core structures. Only recently have Arvidsson and others significantly advanced the field by applying modern catalytic protocols for sulfonimidamide modifications [56–60]. With respect to applications of sulfonimidamides as reagents in synthesis and catalysis, the early work by the groups of Malacria [61–63] and Dodd [64–67] is noteworthy. We made use of sulfonimidamides in organo- [68] and metal catalysis [69, 70], which, in part, required the development of new synthetic protocols [71–75].

Tasisulam (compound 1, LY573636) is an N-acyl sulfonamide with antiproliferative activity developed by Eli Lilly [11, 41, 42]. In various tumor xenograft models, it showed potent efficacy *in vivo* inducing apoptosis and exhibiting antiangiogenesis activity. Being considered as a promising anticancer agent [13, 76], a robust process was introduced providing sufficient material for the initiated clinical trials [77, 78]. Due to safety concerns, however, the compound development was terminated, which also affected a phase 3 study focused on tasisulam treatments of patients with metastatic melanoma [79].

To the best of your knowledge, the sulfonimidamide analog of tasisulam (compound 1), i.e. compound 2, has neither been prepared nor screened on bioactivity. (Note that our first experiments were performed in April 2014. On November 19, 2014, a paper on sulfonimidamide chemistry by Wakchaura et al. [58] appeared online, which seemed to indicate that they were thinking along the same lines.) In the light of the interesting cytotoxic properties of the parent compound and the expertise of our group in sulfur chemistry, we decided to prepare sulfonimidamide compound 2 and to analyze its antiproliferative properties in the melanoma cell lines SKMel23 and A375.

Materials and Methods

Chemistry

General

Flash column chromatography (FCC) was performed with silica gel 60 (63–200 μm) purchased from Merck. For thin-layer chromatography, aluminum sheets with silica gel 60 F₂₅₄ (Merck) were utilized, and the products were visualized by treatment with aqueous potassium permanganate or *n*-butanolic ninhydrin solutions.

Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent VNMRs 400 (¹H-NMR: 400 MHz, ¹³C-NMR: 101 MHz) or an Agilent VNMRs 600 (¹H-NMR: 600 MHz, ¹³C-NMR: 151 MHz) spectrometer. Carbon spectra were recorded decoupled. The chemical shifts δ are given in parts per million (ppm), and the residual solvent peak of the nondeuterated solvent was used as reference [(CH₃)₂CO: ¹H-NMR: δ = 2.05 ppm, ¹³C-NMR: (CD₃)₂CO: δ = 29.84 ppm; (CH₃)₂SO: ¹H-NMR: δ = 2.50 ppm, ¹³C-NMR: (CD₃)₂SO: δ = 39.5 ppm]. The multiplicity was reported with the following abbreviations: brs = broad singlet, s = singlet, d = doublet, m = multiplet, and coupling constants were given in hertz. Note: signals in the carbon spectra recorded on Agilent VNMRs 600 (δ = 78 ppm) are artifacts and do not belong to the molecule.

Infrared spectra (IR) were recorded on a PerkinElmer Spectrum 100 spectrometer using the attenuated total reflectance (ATR) technique. The wave number ν is given per centimeter.

Mass spectra were recorded on a Finnigan SSQ 7000 spectrometer [electron ionization (EI), 70 eV; chemical ionization (CI), 100 eV]. For high-resolution mass spectra (HRMS), a Thermo Fisher Scientific LTQ Orbitrap XL spectrometer [electrospray ionization (ESI) in positive ion mode] was used. The resulting signals are given according to their *m/z* values, and their relative intensity is reported in parentheses. Elementary analysis was performed on an Elementar Vario El instrument.

Anhydrous solvents were freshly distilled in a moisture-free atmosphere with adequate drying reagents or purchased. If not mentioned differently, the common Schlenk technique was applied. Purchased chemicals were used without further purification. DMSO was from Calbiochem (Darmstadt, Germany). Tasisulam was purchased from Selleckchem (Munich, Germany).

Compounds 2 [72], 4 [43], 7 [43] and 8 [72] were synthesized in analogy to reported protocols.

Preparative Protocols and Analytical Data

N-[(5-Bromothiophen-2-yl)sulfinyl]-2,4-dichlorobenzamide (Compound 2). In an oven-dry flask, compound 8 (100 mg, 0.23 mmol) was diluted in dry CH₃CN (10 mL), and hexamethyldisilazane (0.10 mL, 0.46 mmol) was added. The mixture was stirred for 9 h at room temperature, and after the solvent had been removed under reduced pressure the mixture was directly subjected to column chromatography. Sulfonimidamide compound 2 was obtained after purification by FCC (*n*-pentane:ethyl acetate = 3:1) as a white solid. The yield was 78%. MP 129–131°C (decomposition). ¹H-NMR [600 MHz, (CD₃)₂CO]: δ = 7.84 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 4.1 Hz, 1H), 7.59 (brs, 2H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.42 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.30 (d, *J* = 4.1 Hz, 1H). ¹³C-NMR [151 MHz, (CD₃)₂CO]: δ = 171.41, 144.77, 136.80, 135.90, 134.01 (almost covered by the proximate peak, not visible in the edited spectrum), 133.90, 133.04, 131.67, 130.91, 127.72, 120.47. MS (EI): *m/z* = 414

([M]⁺, 3), 211 (13), 174 (100), 145 (57), 109 (52), 96 (17), 82 (56), 74 (65), 64 (45). MS (CI): *m/z* = 415 ([M + H]⁺, 58), 381 (71), 190 (74). IR (ATR): ν = 3,784, 2,923, 2,421, 2,177, 2,116, 1,992, 1,796, 1,716, 1,589, 1,549, 1,465, 1,398, 1,375, 1,319, 1,296, 1,244, 1,154, 1,102, 1,052, 1,020, 957, 892, 861, 832, 799, 769, 557. HRMS (ESI): *m/z* calcd. for C₁₁H₇BrCl₂N₂O₂NaS₂, 434.8402; found, 434.8405. Elemental analysis: calcd. for C₁₁H₇BrCl₂N₂O₂S₂, 31.90 (C), 1.70 (H), 6.76 (N); found, 32.17 (C), 1.90 (H), 6.58 (N).

Sodium 5-Bromothiophene-2-sulfinate (Compound 4). 5-Bromothiophene-2-sulfonylchloride (2.40 g, 9.21 mmol) was added to a solution of Na₂SO₃ (2.55 g, 20.3 mmol) and Na₂CO₃ (1.70 g, 16.6 mmol) in distilled water (20.0 mL). The mixture was heated to 80°C for 3 h, and a white solid was obtained after the water had been removed. The residue was dissolved in ethanol (30.0 mL) and heated to 80°C for 1.5 h. The suspension was filtered, and after evaporation of the filtrate the sodium salt compound 4 was obtained as a white crystalline powder. The yield was 99%. MP: 330–332°C (decomposition). ¹H-NMR [600 MHz, (CD₃)₂SO]: δ = 7.01 (d, *J* = 3.6 Hz, 1H), 6.76 (d, *J* = 3.6 Hz, 1H). ¹³C-NMR [151 MHz, (CD₃)₂SO]: δ = 170.07, 130.17, 123.70, 111.05. MS (ESI[–]): *m/z* = 226 (M[–], 100). IR (ATR): ν = 3,602, 2,947, 2,657, 2,325, 2,175, 2,114, 1,993, 1,761, 1,433, 1,405, 1,246, 1,192, 1,126, 1,050, 952, 879, 798, 731, 693. Elemental analysis: calcd. for C₄H₂BrCl₂NaO₂S₂, 19.29 (C), 0.81 (H); found, 19.15 (C), 0.95 (H).

N-[(5-Bromothiophen-2-yl)sulfinyl]-2,4-dichlorobenzamide (Compound 7). Compound 6 (745 g, 3.00 mmol) was dissolved in a mixture of Et₂O (13.0 mL) and H₂O (13.0 mL), and concentrated HCl (0.5 mL) was added. After the mixture had been stirred for 2 h at 40°C, it was cooled to room temperature, and the organic layer was separated from the aqueous one. After evaporation of the solvent under reduced pressure, the sulfinic acid was obtained (653 mg, 3.0 mmol) and directly treated with SOCl₂ (0.90 mL, 12.0 mmol) at 0°C under argon with dry dichloromethane (12.0 mL). After the mixture had been warmed to room temperature, it was left to stir for 3 h, and the solvent was removed under reduced pressure. 2,4-Dichlorobenzamide was dissolved in dry tetrahydrofuran (THF; 12.0 mL), and *n*-butyllithium (1.6 M, 1.80 mL) was added at –78°C. After the mixture had been stirred for 20 min, a solution of the chloride compound 5 in THF (4 mL) was added dropwise to the mixture. The reaction mixture was stirred for 1 h at –78°C and then left to warm up to room temperature. H₂O (3 mL) was added, and the mixture was extracted with ethyl acetate. After the solvent had been removed under reduced pressure, the mixture was directly subjected to column chromatography. Title compound 7 was isolated after FCC (*n*-pentane:ethyl acetate = 4:1) as a white crystalline powder. The yield was 15%. MP: 138–140°C (decomposition). ¹H-NMR [600 MHz, (CD₃)₂CO]: δ = 10.75 (bs, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.62 (d, *J* = 1.9 Hz, 1H), 7.52 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.50 (d, *J* = 4.0 Hz, 1H), 7.33 (d, *J* = 4.0 Hz, 1H). ¹³C-NMR [151 MHz, (CD₃)₂CO]: δ = 165.99, 147.43, 136.73, 132.34, 131.93, 131.33, 130.53, 130.14, 129.62, 127.40, 117.90. MS (EI): *m/z* = 399 ([M]⁺, 2), 209 (35), 173 (100), 145 (30), 109 (27), 96 (17), 82 (37). MS (ESI): *m/z* = 421 ([M + Na]⁺, 16). IR (ATR): ν = 3,122, 2,935, 2,661, 2,325, 2,180, 2,088, 1,905, 1,671, 1,580, 1,396, 1,240, 1,083, 966, 883, 803, 746. HRMS (ESI): *m/z* calcd. for C₁₁H₆BrCl₂NO₂NaS₂, 419.8293; found, 419.8285.

5-Bromo-N-(2,4-dichlorobenzoyl)thiophene-2-sulfonimidoyl Chloride (Compound 8). By use of *N*-chlorosuccinimide (NCS; Fig. 2, path d), compound 7 (200 mg, 0.50 mmol) was dissolved in acetonitrile (1.70 mL). After the mixture had been cooled to 0°C,

Fig. 2. Synthesis of sulfonimidamide compound 2. Reagents and conditions: (a) Na₂SO₃ (2.2 equiv.), Na₂CO₃ (1.8 equiv.), H₂O, EtOH; (b) first, HCl, Et₂O, H₂O, 40°C, 2 h; second, SOCl₂ (4.0 equiv.), dichloromethane; (c) compound 6 (1.0 equiv.), *n*-butyllithium (1.2 equiv.), THF, -78°C to room temperature; (d) NCS (2.0 equiv.), CH₃CN, 0°C, 1 h; (e) *t*-butyl hypochlorite (1.5 equiv.), dichloromethane, 0°C, 2 h; (f) NH₃ (aq., conc.), MeOH/H₂O (1:1), room temperature, 2 h, then 70°C, 5 h; (g) NH₃ (g.), 15 min, THF, -78°C to room temperature; (h) NH₃ in MeOH (7 M, 0.3 equiv), MeOH, overnight; (i), hexamethyldisilazane (2.0 equiv.), CH₃CN, overnight.

NCS (382 mg, 5.60 mmol) was added. The reaction mixture was left to reach room temperature and stirred for 1 h at room temperature. The mixture was directly suspended to the column, and compound 8 was isolated as white solid after purification by FCC (*n*-pentane:ethyl acetate = 2:1). The yield was 7%. By use of *tert*-butyl hypochlorite (Fig. 2, path e), freshly prepared *t*-butyl hypochlorite [80] (0.04 mL, 0.38 mmol) was added to an iced suspension of compound 7 (100 mg, 0.25 mmol). The solvent was removed under reduced pressure, and compound 8 was isolated as a white solid. The yield was 99%. MP: 205–210°C (decomposition). ¹H-NMR [600 MHz, (CD₃)₂CO]: δ = 8.15 (d, *J* = 4.3 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 2.1 Hz, 1H), 7.59 (d, *J* = 4.3 Hz, 1H), 7.55 (dd, *J* = 8.4, 2.0 Hz, 1H). ¹³C-NMR [151 MHz, (CD₃)₂CO]: δ = 167.85, 141.11, 138.06, 137.14, 134.14, 133.12, 132.71, 131.62, 130.77, 127.40, 126.18. MS (CI): *m/z* = 434 ([*M* + *H*]⁺, 4), 291 (20), 257 (18), 211 (33), 190 (74), 172 (100), 156 65. MS (ESI): *m/z* = 456 ([*M* + *Na*]⁺, 100). IR (ATR): ν = 3,346, 3,178, 3,082, 2,924, 2,855, 2,654, 2,299, 2,097, 2,021, 1,670, 1,585, 1,469, 1,402, 1,365, 1,325, 1,259, 1,233, 1,201, 1,091, 1,045, 963, 804, 765, 689. HRMS (ESI): *m/z* calcd. for C₁₁H₅O₂NBrCl₃NaS₂, 453.7903; found, 453.7909.

Biology

Cell Culture

Melanoma cell lines were cultivated at 37°C in an atmosphere of 5% CO₂. SKMel23 cells were cultured with RPMI 1640 and 2 mM L-glutamine (Invitrogen, Darmstadt, Germany), supplemented with 10% heat-inactivated fetal calf serum (Biochrom, Berlin, Germany) and 0.25 µg/mL puromycin (Invitrogen). A375 cells were grown in RPMI 1640 medium supplemented with 5% fetal calf serum, 100 µg/mL streptomycin (Invitrogen) and 60 µg/mL penicillin (Invitrogen).

Proliferation Assay

Cells (5 × 10³) were seeded in 24-well culture plates. After 18 h the cells were stimulated with various concentrations of the sulfonimidamide-based analog of tasisulam (compound 2) or tasisulam (compound 1). Control cells were treated with DMSO. All treatments were performed in triplicate. Proliferation was assayed 8, 24, 48 and 72 h after stimulation using the alamarBlue® cell proliferation assay (AbD Serotec, Düsseldorf, Germany) according to

the manufacturer's protocol. Absorbance was measured using an Eon™ (BioTek) microplate spectrophotometer at 570 and 600 nm (for reference).

Results

For the synthesis of sulfonimidamide compound 2 (Fig. 2), commercially available sulfonyl chloride compound 3 served as key component, and the corresponding sodium sulfinate compound 4 was generated in an excellent yield (99%). Before the coupling with benzamide compound 6, sodium salt compound 4 was converted to sulfinyl chloride compound 5, which was directly subjected to amide formation with the lithium salt of compound 6 to give *N*-acyl sulfonamide compound 7 in 15% yield (over 3 steps). While chlorination of compound 7 with NCS gave *N*-acyl sulfonimidoyl chloride compound 8 in only 7% yield, the analogous halogenation with freshly prepared *tert*-butyl hypochlorite led to target compound 8 essentially quantitatively. For finalizing the synthesis of compound 2, intermediate compound 8 had to be aminated. To our surprise, this last synthetic step proved challenging. All attempts to use ammonia (in aqueous, concentrated, diluted, or gaseous form) remained unsuccessful, and degradation of compound 8 occurred. Finally, hexamethyldisilazane proved to be a suitable NH₂ equivalent [72], and in this manner, tasisulam analog 2 was obtained in 78% yield. The NMR spectroscopic data suggested the depicted (racemic) tautomeric structure for compound 2. However, the alternative tautomer (with respect to the N=S–NH₂ fragment) could not rigorously be excluded. The antiprolif-

Fig. 3. Antiproliferative effect of the sulfonimidamide analog of tasisulam (compound 2, **a–d**) and the original compound 1 (tasisulam, **e–h**) in the melanoma cell line A375.

erative properties of the sulfonimidamide-based analog of tasisulam (compound 2) were evaluated in comparison to the original compound 1 (tasisulam) by performing a cell proliferation assay in the 2 melanoma cell lines SKMel23 and A375. The results revealed that analog 2 had inhibitory effects on the proliferation comparable to tasisulam (compound 1) in both investigated cell lines. As shown in Figures 3 and 4a, b, e, and f, both analog 2 and the original substance 1 had essentially no effect on cell proliferation 8 and 24 h after treatment of the melanoma cell lines A375 and SKMel23. Also 48 and 72 h after treatment, both substances had no effect on cell proliferation up to a concentration of 10 μmol in the melanoma cell line SKMel23 but exhibited dose-dependent effects in the range of 25–200 μmol (Fig. 4c, d, g, and h).

Seventy-two hours after stimulation of SKMel23 cells with 200 μmol of the sulfonimidamide-based tasisulam analog (compound 2), cell proliferation was strongly reduced by 76% in comparison to DMSO-treated control cells (Fig. 4d). Inhibition of cell proliferation in A375 cells exposed to tasisulam analog 2 (25–200 μmol) was lower than in cells exposed to tasisulam (compound 1) 48 h after stimulation (Fig. 3c, g). Further evaluation revealed that 72 h after stimulation tasisulam analog 2 as well as

the original compound 1 inhibited A375 cell proliferation highly efficiently. In comparison to DMSO-treated control cells, compound 2 reduced cell proliferation down to 40% and tasisulam (compound 1) even down to 25% (Fig. 3c, h).

Discussion

Tasisulam is a promising drug which is used as an antiproliferative agent in patients with metastatic melanoma and other solid tumors [10, 13, 14–16]. A randomized, open-label clinical trial of tasisulam sodium versus paclitaxel as second-line treatment in patients with metastatic melanoma has recently been stopped early because of safety issues in the tasisulam arm. Although it was considered to be unlikely that tasisulam was superior to prior chemotherapies, the importance of pharmacokinetic monitoring of compounds with complex dosing was underscored [43].

Especially in the era before immuno- and targeted therapy, chemotherapy has long been the mainstay in the treatment of advanced melanoma particularly with compounds such as fotemustine, dacarbazine, temozolomide, and platinum-based agents. Much effort has been made

Fig. 4. Antiproliferative effect of the sulfonimidamide analog of tasisulam (compound 2, **a–d**) and the original compound 1 (tasisulam, **e–h**) in the melanoma cell line SKMel23.

to increase response rates and to prolong survival by combined treatment modalities. However, until now there have been no defined and generally accepted standards of systemic treatment in metastatic melanoma [81, 82].

Melanoma is a common type of skin cancer with a high propensity to metastasize. Tyrosine kinase inhibitors targeting the mitogen-activated protein kinase pathway and immune checkpoint blockade have recently revolutionized the management of unresectable and metastatic disease. However, acquired resistance and primary nonresponse to therapy require novel treatment strategies and combinations [7]. The double-blind, randomized phase 3 study COMBI-d compared dabrafenib and trametinib to dabrafenib alone. The objective RR lay at 67% for the combination and at 51% for the monotherapy cohort [83]. In addition to objective clinical benefits such as delayed progression or longer overall survival, patients receiving the combination showed a better health-related quality of life along with significant functional and pain improvements compared with patients receiving monotherapy with dabrafenib. While these targeted therapies can be only applied in approximately 50% of melanoma patients, immunotherapies can be a therapeutic option independent of the mutational status.

The antibody ipilimumab is directed against CTLA-4 and administered intravenously for 4 cycles at 3-weekly intervals, in contrast to the PD-1 inhibitors, which are applied as continuous therapy. These monoclonal antibodies are also called immune checkpoint inhibitors and are crucial in controlling immune responses. Data from several phase 2 and phase 3 trials have recently been collected and analyzed, revealing a median survival time of 11.4 months for ipilimumab monotherapy [84]. According to the studies that have been published thus far, the response rates of ipilimumab as monotherapy are lower than those with PD-1 inhibitors, and the rate of adverse events is higher, which makes ipilimumab uncertain as monotherapy in melanoma in the future.

The combination of a CTLA-4 and a PD-1 inhibitor seems to be superior to the monotherapies, especially in patients with PD-L1 negative tumors [85–87]. Data have already been published and revealed a response rate of 58% for the combination, 44% for nivolumab alone, and 19% for ipilimumab alone [7]. However, the high antitumor efficacy was accompanied by an elevated frequency of severe immune-related adverse events. In the combination cohort, nearly all patients experienced at least 1 side effect, while 57% showed an event of grade 3 or 4. In 39% of all patients the combination therapy had to be

stopped due to side effects such as diarrhea with colitis or hepatitis with elevated liver enzymes. Pembrolizumab and nivolumab show higher response rates compared to ipilimumab and fewer severe immune-related adverse events [7]. Recent advances in targeted and immunotherapy have significantly improved the patient outcome. However, there is still a substantial number of patients with primary or acquired resistance in need of innovative treatment modalities and combination therapies.

As for chemotherapeutic agents, dacarbazine is the most widely used single-agent chemotherapy for metastatic melanoma and has shown low response rates of 6–8% and a median overall survival of 6–12 months across large-scale group trials with follow-up times over 22 months [88–90]. Additionally, in a retrospective chart analysis of metastatic melanoma patients treated with various second-line therapies, the response rate was only 2% [91].

Fotemustine has also been investigated as chemotherapy in advanced melanoma treatment [92]. In a retrospective study it was the second most common agent used as first-line systemic treatment in France and Italy, despite modest clinical activity [93].

Paclitaxel activity in patients with metastatic melanoma showed RRs of 15%, with several patients reported to achieve a complete response [94–96]. A study with single-agent paclitaxel in second-line treatment showed an RR of 5%, without an observation of complete response. Previous studies have suggested that paclitaxel in combination with carboplatin may have a slight additional benefit in the second-line treatment of patients with metastatic melanoma but may also be associated with greater hematological toxicity [97, 98].

Tasisulam is of high interest because of its unique dual antitumor mechanism of action, involving antiangiogenesis and mitotic inhibition [11]. Investigation of the unexpectedly high rate of hematological toxicity revealed a subset of patients with low tasisulam clearance, leading to drug accumulation and high albumin-corrected exposure in cycle 2 [79]. Because of high-affinity albumin binding and rapid, extensive metabolism of free tasisulam in vivo, preclinical studies had failed to identify a clearance mechanism for unbound tasisulam, which is the biologically active form. Tasisulam shows biexponential disposition with a median terminal elimination half-life of approximately 11 days. Therefore, given the extensive albumin binding of tasisulam and its long terminal half-life, a loading dose in cycle 1 followed by a lower chronic dose in subsequent cycles were necessary to decrease the risk of a progressive rise in drug concentrations in later cycles and to reduce the risk of saturating albumin binding. Unbound tasisulam clear-

ance was found to be lower than the population mean in the majority of the patients (27 of 28) who experienced grade 4/5 hematological toxicities. Possible explanations for the lower tasisulam clearance also include CYP2C19 polymorphisms and the use of CYP2C19-inhibiting proton pump inhibitors, which is consistent with subsequent findings that it may be metabolized by CYP2C19. However, none of these factors explain the vast majority of observed grade 4/5 cases of hematological toxicity.

Therefore, we decided to prepare a sulfonimidamide analog of tasisulam in the intention to reduce the toxicity imbalance. Initial in vitro testing revealed similar antiproliferative properties on the melanoma cell lines SKMel23 and A375 tested compared to the original compound. Further preclinical studies will be needed to evaluate the renal clearance rate and hematological toxicity of this sulfonimidamide analog in order to find out whether this compound could be an alternative treatment with less hematological side effects for patients with metastatic melanoma and other solid tumors to improve safety and efficacy of the therapy.

Note Added in Proof

At the proof stage of the manuscript an important overview on medical and agrochemical applications of sulfonimidamides appeared [99].

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Statement of Ethics

Neither human participants nor human cell material were used.

Disclosure Statement

All authors have declared no conflict of interest.

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