Available online at www.sciencedirect.com

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 16 (2006) 3663–3667

Bioorganic &
Medicinal
Chemistry
Letters

Synthesis and in vitro anti-tumor activity of *N*-{1-[(3-thioxo-5,6-dihydroimidazo[2,1-*c*][1,2,4]thiadiazol-7-ylthio)thiocarbonyl]-2-imidazolidene}arylsulfonamides

Jarosław Sączewski,[a] Zdziaław Brzozowski,[a] Franciszek Sączewski,[a,*]
Patrick J. Bednarski,[b] Manuel Liebeke[b] and Maria Gdaniec[c]

[a]*Department of Chemical Technology of Drugs, Medical University of Gdańsk, 80-416 Gdańsk, Poland*
[b]*Department of Pharmaceutical and Medicinal Chemistry, University of Greifswald, 17489 Greifswald, Germany*
[c]*Faculty of Chemistry, A. Mickiewicz University, 60780 Poznań, Poland*

Received 4 April 2006; revised 20 April 2006; accepted 21 April 2006
Available online 8 May 2006

Abstract—A series of *N*-{1-[(3-thioxo-5,6-dihydroimidazo[2,1-*c*][1,2,4]thiadiazol-7-ylthio)thiocarbonyl]-2-imidazolidene}arylsulfonamides (**2a**–**z**) was obtained by reacting 6,7-dihydro-1*H*-imidazo[2,1-*c*][1,2,4]thiadiazol-3-thione (**1**) with arylsulfonyl chlorides. The relationships between structure and anti-tumor activity revealed that compound **2o** with *p*-Cl substituent at the phenyl ring was most active ($-\log \mathrm{GI}_{50} > 8.00$, $-\log \mathrm{TGI} = 7.66$) and was found to exhibit high selectivity toward the leukemia CCRF-CEM cell line ($\Delta^{\mathrm{f}} = 3.08$ and 3.31, respectively).

In recent years 1,2,4-thiadiazole derivatives have been found to possess a broad pharmacological potential. They act as allosteric modulators[1,2] or antagonists[3] of adenosine receptors, melanocortin MC4 receptor agonists,[4] potassium channel openers,[5] cyclogenase 2 inhibitors[6] as well as anti-angiogenic[7] and anti-bacterial agents.[8,9] Promising non-ATP competitive glycogen synthase kinase 3β (GSK-3β) inhibitors[10] and acetylcholinesterase inhibitors[11] with 1,2,4-thiadiazolidinone ring system were also discovered as promising candidates for development of Alzheimer's disease pharmacotherapy.

Moreover, following the finding that 1,2,4-thiadiazoles may act as thiol trapping agents,[12] novel liver transglutaminase (TGase) inhibitors[13] and cathepsin B[14] inhibitors were synthesized. As shown in Figure 1, the proposed enzymatic mechanism involves the nucleophilic attack of the active site cysteine thiol at the sulfur atom leading to the formation of a disulfide bond upon the opening of the 1,2,4-thiadiazole ring.[13]

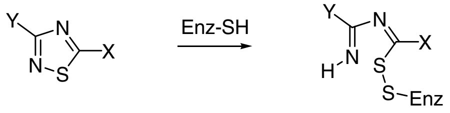


Figure 1.

On the other hand, aromatic and heteroaromatic sulfonamides have emerged as important therapeutic agents for the development of anti-tumor drugs acting as potent inhibitors of tumor-associated carbonic anhydrase isozymes IX and XII.[15–19]

Finally, compounds bearing a dithiocarbamate moiety, such as brassinin and its synthetic analogues, have recently been found to act as potent inhibitors of indoleamine 2,3-dioxygenase which plays a role in undermining immune response to tumor growth, and therefore, they emerge as a new potential anti-cancer therapeutic.[20]

In this paper, we wish to describe a facile method for the preparation of novel class of sulfenamide compounds incorporating all the above-mentioned pharmacophoric groups: the 1,2,4-thiadiazole ring, arylsulfonamide and dithiocarbamate moieties, which exhibit structure dependent in vitro anti-cancer activity.

Keywords: Sulfonamides; Sulfenamides; Synthesis; Anti-tumor activity.

[*] Corresponding author. Tel.: +48 58 3493250; fax: +48 58 3493257; e-mail: saczew@amg.gda.pl

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.04.067

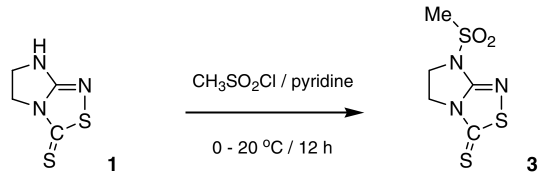
The starting 6,7-dihydro-5*H*-imidazo[2,1-*c*][1,2,4]thiadiazol-3-thione (1) was obtained by reacting 2-hydroxylamino-4,5-dihydroimidazoliun-*O*-sulfonate with carbon disulfide in the presence of triethylamine as described previously.[21] Compound **1** was found to react with alkylating reagents as well as with acetic acid anhydride to give the corresponding N-7 alkylated or acetylated products.[21]

However, as shown in Scheme 1, the reaction of **1** (2 molar equivalents) with variously substituted arylsulfonyl chlorides (1 molar equivalent) carried out in pyridine solution at 0–20 °C, unexpectedly, led to the formation of thiocarbonylsulfenamides **2a**–**z** in 55–90% yield.[22] Structure of these products was confirmed by elemental analyses, IR as well as 1D and 2D NMR spectroscopic data (Supporting Information).

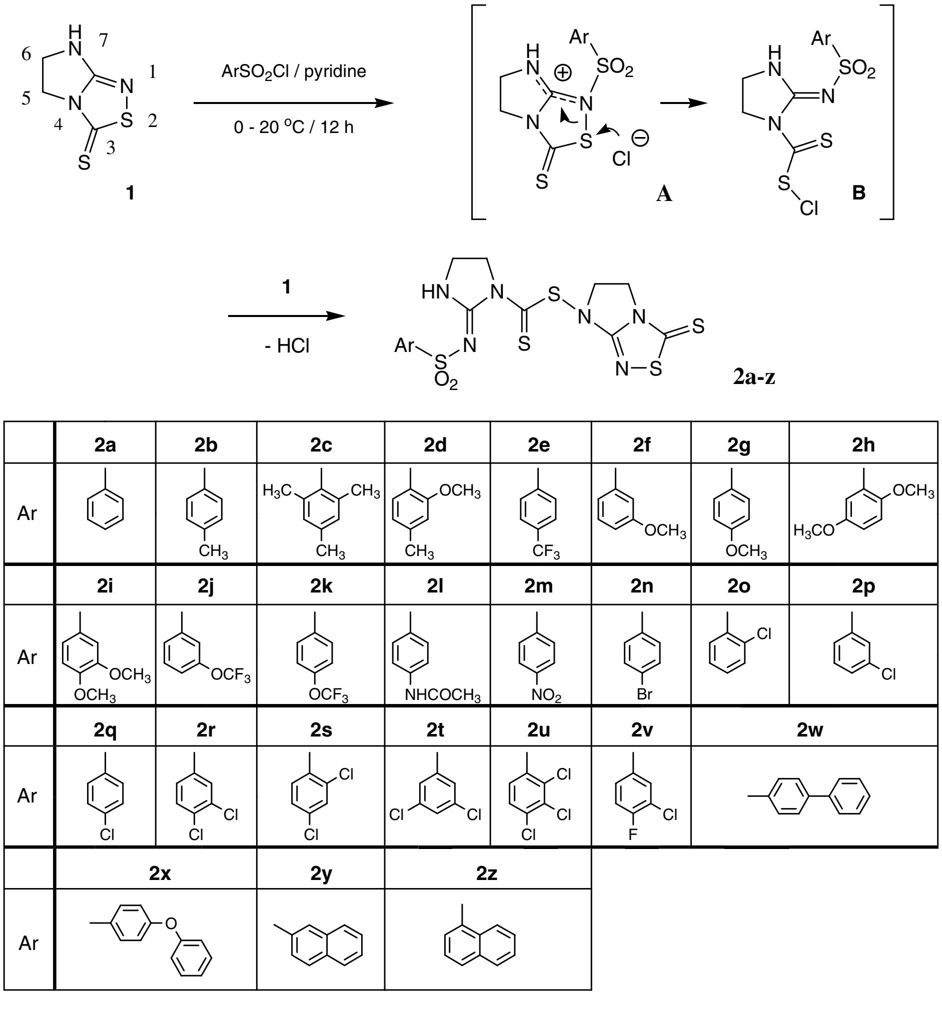
Apparently, at the first step of the reaction sequence the electrophilic attack of *N*-arylsulfonyl pyridinium cation at the N-1 nitrogen atom of **1** takes place to give intermediate **A**, which subsequently upon nucleophilic attack of the chloride anion undergoes ring opening with the formation of thiocarbonyl sulfenyl chloride **B**. The latter compound reacts with a second molecule of 1,2,4-thiadiazole **1** present in the reaction mixture to give the final product **2** incorporating both the sulfenamide and sulfonamide moieties.

It should be pointed out that cleavage of sulfenamide S–N bond upon treatment with hydrobromic acid leading to the formation of corresponding sulfenyl bromides and amines was previously described.[23] However, no information has been found in chemical literature regarding S–N bond scission upon treatment with arylsulfonyl chlorides.

In contrast, the reaction of **1** with methanesulfonyl chloride carried out under similar conditions furnished the expected N-7 substituted product **3** (Scheme 2).



Scheme 2.



Scheme 1.

Structure of sulfonamide **3** was confirmed by X-ray single crystal analysis (Supporting Information). Different reactivity of methanesulfonyl chloride may be explained by initial formation of the sulfene ($CH_2{=}SO_2$) in pyridine solution, which then reacts readily with N7-H group of **1**.

All the compounds **2a**–**z** were tested against a panel of approximately 60 human tumor cell lines (NCI60). Details of this test system and the information, which is encoded by the activity pattern over all cell lines, have previously been published.[24–27]

The systematic modification of substitution pattern at the phenyl ring of the compounds **2** enabled us to elucidate the important structure–activity relationships. First of all, we have found that in this series the potency and selectivity of anti-tumor action is very sensitive to minor structural modifications. Thus, the multiply substituted congeners **2c**, **2d**, **2h**, **2r**, **2s**, **2t**, **2u**, and **2v** as well as compounds with electron-withdrawing substituents such as CF_3 (**2e**), $NHCOCH_3$ (**2l**) or NO_2 (**2m**) were inactive. Also analogues with bulky substituents such as phenyl (**2w**) and naphthyl (**2y**) did not pass the preliminary tests, whereas other compounds exhibited reasonable activity against one or more tumor cell lines (Table 1).

Relatively highest sensitivity to the compounds described here was found for cell lines of leukemia and renal cancer (Table 2, Supporting Information). Thus, compounds **2f**, **2g**, and **2i** bearing strong electron-donating OCH_3 group showed rather marked inhibitory activity ($-\log GI_{50} = 4.63$–5.79) against renal RXF393 and UO-31 cell lines. However, the highest activity against tested tumor cell lines was exhibited by compounds **2o**, **2p**, and **2q** with electron-withdrawing Cl substituent at positions *o*-, *p*-, and *m*- of phenyl ring, respectively. The most active compound **2o** ($-\log GI_{50} = 8.00$, $-\log$ TGI = 7.66) was also found to exhibit high selectivity toward leukemia CCRF-CEM cell line (Δ[f] = 3.08 and 3.31, respectively; Table 2, Supporting Information).

We further tested the abilities of the compounds to inhibit cell growth in the human bladder cancer cell lines 5637 and RT-4, the human pancreas cell line YAPC, the human breast cancer line MCF-7, the human cervix

Table 1. Overview of the results of the in vitro anti-tumor screening for compounds **2b**, **2f**, **2g**, **2i**, **2j**, **2k**, **2n**, **2o**, **2p**, **2q**, **2x**, and **2z**[a]

Compound	No. of the cell lines giving positive log GI_{50}[b], log TGI[c] and log LC_{50}[d]						MG_MID[e] and Δ[f] for		Most sensible cell lines
	log GI_{50}		log TGI		log LC_{50}				
	No	Range	No	Range	No	Range	log GI_{50}	log TGI	
2b	57	−6.94 to −4.49	49	−5.37 to −4.01	21	−5.03 to −4.02	−4.83 2.11	−4.36 1.01	Melanoma LOXIMVI, ovarian cancer SK-OV-3
2f	55	−4.88 to −4.01	24	−4.58 to −4.01	5	−4.27 to −4.05	−4.46 0.42	−4.1 0.48	Melanoma LOXIMVI, renal cancer ACHN
2g	56	−5.79 to −4.49	48	−5.47 to −4.10	30	−5.14 to −4.03	−4.78 1.01	−4.37 1.1	Renal cancer UO-31, RXF 393
2i	58	−5.51 to −4.21	37	−4.89 to −4.02	12	−4.17 to −4.07	−4.65 0.86	−4.19 0.7	Renal cancer RXF 393
2j	58	−5.69 to −4.36	51	−5.37 to −4.19	37	−5.08 to −4.04	−4.96 0.73	−4.49 0.88	Leukemia CCRF-CEM, RPMI-8226
2k	58	−5.88 to −4.59	53	−5.24 to −4.01	37	−4.48 to −4.02	−5.03 0.85	−4.52 0.72	Leukemia SR, CNS cancer SF-539
2n	53	−7.56 to −4.14	23	−4.41 to −4.02	3	−4.09 to −4.04	−4.57 2.99	−4.08 0.35	Leukemia RPMI-8226, non-small cell lung cancer HOP-92
2o	56	<−8.00 to −4.24	39	−7.66 to −4.06	15	−6.15 to −4.04	−4.92 3.08	−4.35 3.31	Leukemia CCRF-CEM, HL-60(TB)
2p	56	−5.42 to −4.41	48	−4.73 to −4.09	25	−4.30 to −4.01	−4.81 0.61	−4.33 0.4	Leukemia CCRF-CEM, renal cancer RXF 393
2q	56	−5.83 to −4.54	52	−4.51 to −4.12	29	−5.18 to −4.02	−5.14 0.69	−4.59 0.92	CNS cancer SF-539, renal cancer UO-31
2x	39	−5.56 to −4.09	7	−4.95 to −4.01	0	>−4.00	−4.27 1.29	−4.03 0.92	Leukemia HL-60(TB), SR
2z	57	−5.60 to −4.21	34	−4.49 to −4.01	6	−4.14 to −4.01	−4.68 0.92	−4.16 0.33	Leukemia CCRF-CEM, RPMI-8226

[a] Data obtained from the NCI's in vitro disease—oriented human tumor cell screen (see Refs. 5–7 for details).

[b] The log of the molar concentration that inhibits 50% net cell growth.

[c] The log of the molar concentration leading to total growth inhibition.

[d] The log of the molar concentration leading to 50% net cell death.

[e] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

[f] The reported data represent the logarithmic difference between the parameter value referred to the most sensible cell line and the same mean parameter. Delta is considered low if <1, moderate >1 and <3, high if >3.

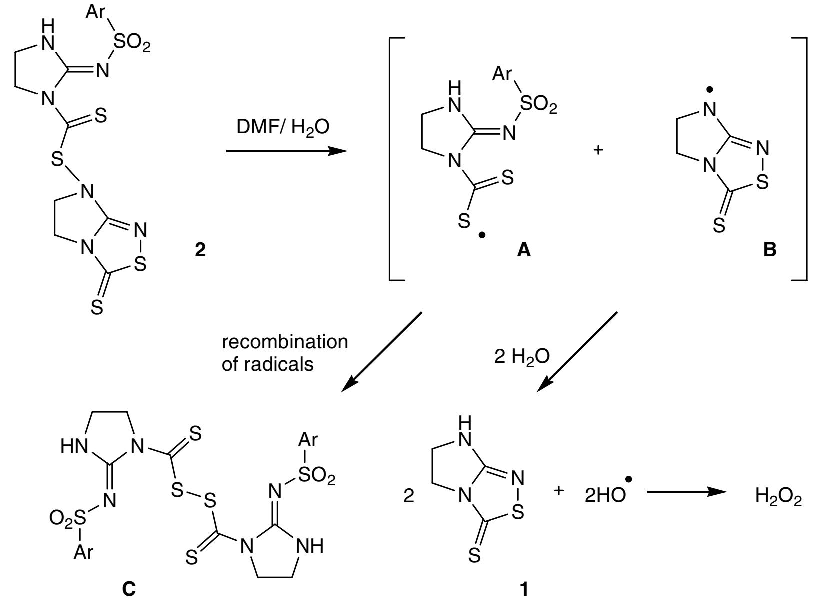


Figure 2.

cancer line SISO, and the human esophagus cancer cell line KYSE-510. Full details on the method of testing have been described elsewhere,[28] but unlike the NCI testing method,[26] which exposes cells to compound for 48 h, this method exposes cells to the test compound for 96 h. Furthermore, our method is based on staining the cells with crystal violet instead of sulforhodamine B (SRB).

Five of the six cell lines show about an equal average sensitivity to the compounds, while the KYSE-510 cell line is particularly resistant to the compounds and no compound gives a GI_{50} value below 20 μM (Table 3, Supporting Information). Overall, the new compounds have an in vitro activity comparable in potency to chlorambucil. The 5637 cell line is the most sensitive to any one single agent, with **2 m** showing a GI_{50} value of 3.67 μM. However, this compound is not the most active in all cell lines; in fact in the YAPC and RT-4 it is one of the weakest. The 5637 cell line shows the greatest difference in the range of activities, with GI_{50} values ranging from 3.67 to >20 μM; a 51% relative standard deviation between the compounds over all 21 compounds is noted. The other cell lines show considerably less dependency on the structure of the compounds to inhibit cell growth. Thus, a discussion of structure activity relationships is limited to just the 5637 cell line.

The compounds with the best growth inhibitory activity against the 5637 cell line possess electronegative (i.e., -I) substituents in the phenyl ring, in particular NO_2, OCF_3, and Cl. The position in the ring does not appear to be a factor that influences growth inhibition since *o*- (**2o**), *m*- (**2p**), and *p*- (**2q**) substituted Cl-derivatives all have about the same activity. The presence of a second chloro-substitutent increases activity slightly. The presence of three methyl groups (**2c**) gives one of the most active compounds. A single methoxy group in the phenyl ring has no effect on activity (compare **2a** with **2g** or **2f**) but a *p*-phenoxy group (**2x**) leads to an activity reduction. On the other hand, the presence of a *p*-phenyl group (**2w**) increases activity slightly.

In conclusion, variations in the substitutents in the phenyl ring of arylsulfenamides lead to changes in the potency of in vitro anti-tumor activity; however, the relative changes are dependent on the cell line investigated. Interestingly, the KYSE-510 cell line is insensitive to all of the tested arylsulfenamides, suggesting an intrinsic mechanism of resistance to these compounds.

The mechanism of anti-proliferative action of the compounds **2** has not been investigated in detail. However, we have recently found that upon heating in aqueous DMF a facile cleavage of the sulfonamide S-N bond of **2** takes place leading to the radicals **A** and **B**, as shown in Figure 2.[29] Recombination of sulfur radials **B** gives rise to the formation of disulfide **C**, while radical **B** may react further with water molecule to give 6,7-dihydro-5*H*-imidazo[2,1-*c*][1,2,4]thiadiazol-3-thione (1) and hydroxyl radical which upon subsequent recombination may form hydrogen peroxide. Thus, the reactive oxygen species which play important role in apoptosis may be responsible for anti-proliferative action of the compounds **2**.

Further experiments aimed at confirming the above mechanism are planned and will be reported in due time.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.067.

References and notes

1. van den Nieuwendijk, A. M. C. H.; Pietra, D.; Heltman, L.; Goblyos, A.; IJzerman, A. P. *J. Med. Chem.* **2004**, *47*, 663.
2. Goblyos, A.; de Vries, H.; Brussee, J.; IJzerman, A. P. *J. Med. Chem.* **2005**, *48*, 1145.
3. Jung, K.-Y.; Kim, S.-Y.; Gao, Z.-G.; Gross, A. S.; Melman, N.; Jacobsen, K. A.; Kim, Y.-Ch. *Bioorg. Med. Chem.* **2004**, *12*, 613.
4. Pan, K.; Scott, M. K.; Lee, D. H. S.; Fitzpatrick, L. J.; Crooke, J. J.; Rivero, R. A.; Rosenthal, D. I.; Vaidya, A. H.; ZXhao, B.; Reitz, A. B. *Bioorg. Med. Chem.* **2003**, *11*, 185.
5. Martinez, A.; Castro, A.; Cardelus, I.; Llenas, J.; Palacios, J. M. *Bioorg. Med. Chem.* **1997**, *5*, 1275.
6. Song, Y.; Connor, D. T.; Sercel, A. D.; Sorenson, R. J.; Doubleday, R.; Unangst, P. C.; Roth, B. D.; Beylin, V. C.; Gilbertson, R. B.; Chan, K.; Schrier, D. J.; Guglietta, A.; Bornemeier, D. A.; Dyer, R. D. *J. Med. Chem.* **1999**, *42*, 1161.
7. Bongartz, J.-P.; Stokbroekx, R.; Van der Aa, M.; Luyckx, M.; Willems, M.; Ceusters, M.; Meerpoel, L.; Smets, G.; Jansen, T.; Wouters, W.; Bowden, C.; Valletta, L.; Herb, M.; Tominovich, R.; Tuman, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 589.
8. Yoshizawa, H.; Kubota, T.; Itani, H.; Ishitobi, H.; Miwa, H.; Nishitani, Y. *Bioorg. Med. Chem.* **2004**, *12*, 4211.
9. Piskala, A.; Vachalkova, A.; Masojidkova, M.; Horvathova, K.; Olesna, Z.; Paces, V.; Nowotny, L. *Pharmazie* **2004**, *59*, 756.
10. Martinez, A.; Alonso, M.; Castro, A.; Perez, C.; Morena, F. J. *J. Med. Chem.* **2002**, *45*, 1292.
11. Martinez, A.; Fernandez, E.; Castro, A.; Conode, S.; Rodriguez-Franco, I.; Banos, J.-E.; Badia, A. *Eur. J. Med. Chem.* **2000**, *35*, 913.
12. Marrano, C.; de Macedo, P.; Gagnon, P.; Lapierre, D.; Gravel, C.; Keillor, J. W. *Bioorg. Med. Chem.* **2001**, *9*, 3231.
13. Karimian, K.; Tam, T. F.; Desilets, D.; Lee, S.; Cappeltto, T.; Li, W. Apotex Research Inc., U.S. Patent 1997, 5, 677, 302, October 14.
14. Leung-Toung, R.; Wodzinska, J.; Li, W.; Lowrie, J.; Kukreja, R.; Desilets, D.; Karimian, K.; Tam, T. F. *Bioorg. Med. Chem.* **2003**, *11*, 5529.
15. Almajan, G. L.; Innoccenti, A.; Puccetti, L.; Manole, G.; Barbuceanu, S.; Saramet, I.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2347.
16. Winum, J.-Y.; Innoccenti, A.; Nasr, J.; Montero, J.-L.; Scozzafava, A.; Vullo, D.; Supuran, C. T. *Bioorg, Med. Chem. Lett.* **2005**, *15*, 2353.
17. Puccetti, L.; Fasolis, G.; Cecchi, A.; Winum, J.-Y.; Gamberi, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2359.
18. Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.* **2003**, *10*, 925.
19. Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199.
20. Gaspari, P.; Banerjee, T.; Malachowski, W. P.; Muller, A. J.; Prendergast, G. C.; DuHadway, J.; Bennett, S.; Donovan, A. M. *J. Med. Chem.* **2006**, *49*, 684.
21. Sączewski, F.; Sączewski, J.; Gdaniec, M. *J. Org. Chem.* **2003**, *68*, 4791.
22. General procedure for the synthesis of*N*-{1-[(3-thioxo-5,6-dihydroimidazo[2,1-*c*][1,2,4]thiadiazol-7-lythio)-thiocarbonyl]2-imidazolidene}arylsulfonamides (**2a**–**z**). 6,7-Dihydro-5*H*-imidazo[2,1-*c*][1,2,4]thiadiazole-3-thione (**1**) (0.5 g, 3.14 mmol) was dissolved in pyridine (10 mL). The resulting solution was cooled to 0 °C and a corresponding sulfonyl chloride (1.89 mmol) was added. The reaction mixture was stirred for 12 h at room temperature. Then, water was added (15 mL) and the solution was concentrated under reduced pressure to half of its initial volume. The oily product **2** thus obtained was purified by either crystallization or column chromatography (see Supplementary material).
23. Benati, L.; Montevecchi, P. C.; Spagnolo, P. *Tetrahedron* **1993**, *49*, 5365.
24. The anti-tumor activity of a test compound is given by three parameters for each cell line: log GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), log TGI value (TGI = molar concentration of the compound leading to total inhibition of net cell growth), and log LC_{50} (LC_{50} = molar concentration of the compound leading to 50% net cell death). Furthermore, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an averaged activity parameter over cell lines. Selectivity of a compound with respect to one or more cell lines of the screen is characterized by a high deviation of the particular cell line parameter compared to the MG_MID value.
25. Boyd, M. R. *Am. Assoc. Cancer Res.* **1989**, *30*, 652.
26. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Poull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolf, A.; Gray-Goodrich, M.; Cambell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
27. Weinsten, J. N.; Myers, T. C.; O'Connor, P. M.; Friend, S. H., ; Fornance, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sansiville, E. A.; Zaharewitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. *Science* **1997**, *275*, 343.
28. Bracht, K.; Boubakari; Grünert, R.; Bednarski, P. J. *Anticancer Drugs* **2006**, *17*, 41.
29. Sączewski, J.; Frontera, A.; Gdaniec, M.; Brzozowski, Z.; Sączewski, F.; Tabin, P.; Quinonero, D.; Deya, P. M. *Chem. Phys. Lett.* **2006**, *422*, 234.