Bioorg. Med. Chem. Lett. 43 (2021) 128078

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and evaluation of 1,2,3-dithiazoles and fused 1,2,4-dithiazines as anti-cancer agents

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ARTICLE INFO

Keywords:
1,2,3-Dithiazole
Breast cancer
Bladder cancer
Prostate cancer
Pancreatic cancer
Chordoma

ABSTRACT

Heteroatom rich 1,2,3-dithiazoles are relatively underexplored in medicinal chemistry. We now report screening data on a series of structurally diverse 1,2,3-dithiazoles and electronically related 1,2,4-dithiazines with the aim of identifying interesting starting points for potential future optimisation. The 1,2,3-dithiazoles, were obtained via a number of different syntheses and screened on a series of cancer cell lines. These included breast, bladder, prostate, pancreatic, chordoma and lung cancer cell lines with an additional skin fibroblast cell line as a toxicity control. Several low single digit micromolar compounds with promising therapeutic windows were identified for breast, bladder and prostate cancer. Furthermore, key structural features of 1,2,3-dithiazoles are discussed, that show encouraging scope for future refinement.

Heteroatom rich 1,2,3-dithiazoles are relatively underexplored in medicinal chemistry with the 1,2,4-dithiazine core even rarer.[1] This despite a number of reports on interesting biological applications (Fig. 1) including activity against bacteria,[2–5] fungi/weeds,[6–11] viruses[12,13] and cancers.[14–16] Most 1,2,3-dithiazole chemistry revolves around 4,5-dichloro-1,2,3-dithiazolium chloride, known as 'Appel salt', which can be readily prepared from chloroacetonitrile and disulfur dichloride.[17]

The versatile chemistry of Appel salt and the interesting physical/biological properties of its derivatives, have led to a diverse series of compounds being produced but generally around small, focused libraries. To date, 1,2,3-dithiazole chemistry has centered around the ring systems susceptibility to attack by nucleophiles at the S-1, S-2, and C-5 atoms.[18] These reaction pathways also present opportunities to target biological processes that are historically difficult to target, including proteins, protein–protein interactions and attempt to address issues of selectivity within protein families by irreversible binding to cysteine.[19–23]

To further explore the structural requirements for anti-cancer activity within the 1,2,3-dithiazole scaffold, we profiled several focused arrays of compounds on a series of cancer cell lines. These included breast, bladder, prostate, pancreatic, chordoma and lung cancer cell lines with an additional skin fibroblast cell line as a toxicity control.[24–27] These cancer cell lines each presents a different drug resistance profiles and when combined in a panel, each represents a distinct therapeutic challenge to overcome.[28] We synthesized a series of more common 5*H*-1,2,3-dithiazolimines (**8**–**25**) *via* a nucleophilic displacement of the C-5 chloride of Appel salt **7** using various substituted anilines (Scheme 1).[29–32]

We then converted three 1,2,3-dithiazoles **24**, **26** & **27** using diethylamine and Hünig's base in acetonitrile at *ca.* 20 °C, followed by treatment with concentrated sulfuric acid to afford their respective 1,2,4-dithiazines **28**–**30** in good yields (Scheme 2).[33] Additional structural diversity was achieved by modifying the C-4 position of dithiazolimines **31**–**34** (Scheme 3).[34–37] Treatment of dithiazolimines **31**–**34** with DABCO in chlorobenzene at *ca.* 131 °C to afford a series of *N*-(2-chloroethyl)-piperazin-1-yl-substituted 1,2,3-dithiazoles **35**–**38**.[38] The available *N*-chloroethyl moiety was then further modified by nucleophilic substitution of the chloride using a variety of oxygen, sulfur and nitrogen based nucleophiles to give a series of structurally diverse

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https://doi.org/10.1016/j.bmcl.2021.128078
Received 25 February 2021; Received in revised form 18 April 2021; Accepted 26 April 2021
Available online 2 May 2021

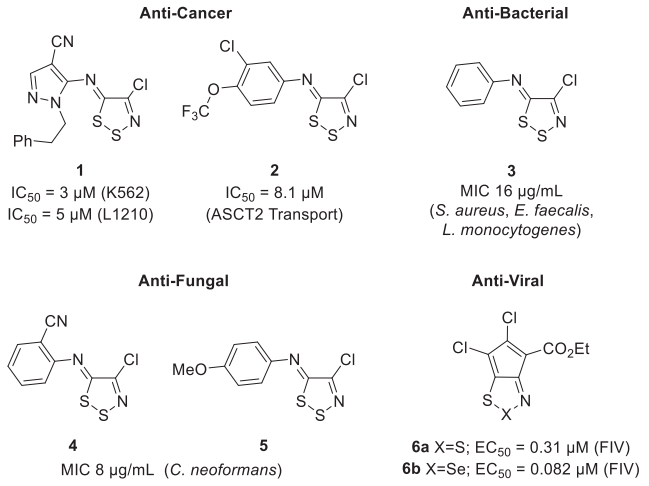
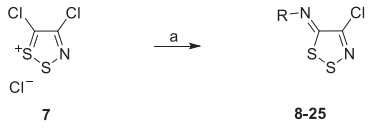
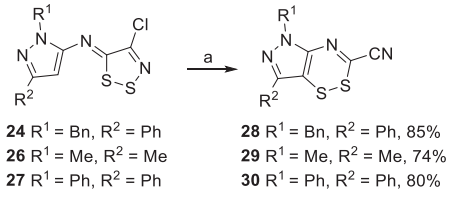


Fig. 1. Examples of biologically active 1,2,3-dithiazoles.



Scheme 1. Nucleophic displacement from Appel salt (**7**) to afford **8**–**25**. *Reagent and Conditions*: for **8**–**21**: a) $ArNH_2$ (1 equiv), DCM, rt, 2 h; b) pyridine (2 equiv), rt, 1 h; 23–98%. Then for **21**–**25**: a) $ArNH_2$ (1 equiv), HCl (g), DCM, rt, 12 h; b) lutidine (2 equiv), rt, 3 h; 17–92%.

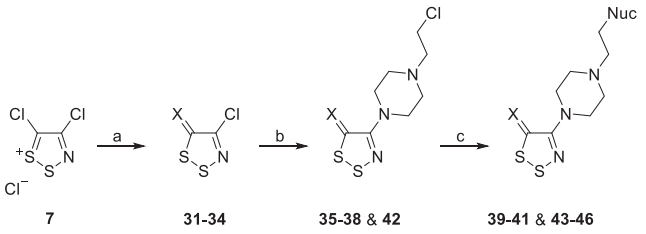


Scheme 2. Synthetic procedure to afford **28**–**30**. *Reagent and Conditions*: a) Et_2NH (3 equiv), *i*-Pr_2NEt (1 equiv), MeCN, rt, 25 min; b) concd H_2SO_4 (5 equiv), MeCN, rt, 5 min.

analogues **39**–**46**.[38]

Initial screening focused on the simpler substituted phenyl analogues based on the (*Z*)-4-chloro-*N*-phenyl-5*H*-1,2,3-dithiazol-5-imines scaffold **8**–**20** (Table 1). The first compound screened, the (*Z*)-*N*-[4-(benzyloxy) phenyl]-4-chloro-5*H*-1,2,3-dithiazol-5-imine (**8**) is known to cause a loss of pigmentation in melanophores and the retinal pigment epithelium (RPE) of developing *Xenopus laevis* embryos.[39] In our cell line screening, dithiazolimine **8** showed limited activity across the cancer cell lines, the most potent activity was an IC_{50} of 13 μM against bladder cancer, with no associated toxicity (WS-1; IC_{50} = >100 μM). Interestingly, the corresponding analogue switching from the benzyl **8** to the phenyl **9** in a previously reported study yielded a toxic compound,[39] whereas no toxicity (WS-1; IC_{50} = >100 μM) was observed in our screening and only limited anti-cancer activity across the cell line panel. The 4-(*n*-butyl)-substituted dithiazolimine **10** showed weaker activity (IC_{50} = 18 μM) on the bladder cancer cell line, but still without any associated toxicity.

The 4-trifluoromethoxy analogue with the additional diversity of a 2-methyl group (**11**) showed a 4-fold drop in bladder cancer inhibition but an increase of 4-fold for prostate cancer inhibition. Additional modification of the 4-trifluoromethoxyphenyl **11** included analogues that lacked the 2-methyl group but included halo substitution at the 3-position (**12**–**13**) but these showed no improvement. Interestingly, the fused methylene 3,4-catechol **14** showed the same potency as the dithiazole **11** on the prostate cancer cell line and a small hint of inhibition on the two chordoma lines.



Scheme 3. Synthetic procedure to afford **39**–**46**.[17,38] *Reagent and Conditions*: **31**–**32** (X = ArN): a) $ArNH_2$ (2 equiv), DCM, rt, 2 h; b) pyridine (2 equiv), rt, 1 h. **33** (X = S): H_2S (g), MeCN, rt. **34** (X = O): HCO_2H, rt, 2 h. b) DABCO (2 equiv), PhCl, 131 °C, 76–85%. c) NucH, MeCN, reflux, 72–98%.

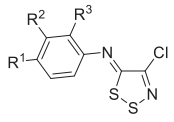
The 4-methylthiophenyl **15** was toxic with non-specific inhibition of the toxicity control, this was consistent with findings in the previously reported *in vivo* pigment study.[39] The 4-bromophenyl **16** showed inhibition of breast cancer (IC_{50} = 11 μM) with weaker activity across the other cancer cell lines apart from lung cancer cell line. The 3-bromophenyl **17** had a similar profile to the 4-bromophenyl **16**, but showed just under a 2-fold increase in potency against breast cancer (IC_{50} = 6.7 μM) with no increase in toxicity. The introduction of an *ortho*-nitrile to the 3-bromophenyl analogue **18** reduced activity on the breast cancer cell line by 4-fold but demonstrated a 3- and 4-fold increase activity on bladder and pancreatic cancer, respectively. The final two analogues of this series **19** and **20** contained *para*-substituted sulfonamide groups. The *N*-(thiazol-2-yl)sulfonamide **19** showed only limited activity, but the *N*-(pyrimid-2-yl)sulfonamide **20** showed potent activity against breast cancer (IC_{50} = 3.0 μM) and some activity against the difficult to treat,[25–26,40] patient-derived chordoma cell line UCH-2 (IC_{50} = 19 μM). It is currently unclear if this activity is related to the pyrimidine substituent, the 1,2,3-dithiazole or both parts of the molecule.

We then made a small series of small heteroaryl amine derivatives **21**–**25** (Table 2). The first analogue with a *N*-(thiazol-2-yl) **21**, an analogue of **19** was toxic across all cell lines including WS-1 with little differentiated activity between the cell lines. The second analogue *N*-(3-methyl-1*H*-pyrazol-5-yl) **22**, also showed some toxicity (WS-1; IC_{50} = 40 μM), but showed a 2-fold improvement over **21**, with some activity against the panel of cancer cell lines. However, with an increase in size of the C-3 substituent from methyl **22** to phenyl **23**, the toxicity can be tuned out, as in the case of **23** (WS-1; IC_{50} = >100 μM). The reduction in toxicity did not compromise inhibition activity with breast, bladder, prostate, pancreatic cancers, all having IC_{50}'s below 25 μM and breast cancer inhibition in the single digit micromolar range (IC_{50} = 9.3 μM). The blocking of the N—H of pyrazolyl **23** with a benzyl group **24**, reduced activity against breast cancer by 4-fold, but maintained activity across bladder, prostate, pancreatic cancers, while showing no toxicity. However, reverting to a methyl group on C-3 **25**, led to a potent a compound against bladder cancer (IC_{50} = 2.1 μM) and prostate cancer (IC_{50} = 10 μM), but also resulted in a compound with increased toxicity in WS-1 (IC_{50} = 15 μM).

We then converted three 1,2,3-dithiazoles to fused 1,2,4-dithiazines **28**–**30** to look at the effect of shifting to an electronically and chemicaly different disulfide bridge system (Table 3).[38] The 5,7-dimethyl-5*H*-pyrazolo[3,4-*e*][1,2,4]dithiazine-3-carbonitrile (**28**) was near inactive. However, the extension of the pendant substituent pattern of the 5-benzyl-7-phenyl-substituted dithiazine **29** allowed for a 10-fold increase of inhibition on the bladder cancer cell line to single digit micromolar with only mild associated toxicity (WS-1; IC_{50} = 51 μM) with respect to **28**. Interestingly, the symmetrical 5,7-diphenyl-substituted dithiazine **30** modulated the anti-cancer activity with improvements on both breast and prostate cancer inhibition potency (IC_{50} = <20 μM) and maintained bladder cancer inhibition (IC_{50} = 14 μM) with no tocivty in WS-1 (IC_{50} = >100 μM).

The encouraging preliminary data from the earlier screening, led us

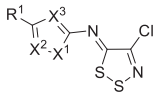
Table 1
Results of a series of *N*-(aryl)-4-chloro-5*H*-1,2,3-dithiazol-5-imines (**8**–**20**).[a]



Name	R^1	R^2	R^3	MCF7	5637	DU145	PANC1	UCH1	UCH2	A431	WS-1
8	BnO	H	H	50	13	45	>100	44	>100	>100	>100
9	PhO	H	H	35	35	>100	55	30	33	>100	>100
10	*n*-BuO	H	H	42	18	52	>100	54	48	>100	>100
11	F_3CO	H	Me	43	47	18	76	57	87	>100	>100
12	F_3CO	Cl	H	88	33	32	>100	36	43	>100	>100
13	F_3CO	Br	H	73	36	52	>100	34	85	100	>100
14	$-OCH_2O-$		H	19	32	17	24	28	22	95	84
15	MeS	H	H	36	34	14	86	38	37	51	66
16	Br	H	H	11	36	48	35	43	51	>100	>100
17	H	Br	H	6.7	53	27	38	40	59	>100	94
18	H	Br	CN	28	14	42	13	39	65	>100	>100
19	2-thiazolyl-$NHSO_2$	H	H	49	27	92	97	30	36	>100	>100
20	2-pyrimidyl-$NHSO_2$	H	H	3.0	39	29	37	34	19	>100	>100

[a] Results are a mean average of 4 replicates.

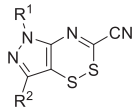
Table 2
Results of a small series 5-membered *N*-hetaryl-substituted 1,2,3-dithiazolimines (**21**–**25**).[a]



Name	X^1	X^2	R^1	X^3	MCF7	5637	DU145	PANC1	UCH1	UCH2	A431	WS-1
21	S	H	H	N	45	15	8.1	24	10	22	17	24
22	NH	N	Me	H	18	20	14	27	33	18	69	40
23	NH	N	Ph	H	9.3	16	11	23	59	38	>100	>100
24	*N*-Bn	N	Ph	H	30	16	14	18	30	52	>100	>100
25	*N*-Bn	N	Me	H	18	2.1	10	26	33	30	27	15

[a] Results are a mean average of 4 replicates.

Table 3
Results of a small series of 1,2,4-dithiazines (**28**–**30**).[a]



Name	R^1	R^2	MCF7	5637	DU145	PANC1	UCH1	UCH2	A431	WS-1
28	Me	Me	>100	81	40	>100	>100	>100	>100	>100
29	Bn	Ph	54	8.7	32	>100	>100	>100	54	51
30	Ph	Ph	36	14	16	75	55	68	72	>100

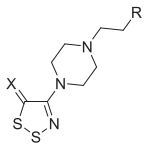
[a] Results are a mean average of 4 replicates.

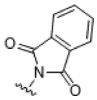
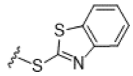
to test more divergent substitution patterns, changing both the 5- and 4-position of the 1,2,3-dithiazole. We reacted a selection of 5-substituted 1,2,3-dithiazoles with DABCO to gain an additional point of variation. The ring opened *N*-(2-chloro-ethyl)piperazin-1-yl derivatives **31**–**34** were then reacted with nucelophies to afford a series of diverse analogues. The final compounds **39**–**46** afford a range of results (Table 4). The first analogue an ester, (*Z*)-2-{4-[5-(phenylimino)-5*H*-1,2,3-dithiazol-4-yl]piperazin-1-yl}ethyl benzoate (**39**), had low micromolar activity against breast and bladder cancer in addition to chordoma. However, **39** also unfortunately, displayed some mild toxicity (WS-1; IC_{50} = 38 μM). The corresponding anilino derivative **40**, slightly reduced this toxicity, while maintaining potency against breast, bladder cancer and both chordoma cell lines. The thiocyanate analogue **41**, showed broadly similar activity with no toxicity. There was one anomalous difference, compound **41** showed reduced inhibition on the bladder cancer cell line, the reason for this is currently unclear.

We then tested (*Z*)-5-bromo-2-({4-[4-(2-chloroethyl)-piperazin-1-yl]-5*H*-1,2,3-dithiazol-5-ylidene}amino)benzonitrile (**42**), that contained several structural point changes compared with **39**–**41**. Compound **42** showed encouraging results particularly towards inhibition of breast (IC_{50} = 4.1 μM) and bladder (IC_{50} = 8.0 μM) cancers with lower single digit micromolar potencies.[41]

Compound **42** also showed good potency on prostate and chordoma cancer with no toxicity observed (WS-1; IC_{50} = >100 μM). This 20- to

Table 4
Results of a series of ring opened *N*-(2-chloroethyl)piperazin-1-yl derivatives (**39**–**46**).[a]



Name	X	R	MCF7	5637	DU145	PANC1	UCH1	UCH2	A431	WS-1
39	*N*-Ph	OBz	17	13	30	60	23	34	36	38
40	*N*-Ph	NH-Ph	10	18	55	67	25	21	87	55
41	*N*-Ph	SCN	32	93	14	16	38	38	>100	>100
42	*N*-Ph(2-CN, 4-Br)	Cl	4.1	8.0	15	18	32	23	>100	>100
43	S		51	25	23	73	46	>100	>100	>100
44	O		9.9	33	22	77	25	31	90	92
45	O	N(Me)-Ph	2.2	12	4.4	21	12	17	17	20
46	O	SCN	5.4	39	3.3	11	12	14	25	17

[a] Results are a mean average of 4 replicates.

25-fold toxicity window was encouraging and demonstrated that the activities observed were not the result of a generic toxicity phenotype. Switching to the 5-thione 4-phthalimido derivative 2-{2-[4-(5-thioxo-5*H*-1,2,3-dithiazol-4-yl)piperazin-1-yl]ethyl}isoindoline-1,3-dione (**43**), led to a net drop in inhibition across the panel of cell lines, with no toxicity observed. The ketone derivative 4-{4-[2-(benzo[*d*]thiazol-2-ylthio)ethyl]piperazin-1-yl}-5*H*-1,2,3-dithiazol-5-one (**44**), showed mild inhibition across all cancer cell lines with no toxicity observed. The switch to the *N*-methylaniline analogue **45** and thiocyanate analogue **46** both had similar biological profiles, potent across the cancer cell line panel with a moderate amount of toxicity observed.

The 1,2,3-dithiazole core have previously been shown to have some activity against breast cancer, but this is the first report of a broader screening against multiple cancer cell lines.[14] Within this screening we have identified several low single digit micromolar compounds for breast, bladder and prostate cancer all with low relative toxicity. We have highlighted a series of modifications and demonstrated the effect of key structural features on the 1,2,3-dithiazole scaffold.

A key finding within this array of results was that the relationship between inhibition of cancer cell proliferation and toxicity was not linear. This was particularly relevant when looking at the more complex analogues **39**–**46**, where the therapeutic index ranged widely from >24-fold for **42** to 3-fold for **46**. Compound **42** could prove to be an interesting starting point for future development, particularly with no toxicity observed in WS-1 cells. The present results help to define a de-risked medicinal chemistry trajectory towards utilizing this under-studied scaffold in new and exciting applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Cyprus Research Promotion Foundation (Grants No. NEAYPODOMH/NEKYP/0308/02) and the following organizations and companies in Cyprus for generous donations of chemicals and glassware: the State General Laboratory, the Agricultural Research Institute, the Ministry of Agriculture, MedoChemie Ltd., Medisell Ltd., and Biotronics Ltd. Furthermore, we thank the A. G. Leventis Foundation for helping to establish the NMR facility at the University of Cyprus. We also thank Dr. Brandie Ehrmann and Ms. Diane E. Wallace for for LC-MS/HRMS support provided by in the Mass Spectrometry Core Laboratory at the University of North Carolina at Chapel Hill. The core is supported by the National Science Foundation under Grant No. (CHE-1726291).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128078.

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27 **Cell culture method**: U-CH1 and U-CH2 cell lines were cultured in 4:1 IMDM:RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin in gel-coated flasks. A-431, PANC-1, and WS1 cell lines were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin. DU145 and MCF-7 cells were cultured in MEM medium supplemented with 10% FBS and 1% penicillin/streptomycin. 5637 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were seeded in 384 well plates and were treated with test compound in quadruplicate 24 hours after plating. Cell viability was assessed at 72 hours using alamarBlue (ThermoFisher, USA). Fluorescence was measured using Tecan Infinite 200 PRO plate reader with an excitation at 535 nM and emission at 590 nM. IC_{50} values were determined by nonlinear regression using GraphPad PrismTM software. (Positive controls: MCF7, lapatinib IC_{50} = 6.3 μM[42]; 5637, pazopanib IC_{50} = 15 μM[43]; PANC1, panobinostat IC_{50} = 1.0 μM[44]; DU145, docetaxel IC_{50} = 5 nM[45]; UCH1, gefitinib IC_{50} = 1.4 μM[25]; UCH2, gefitinib IC_{50} = 23 μM[25]; A431, lapatinib IC_{50} = 100 nM[46]; WS-1, lapatinib IC_{50} = 13 μM[25]).
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41 **General procdure for [(4-chloro-5 *H*-1,2,3-dithiazol-5-ylidene)amino] aren**es: Aminoarene (0.48 mmol) was added to a stirred suspension of 4,5-dichloro-1,2,3-dithiazolium chloride (100 mg, 0.48 mmol) in dichloromethane (4 mL) at ca. 20 °C. After 1 hour 2,6-lutidine (111.2 μL, 0.96 mmol) was added dropwise. The reaction mixture was adsorbed onto silica after a further 2 hours and purified by flash chromatography to afford the corresponding [(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene)amino]arene. Represenative example: **(Z)-5-Bromo-2- [(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene)amino] benzonitrile** (**32**). Yellow needles (72%), mp (DSC) onset: 167.5 °C, peak max: 168.3 °C (from cyclohexane/DCE); (found: C, 32.6; H, 1.0; N, 12.6. $C_9H_3BrClN_3S_2$ requires C, 32.5; H, 0.9; N, 12.6%); λ_{max}(DCM)/nm 230 (log ε 3.14), 250 (3.08), 314 (2.50), 383 (2.88), 419 inf (2.65); ν_{max}/cm^{-1} 3080w (Ar CH), 3063w, 2232w (C≡N), 1585m, 1560s, 1545m, 1489s, 1476s, 1458m, 1389w, 1269w, 1233w, 1182w, 1148m, 1126m, 1074m, 880m, 864s, 812m, 795m, 770m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, J 2.0, H-6), 7.78 (1H, dd, J 8.5, 2.0, H-4), 7.21 (1H, d, J 9.0, H-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.9 (s), 125.1 (s), 148.2 (s), 137.5 (d), 136.4 (d), 119.1 (d), 118.8 (s), 114.9 (s), 108.1 (s); *m/z* (EI) 335 (M^++4, 7%), 333 (M^++2, 24), 331 (M^+, 17), 272 (22), 270 (22), 240 (4), 238 (4), 234 (3), 232 (3), 208 (3), 206 (3), 182 (3), 180 (3), 159 (8), 127 (12), 125 (6), 115 (4), 100 (20), 93 (5), 88 (4), 75 (9), 70 (4), 66 (9), 64 (100), 50 (8). **(*Z*)-5-Bromo-2-{{4-[4-(2-chloroethyl)piperazin-1-yl] -5*H*-1,2,3-dithiazol-5-ylidene}amino) benzonitrile (42).** To a stirred solution of 5-bromo-[*N*-(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene)amino]benzonitrile (33.3 mg, 0.1 mmol) in PhCl (1 mL) at *ca.* 20 °C was added in one portion DABCO (13.5 mg, 0.12 mmol). The mixture was then heated at *ca.* 131 °C for 1 h and then left to cool to *ca.* 20 °C. The mixture was poured onto a packed column of silica and eluted with *n*-hexane. Subsequent elution (*n*-hexane/Et_2O, 70:30) gave **42** as yellow needles (37.5 mg, 84%), mp (DSC) onset: 144.4 °C, peak max: 145.4 °C (from *c*-hexane); λ_{max} (DCM)/nm 321 (log ε 2.86), 321 inf (2.73); ν_{max}/cm^{-1} 3026w, 2953w, 2882w, 2833w, 2778w, 2228w (C≡N), 1575s, 1547m, 1504m, 1470m, 1462m, 1437m, 1385m, 1369m, 1350w, 1333w, 1306m, 1294m, 1273m, 1252m, 1223m, 1200w, 1170m, 1142m, 1128m, 1121m, 1099w, 1076m, 1061w, 1051w, 1032w, 997s, 953m, 881m, 872m, 851m, 835m, 824s, 797m, 766m, 729m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (1H, d, *J* 2.0), 7.75 (1H, dd, *J* 8.8, 2.3), 7.30 (1H, d, *J* 9.0), 3.81 (4H, t, *J* 4.8), 3.61 (2H, t, *J* 6.8), 2.78 (2H, t, *J* 7.0), 2.69 (4H, t, *J* 5.0); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1 (s), 158.5 (s), 153.0 (s), 137.4 (d), 136.1 (d), 119.4 (d), 117.8 (s), 115.7 (s), 108.5 (s), 59.7 (t), 52.8 (t), 48.6 (t), 40.8 (t); MALDI-TOF MS (*m/z*): 448 (MH^++4, 52%), 446 (MH^++2, 100), 444 (MH^+, 86), 410 (MH^+-Cl, 30), 339 (80), 105 (57). HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{16}N_5S_2ClBr$: 443.9719, found 443.9707.
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