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Original article

Synthesis, structural characterization and in vitro antitumor activity of 4-dimethylaminopyridinium (6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides

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Abstract

Previously, we have described a novel series of low molecular weight cancer-specific antitumor agents with aminium *N*-(1,1-dioxo-1,4,2-benzodithiazin-3-yl)arylsulfonamidate structure. In an attempt to determine some of the structural features that account for the cytotoxic activity of such aminium salts, a novel series of 4-dimethylaminopyridinium (1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides (**6**–**19**) has been synthesized by the reactions of 3-methylthio-1,4,2-benzodithiazine1,1-dioxides with 4-DMAP and some active methylene compounds. The in vitro antitumor activity of these compounds has been tested in the National Cancer Institute (NCI), and relationships between structure and antitumor activity are discussed. Among the aminium salts 4-dimethylamino-pyridinium 4-chlorobenzoyl cyano (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**9**) was superior to other pyridinium salts in terms of both remarkable activity (log GI_{50} and log TGI < -8.00) and high selectivity for the lung HOP-92 and melanoma UACC-257 cell lines.

Keywords: Cancer-specific antitumor agents; Structural characterization; Aminium salts

1. Introduction

Recently, arylosulfonamides have attracted attention as anticancer [1–5] and antiviral agents [2,3]. We also described the syntheses of various 4-chloro-2-mercapto-benzenesulfonamide derivatives (**I**) (MBS As, Fig. 1) with the nitrogen atom of sulfonamide moiety attached to a variety of heteroaromatic ring systems. These compounds, depending on their structure, exhibited either anticancer or anti-HIV activities and have been described by Neamati et al. [6] as a novel class of potent HIV-1 integrase inhibitors. We further found that analogues of 2-mercaptobenzenesulfonamides (**II**, **III** and **IV**, Fig. 1) also showed anticancer properties [7,8].

Our recent investigations demonstrated that the reactions of 3-methylthio-1,4,2-benzodithiazine 1,1-dioxides with benzenesulfonamides in the presence of tertiary amines led to the formation of nonconventional aminium *N*-(1,1-dioxo-1,4,2-benzodithiazin-3-yl)-arylsulfonamidates (**IV**) (Fig. 1), some of which exhibited a pronounced anticancer activity [9]. Hence, in search for even more potent derivatives and for structure–activity relationship studies we synthesized a new series of aminium salts based on 1,1-dioxo-1,4,2-benzodithiazine ring system, namely 4-dimethylaminopyridinium (1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides of type **V** and **VI** (Fig. 1).

2. Results and discussion

The starting methylthiobenzodithiazines **1** [10,11], **2** [12], **3** [9] and **4** [11] were prepared according to the known methods. Analogous procedure [11] was applied for the synthesis of **5** (Scheme 1).

Upon treatment of **2**–**5** with 4-dimethylaminopyridine (4-DMAP) and compounds containing active

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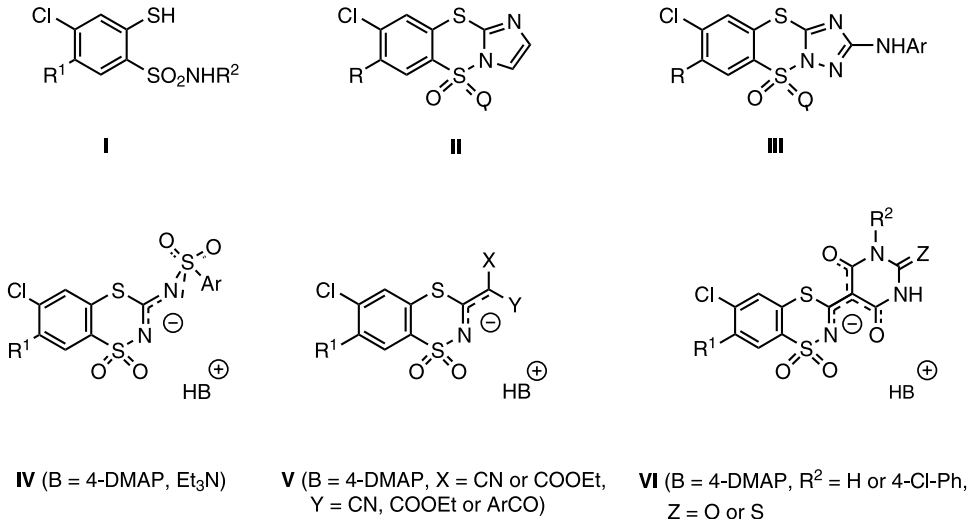
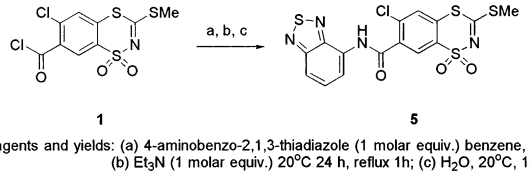
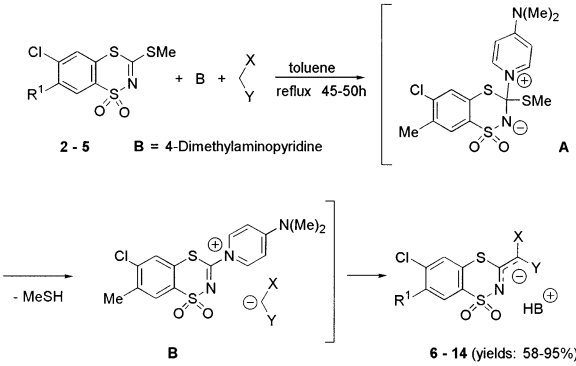


Fig. 1.

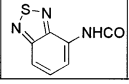


Scheme 1. Synthesis of *N*-(benzo-2,1,3-thiadiazol-5-yl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (**5**).

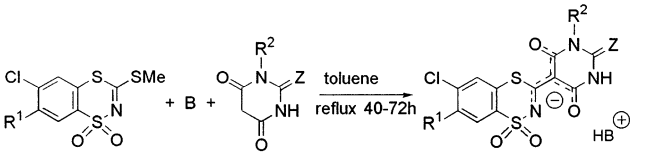
methylene group in toluene under reflux, the desired 4-dimethylaminopyridinium (1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides (**6**–**18**) were obtained (Schemes 2 and 3). The formation of these methanide salts is believed to arise from the following three-step process: addition of 4-DMAP to the C3–N2 double bond of



Compds	R^1	X	Y
2, 6	Me	CN	CN
2, 7	Me	CN	COOEt
2, 8	Me	CN	PhCO
2, 9	Me	CN	4-ClPhCO
2, 10	Me	4-O_2NPhCO	COOEt

Compds	R^1	X	Y
3, 11	H	CN	4-ClPhCO
4, 12	4-ClPhNHCO	CN	PhCO
4, 13	4-ClPhNHCO	CN	4-ClPhCO
5, 14		CN	4-ClPhCO

Scheme 2. Syntheses of the 4-dimethyaminopyridinium (6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides derivative (**6**–**14**).

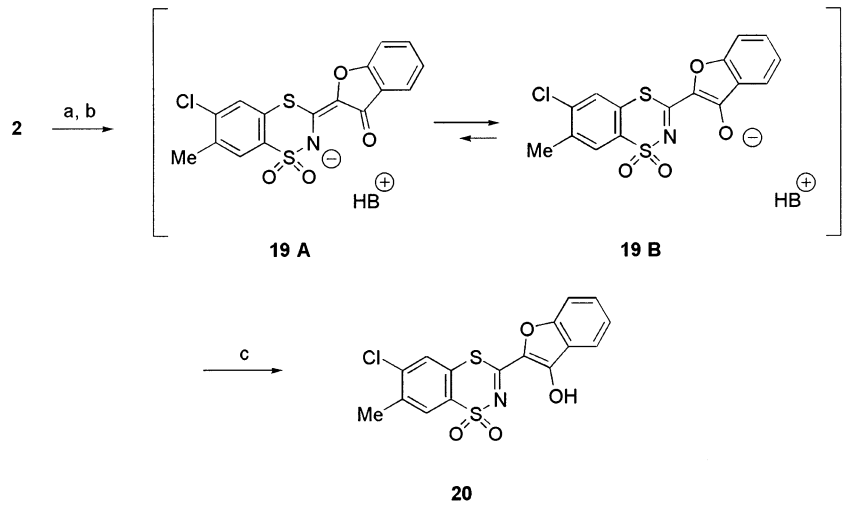


B = 4-Dimethylaminopyridine

Compds	R^1	R^2	Z
2, 15	Me	H	O
3, 16	H	H	O
2, 17	Me	4-ClPh	O
2, 18	Me	H	S

Scheme 3. Syntheses of the 4-dimethyaminopyridinium 5-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-perhydro-5-pyrimidinide derivatives (**15**–**18**).

benzodithiazine resulting in the intermediate **A**, elimination of methyl mercaptane with formation of pyridinium salt **B**, and finally nucleophilic displacement of the pyridinium moiety with active methylene compound (Scheme 2). A similar mechanism was proposed for the reactions of corresponding methylbenzodithiazines with benzenesulfonamides and a relatively stable adduct of type **A** was isolated [9]. Similarly to the previously described *N*-(1,1-dioxo-1,4,2-benzodithiazin-3-yl)-arylsulfonamidates [9], the aminium salts **6**–**18** proved to be very stable under acidic conditions due to conjugation of the unshared electron pair with π electrons of unsaturated bonds located α to the carbanionic carbon (Schemes 2 and 3). However, in case of the aminium salt **19** (Scheme 4) the oxygen atom of benzofurane moiety exerts destabilizing effect on adjacent carbanion, and the enolate **19B** contributes more to the hybrid than the amidate **19A**. Therefore, the compound **19** can be easily converted to the enol **20** on treatment with acetic acid under reflux for 1 min.



[a] Reagents and yields: (a) B = 4-dimethylaminopyridine (1 molar equiv.); (b) 2,3-dihydrobenzofuran-3-one, (1 molar equiv.), benzene, reflux 60h, 69%; (c) acetic acid, reflux 1 minute, 87%.

Scheme 4. Synthesis of the 4-dimethylaminopyridinium 2-(4-chloro-5-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)benzofuran-3-olate (**19**) and 6-chloro-3-(3-hydroxybenzofuran-2-yl)-7-methyl-1,4,2-benzodithiazine 1,1-dioxide (**20**).

The structures of the compounds **6**–**20** were confirmed by elemental analysis (C, H, N) and spectroscopic data presented in experimental section.

X-ray crystallography was undertaken on representative aminium salt **9** with a view to reveal some more discrete structural features of these compounds. The anion shows the E and Z configurations at the partially double C3–C15 and C15–C18 bonds, respectively (Fig. 2). This form of the molecule is stabilized by hypervalent S···O interaction with S4···O19 distance of 2.645(2) Å and C10–S4···O19 angle of 168.7(1)°. The 1,4,2–dithiazine ring adopts a conformation intermediate between boat and sofa.

The cation is hydrogen bonded to the anion via N1a–H···O12 interaction. Its nearly coplanar arrangement with the SO_2 group is due to another weak hydrogen bond C6a–H···O11.

Compounds **6**–**18** and **20** were submitted to the National Cancer Institute (Bethesda, MD) for testing against a panel of approximately 60 human tumor cell lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Details of this test system, and the information which is encoded by the activity pattern over all cell lines, have been published [13–15]. The antitumor 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), and log LC_{50}

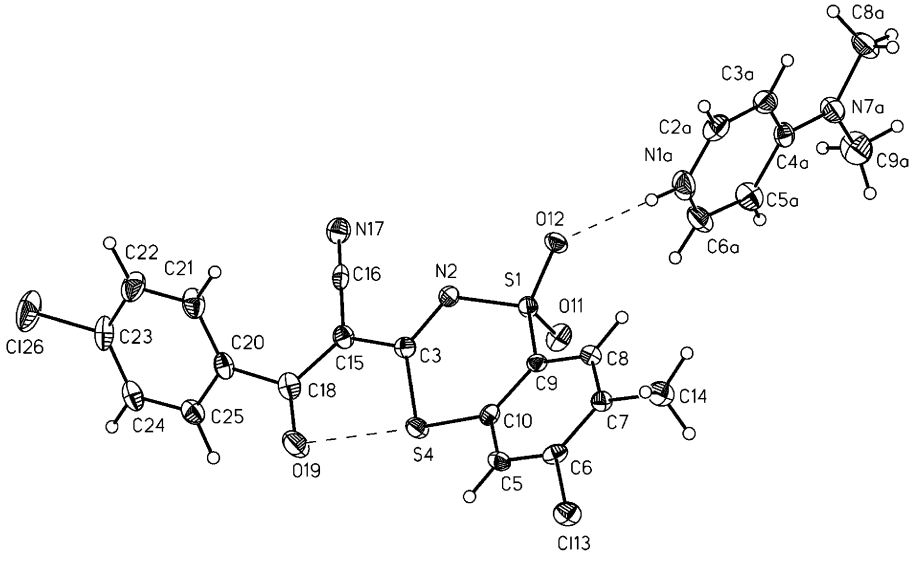


Fig. 2. ORTEP drawing showing atom labeling for **9**.

value (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 all cell lines. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. 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.

For a series of aminium salts obtained, the effects of structural modifications on antitumor activity were explored within two structural domains: benzodithiazine ring (substituents R^1), and substituents at methanide moiety. First, it was found that incorporation of methanide moiety into a barbituric acid derivative (general structure **VI**, Fig. 1) produced inactive compounds **15**–**18**. Within the 'open chain' analogues (general structure **V**, Fig. 1), compounds with R^1 substituents at position 7 of benzodithiazine ring other than CH_3 were also lacking any significant activity (**11**–**14**, $R^1 = H$, $\log GI_{50} < -4$), whereas the compounds **6**–**10** ($R^1 = CH_3$) exhibited moderate or fairly high activity against one or more human tumor cell lines (Table 1). The most active compounds were **8** and **9** indicating that combination of X = benzoyl and Y = cyano groups attached to methanide moiety results in compounds with optimal properties. The compound **9** (X = 4-ClPhCO) exhibited higher activity than **8** (X = PhCO), and showed higher selectivity for cell lines from the lung and melanoma subpanels (Table 2), namely this compound acted selectively as potent inhibitor ($\log GI_{50} < -8$, $\log TGI < -8$) against HOP-92 lung cancer line ($\Delta \log GI_{50} = 3.12$, $\Delta \log TGI = 3.40$) and UACC-257 melanoma cell line ($\Delta \log GI_{50} = 2{,}89$, $\Delta \log TGI = 3.40$).

3. Conclusion

We have developed a new class of potent anticancer agents, namely 4-dimethylamino-pyridinium 1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides some of which show a selectivity for lung HOP-92 and melanoma UACC-257 cancer cell lines. 4-Dimethylaminopyridinium 4-chlorobenzoyl cyano (6-chloro-7-methyl)-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**9**) may serve as a useful lead compound for the search for powerful and selective antineoplastic agents. Moreover, the combined data obtained for both sulfonamidate [9] and methanide aminium salts suggest that further modifications of an anionic moiety of these salts may lead to discovery of highly active compounds with selectivity against various cancer types.

4. Experimental

The following instruments and parameters were used; (melting points) Büchi 535 apparatus; (IR spectra) KBr pellets, 400–4000 cm^{-1} Perkin Elmer 1600 FTIR spectrometer; (^{1}H- and ^{13}C-NMR) Varian Gemini 200 apparatus at 200 and 50 MHz, respectively (chemical shifts are expressed as δ values relative to Me_4Si as standard). Analyses of C, H, N were within $\pm 0.4\%$ of the theoretical values.

4.1. Synthesis of N-(benzo-2,1,3-thiadiazol-5-yl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (5)

6-Chloro-7-chloroformyl-3-methylthio-1,1-dioxo-1,4,2-benzodithiazie (**1**) (8.1 g, 0.025 mol) was added portionwise to a solution of 4-amino-benzo-2,1,3-thiadiazole (3.8 g, 0.025 mol) in dry benzene (60 mL), and the reaction mixture was stirred at room temperature (r.t.) for 24 h. Then, a solution of Et_3N (2.53 g, 0.025 mol) in dry benzene (10 mL) was added dropwise and stirring was continued at r.t. for 12 h, followed by reflux for 1 h. After cooling to r.t., the solid that precipitated was collected by filtration, and then triturated with water (150 mL). The suspension thus obtained was stirred at r.t. for 1 h and the insoluble product **5** was separated by suction, and washed successively with water, 50% methanol (4 × 5 mL) and methanol (2 × 3 mL), (7.9 g, 69%); m.p. 208–210 °C. IR (KBr) 3330 (NH), 1660 (C=O), 1610 (C=N), 1330, 1160 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.73 (s, 3H, CH_3S), 7.73–7.91 (m, 2H, arom.), 8.18 (s, 1H, H-5, benzodithiazine), 8.33 (s, 1H, H-8, benzodithiazine), 8.42 (d, $J = 7.0$ Hz, 1H, arom.), 11.35 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6) δ 16.25 (CH_3S), 117.00, 118.34, 126.07, 128.12, 128.51, 129.79, 130.71, 132.41, 135.01, 137.89, 148.13, 154.70, 163.50, 181.47 ppm. Anal. Calc. ($C_{15}H_9ClN_4O_3S_4$): C, 39.42; H, 1.98; N, 12.26. Found: C, 39.54; H, 2.00; N, 12.52%.

4.2. General procedure for the preparation of 4-dimethyaminopyridinium (6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanides (6–14) and 5-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-2,4,6-trioxo(or 4,6-dioxo-2-thioxo)perhydro-5-pyrimidinides (15–18)

A mixture of 4-dimethylaminopyridine (1.92 g, 0.0157 mol), the corresponding methylthiobenzodithiazine **2**, **3**, **4** or **5** (0.015 mol) and the appropriate active methylene compound (0.016 mol) in dry toluene (50 mL for the reagents **2** and **3** or 200 mL for the reagents **4** and **5**) was refluxed with stirring until the evolution of CH_3SH had ceased (45–60 h) (caution: due to a high toxicity, MeSH should be trapped into an aqueous NaOH solution). Then, the reaction mixture was left overnight at r.t. and

Table 1
Overview of the results of the in vitro antitumor screening for compounds **6**–**18** and **20** [a]

Compd.	No. of the cell lines investigated	No. of the cell lines giving positive log GI_{50}, log TGI and log LC_{50}						MG_MID [e] and Δ [f] for		Most sensible cell lines
		log GI_{50} [b] [M]		log TGI [c]		log LC_{50} [M] [d]		log GI_{50}	log TGI	
		No	Range	No	Range	No	Range			
6	57	3	−4.54 to −4.00	0	> −4.00	0	> −4.00	−4.02 0.52	> −4.00 0.00	Lung cancer: HOP-92
7	57	1	−4.56	1	−4.04	0	> −4.00	−4.01 0.55	> −4.0 0.00	Lung cancer: HOP-92
8	57	13	−6.55 to −4.08	2	−6.00 to −4.06	0	> −4.00	−4.16 2.39	−4.00 1.97	Lung cancer: HOP-92 NCI-H322M
9	53	29	< −8.00 to −4.08	9	< −8.00 to −4.01	3	−4.18 to −4.15	−4.39 3.61	−4.17 3.83	Lung cancer: HOP-92 Melonoma: UACC-257
10	53	53	−5.49 to −4.19	27	−4.60 to −4.00	4	−4.15 to −4.01	−4.56 0.93	−4.12 0.50	Lung cancer: HOP-92 Melanoma: SK-MEL-2

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen (see Refs. [13,14] for details). Compounds **11**–**18** and **20** were inactive (log GI_{50} [M] > −4).
[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 is <1, moderate >1 and <3, high if >3.

Table 2
Inhibition of in vitro lung cancer and melanoma cell lines by selected compounds **8**, **9** and **10** [a]

Panel cell line MG_MID [e] and Δ [f]	Compd. **8**			Compd. **9**			Compd. **10**		
	log GI_{50} [b] [M]	log TGI [c] [M]	log LC_{50} [d] [M]	log GI_{50} [b] [M]	log TGI [c] [M]	log LC_{50} [d] [M]	log GI_{50} [b] [M]	log TGI [c] [M]	log LC_{50} [d] [M]
Non-small cell lung cancer									
A549/ATCC	[g]	[g]	[g]	−4.42	[g]	[g]	−4.41	[g]	[g]
HOP-62	[g]	[g]	[g]	−4.42	[g]	[g]	−4.53	−4.10	[g]
HOP-92	−6.48	−6.01	[g]	< −8.00	< −8.00	−4.18	−5.49	−4.62	−4.05
NCI–H 226	[g]	[g]	[g]	[g]	[g]	[g]	[g]	−4.29	[g]
NCI–H 322 M	−6.55	[g]	[g]	−4.37	−4.24	[g]	−4.96	−4.44	[g]
NCI–H 460	[g]	[g]	[g]	−4.37	[g]	[g]	4.46	[g]	[g]
NCI–H 522	−4.45	[g]	[g]	−4.54	[g]	[g]	−4.91	−4.46	−4.01
MG_MID [e]	−4.78	−4.28	−4.00	−4.87	−4.60	−4.02	4.68	4.27	4.01
Δ [f]	1.77	1.73	0.00	3.12	3.40	0.16	0.81	0.35	0.04
Melanoma									
MALM-3M	−4.68	−4.03	[g]	−6.08	−4.10	[g]	−4.84	−4.39	[g]
M 14	[g]	[g]	[g]	−4.38	[g]	[g]	−4.51	−4.02	[g]
SK-MEL-2	−4.08	[g]	[g]	−5.32	−4.08	[g]	−5.42	−4.59	[g]
SK-MEL-28	[g]	[g]	[g]	[g]	[g]	[g]	−4.55	−4.08	[g]
SK-MEL-5	[g]	[g]	[g]	[g]	[g]	[g]	−4.37	[g]	[g]
UACC-257	[g]	[g]	[g]	< −8.00	< −8.00	−4.15	−5.19	−4.60	[g]
UACC-62	[g]	[g]	[g]	[g]	[g]	[g]	−4.31	[g]	[g]
MG_MID [e]	−4.11	−4.00	−4.00	5.11	−4.60	−4.02	−4.74	−4.24	−4.00
? [f]	0.57	0.03	0.00	2.89	3.40	0.13	0.68	0.36	0.00

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen (see Refs. [13,14] for details). Compounds **11**–**18** and **20** were inactive (log GI_{50} [M] > −4).
[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 is <1, moderate >1 and <3, high if >3.
[g] The values of log GI_{50}, log TGI or log LC_{50} > −4.

the product that precipitated was collected by filtration, washed with toluene, dried an purified by crystallization from acetic acid.

In this manner the following products were obtained.

4.3. 4-Dimethylaminopyridinium dicyano (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**6**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and malononitrile (1.06 g), the title compound **6** was obtained (6.2g, 95%); m.p. 187–189 °C. IR (KBr) 3280 (NH); 2665, 1940, 1920 (NH^+), 2200 (C≡N), 1650 (C=N), 1345, 1335, 1150 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3-7), 3.17 (s, 6H, CH_3NCH_3), 6.95 (d, $J = 7.1$ Hz, 2H, β-pyrid.), 7.81 (s, 1H, H-5), 7.89 (s, 1H, H-8), 8.19 (d, $J = 7.1$ Hz, 2H, α-pyrid), 13.15 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 19.30, 50.07, 106.89, 107.10, 116.27, 117.31, 126.00, 127.73, 129.01, 131.52, 136.54, 136.94, 139.02, 156.91, 169.74 ppm. Anal. Calc. ($C_{18}H_{16}ClN_5O_2S_2$): C, 49.82; H, 3.72; N, 16.14. Found: C, 50.03; H, 3.70; N, 16.11%.

4.4. 4-Dimethylaminopyridinium cyano ethoxarbonyl (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**7**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and ethyl cyanoacetate (1.81 g), the title compound **7** was obtained (6.2 g, 86%); m.p. 167–168 °C; (IR (KBr) 3260 (NH), 2675, 2575, 1950 (NH^+), 2190 (C≡N), 1680 (C=O), 1650(C=N), 1345, 1145 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.19 (t, 3H, CH_3CH_2), 2.39 (s, 3H, CH_3-7), 3.17 (s, 6H, CH_3NCH_3), 4.07 (q, 2H, CH_3CH_2), 6.96 (d, $J = 7.7$ Hz, 2H, β-pyrid.), 7.67 (s, 1H, H-5), 7.83 (s, 1H, H-8), 8.20 (d, $J = 7.7$ Hz, 2H, α-pyrid.), 13.15 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 14.53, 19.25, 58.98, 74.90, 106.87, 118.60, 125.33, 127.23, 132.11, 132.22, 135.94, 136.06, 138.99, 156.87, 166.11, 170.38

ppm. Anal. Calc. ($C_{20}H_{21}ClN_4O_2S_2$): C, 49.49; H, 4.40; N, 11.65. Found: C, 49.64; H, 4.58; N, 11.81.%

4.5. 4-Dimethylaminopyridinium benzoyl cyano (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**8**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and benzoylacetonitrile (2.3 g), the tile compound **8** was obtained (7.1 g, 92%); m.p. 190–191 °C. IR (KBr) 3250 (NH), 2810, 2695 (NH^+), 2195 (C≡N), 1650 (C=O), 1595, (C=N), 1360, 1345 (SO_2) cm^{-1}. ^{1} H-NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3-7), 3.17 (s, 6H, CH_3NCH_3), 6.95 (d, $J = 7.1$ Hz, 2H, β-pyrid.), 7.40–7.68 (m, 5H, Ph), 7.74 (s, 1H, H-5), 7.87 (s, 1H, H-8), 8.19 (d, $J = 7.1$ Hz, 2H, α-pyrid.), 13.15 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{24}H_{21}ClN_4O_3S_2$): C, 56.18; H, 4.13; N, 10.92. Found: C, 56.05; H, 4.30; N, 11.07%.

4.6. 4-Dimethylaminopyridinium 4-chlorobenzoyl cyano (6-chloro-7-methyl)-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**9**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and 4-chlorobenzoylacetonitrile (2.87 g), the title compound **9** was obtained (7.7 g, 93%); m.p. 225–226 °C. IR (KBr) 3255 (NH), 2845, 2675,1955, 1925 (NH^+), 1650 (C=O), 1605 (C=N), 1345, 1145 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3-7), 3.17 (s, 6H, CH_3–N–CH_3), 6.96 (d, $J = 7.7$ Hz, 2H, β-pyrid.), 7.50 (d, $J = 8.6$ Hz, 2H, Ph), 7.68 (d, $J = 8.6$ Hz 2H, Ph), 7.75 (s, 1H, H-5), 7.88 (s, 1H, H-8), 8.20 (d, $J = 7.7$ Hz, 2H, α-pyrid.), 13.18 (br, s, 1H, NH^+), ppm. ^{13}C-NMR (DMSO-d_6) δ 19.28, 85.75, 106.89, 107.10, 120.59, 125.37, 127.35, 127.95, 129.57, 131.90, 132.61, 135.14, 136.25, 136.58, 138.75, 139.05, 156.89, 172.30, 186.73 ppm. Anal. Calc. ($C_{24}H_{20}Cl_2N_4O_3S_2$): C, 52.65; H, 3.68; N, 10.23. Found: C, 52.78; H, 3.81; N, 10.11%.

4.7. 4-Dimethylaminopyridinium ethoxycarbonyl 4-nitrobenzoyl (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanidie (**10**)

Starting from 6-chloro-7methyl-3-metylthio-1,4,2-benzodithiazine 1,1,-dioxide (**2**) (4.41 g) and ethyl 4-nitrobenzoylacetate (3.79 g), the title compound **10** was obtained (5.3 g, 58%); m.p. 199–200 °C dec. IR (KBr) 3185 (NH), 2830, 2670, 2440, 2375 (NH^+), 1670 (C=O), 1625 (C=N), 1590 (C=N), 1340, 1145 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 0.87 (t, 3H, CH_3CH_2), 2.36 (s, 3H, CH_3-7), 3.16 (s, 6H, CH_3–N–CH_3), 3.87 (q, 2H, CH_3CH_2), 6.94 (d, $J = 7$ Hz, 2H, β-pyrid.), 7.61-7.79 (m, 4H, arom.), 8.17–8.26 (m, 4H, arom.), 13.15 (b, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 13.88, 19.21, 58.89, 105.19, 106.89, 123.36, 124.97, 127.28, 128.69, 133.27, 133.52, 135.29, 135.52, 139.04, 146.75, 148.21, 156.90, 164.96, 166.72, 188.69 ppm. Anal. Calc. ($C_{26}H_{25}ClN_4O_7S_2$): C, 51.60; H, 4.16; N, 9.26. Found: C, 51.67; H, 4.10; N, 9.30%.

4.8. 4-Dimethylaminopyridinium 4-chlorobenzyl cyano (6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)methanide (**11**)

Starting from 6-chloro-3-methylthio-1,4,2-benzodithiazine-1,1-dioxide (**3**) (4.2 g) and 4-chlorobenzoylacetonitrile (2.87 g), the title compound **11** was obtained (7.0 g, 87%); m.p. 216.5–217.5 °C. IR (KBr) 3230 (NH), 2840, 2670, 2.500, 2360, 1930 (NH^+), 2195 (C≡N), 1650 (C=O), 1590 (C=N), 1360, 1160, (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.17 (s, 6H, CH_3–N–CH_3), 6.95 (d, $J =$ 7.1.Hz, 2H, β-pyrid.) 7.48–7.89 (m. 7H, arom.), 8.19 (d, $J = 7.1$ Hz, 2H, α-pyrid.), 13.15 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{23}H_{18}Cl_2N_4O_3S_2$): C, 51.78; H, 3.40; N, 10.50. Found: C, 51.60; H, 3.56; N, 10.79%.

4.9. 4-Dimethylaminopyridinium benzoyl cyano [6-chloro-7-(4-chlorophenyl-carbamoyl)-1,1-dioxo-1,4,2-benzodithiazin-3-yl]methanide (**12**)

Starting from *N*-(4-chlorophenyl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide 4 (6.5 g) and benzoylacetonitrile (2.3 g), the title compound **12** was obtained (6.9 g, 70%); m.p. 151–153 °C. IR (KBr) 3240 (NH), 2840, 2670 (NH^+), 2196 (C≡N), 1650 (C=O), 1595 (C=N), 1365, 1150 (SO2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.16 (s, 6H, CH_3NCH_3), 6.95 (d, $J = 7.7$ Hz, 2H, β-pyrid.), 7.41–7.51 (m, 4H, arom.), 7.96 (s, 1H, H-5), 8.02 (s, 1H, H-8), 8.19 (d, $J =$ 7.7 Hz, 2H, α-pyrid.), 10.83 (s, 1H, NH), 13,10 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 86.62, 107.21, 107.36, 107.40, 120.77, 121.66, 123.77, 128.01, 128.19, 128.83, 129.05, 130.98, 132.41, 133.05, 136.33, 137.46, 137.86, 139.37, 140.13, 157.20, 163.51, 188.66 ppm. Anal. Calc. ($C_{30}H_{23}Cl_2N_5O_4S_2$): C, 55.21; H, 3.55; N, 10.73. Found: C, 55.12, H, 3.72; N, 10.59%.

4.10. 4-Dimethylaminopyridinium 4-chlorobenzoyl cyano [6-chloro-7-(4-chloro-phenylcarbamoyl)-1,1-dioxo-1,4,2-benzodithiazin-3-yl]metanide (**13**)

Starting from *N*-(4-chlorophenyl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (**4**) (6.5 g) and 4-chlorobenzoylacetonitrile (2.87 g), the title compound **13** was obtained (6.9 g, 67%); m.p. 211–213 °C. IR (KBr) 3265 (NH) 2850, 2690 (NH^+), 2195 (C≡N), 1680, 1650, (C=O), 1580 (C=N), 1355, 1155 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.16 (s, 6H. CH_3–N–CH_3), 6.95 (d, $J = 7.1$ Hz, 2H, β-pyrid.), 7.40–7,75 (m, 8H, arom), 7.97 (s, 1H, H-5), 8.01 (s, 1H, H-8),

8.19 (d, $J = 7.1$ Hz, 2H, α-pyrid.), 10.82 (s, 1H, NHCO), 13.15 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{30}H_{22}Cl_3N_5O_4S_2$): C, 52.44; H, 3.23; N, 10.19. Found: C, 52.59; H, 3.09; N, 10.39%.

4.11. 4-Dimethylaminopyridinium 4-chlorobenzoyl cyano [6-chloro-7-(benzo-2,1,3-thiadiazol-5-ylcarbamoyl)-1,1-dioxo-1,4,2-benzodithiazin-3-yl]methanide (**14**)

Staring from *N*-(Benzo-2,1,3-thiadiazol-5-yl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (**5**) (6.85 g) and 4-chlorobenzoylacetonitrile (2.87 g,) the title compound **14** was obtained (9.9 g, 91%); m.p. 205–206 °C. IR (KBr) 3240 (NH), 2850, 2675 (NH^+), 2195 (C≡N), 1670, 1650 (C=O), 1600 (C=N), 1365, 1150 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.17 (s, 6H, CH_3NCH_3), 6.96 (d, $J = 6.1$ Hz, 2H, β-pyrid.), 7.08–7.25 (m, 1H, arom), 7.45–7.56 (m, 2H, arom), 7.70–7.97 (m, 4H, arom), 8.08 (s, 1H, H-8), 8.20 (d, $J = 6$.1 Hz, 2H, α-pyrid.), 8.39 (d, $J = 7$ Hz, 1H, arom.), 11.29 (s, 1H, NHCO), 13.18 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{30}H_{21}Cl_2N_7O_4S_3$): C, 50.07; H, 2.94; N, 13.62. Found: C, 49.96; H, 3.16; N, 13.42%.

4.12. 4-Dimethylaminopyridinium 5-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodi-thiazin-3-yl)-2,4,6-trioxoperyhydro-5-pyrimidinide (**15**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and barbituric acid (2.04 g), the title compound **15** was obtained (4.2 g, 56%); m.p. 289–290 °C dec. IR (KBr) 3225, 3180 (NH), 2840, 2660, 2300, 2115, 1960 (NH^+), 1730, 1705, (C=O), 1630 (C=N), 1370, 1345, 1150 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.51 (s, 3H, CH_3-7), 3.28 (s, 6H, CH_3NCH_3), 7.07 (d, $J = 7.4$ Hz, 2H, β-pyrid.), 7.77 (s, 1H, H-5), 7.95 (s, 1H, H-8), 8.30 (d, 2H, $J = 7.4$ Hz, α-pyrid.), 10.22 (s, 2H, NH–CO–NH), 13.25 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 19.54, 93.42, 107.14, 125.72, 127.47, 130.91, 134.74, 136.38, 139.29, 150.44, 157.15, 163.37, 170.07 ppm. Anal. Calc. ($C_{19}H_{18}ClN_5O_5S_2$): C, 46.01; H, 3.66; N, 14.12. Found: C, 46.15; H, 3.42; N, 14.25%.

4.13. 4-Dimethylaminopyridinium 5-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-2,4,6-trioxoperhydro-5-pyrimidinide (**16**)

Starting from 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine (**3**) (4.2 g) and barbituric acid (2.04 g) the title compound **16** was obtained (4.1 g, 58%); m.p. 283–284 °C dec. IR (KBr) 3400, 3290 (NH), 2825, 2635, 2165, 2100, 1980, 1950 (NH^+), 1705, 1675, (C=O), 1640 (C=N), 1375, 1355, 1155, 1130 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3,17 (s, 6H, $CH_3N–CH_3$), 6.95 (d, $J = 7.4$ Hz, 2H, β-pyrid.), 7.55 (d, $J = 8.3$ Hz, 1H, H-7), 7.70 (s, 1H, H-5), 7.84 (d, $J = 8.3$ Hz, 1H, H-8), 8.19 (d, $J = 7.4$ Hz, 2H, α-pyrid.), 10.13 (s, 2H, NH–CO–NH), 13.12 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 93.16, 106.84, 125.20, 126.88, 128.21, 130.82, 135.57, 137.83, 139.05, 150.14, 156.87, 163.09, 169.60 ppm. Anal. Calc. ($C_{17}H_{16}ClN_5O_6S_2$): C, 43.45; H, 3.43; N, 14.90. Found: C, 43.60; H, 3.57; N, 14.86%.

4.14. 4-Dimethylaminopyridinium 1-(4-chlorophenyl)-5-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-2,4,6-trioxoperhydro-5-pyrimidinide (**17**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and 1-(4-chlorophenyl)barbituric acid (3.8 g), the title compound **17** was obtained (6.9 g, 75%); m.p. 178–180 °C. IR (KBr) 3225, 3175, (NH), 2850, 2680 (NH^+), 1705, 1675, 1645 (C=O), 1620 (C=N), 1345, 1150, (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.40 (s, 3H, H-7), 3.17 (s, 6H, CH_3–N–CH_3), 6.95 (d, $J = 7.7$ Hz, 2H, β-pyrid.), 7.24 (d, $J = 8.6$ Hz, 2H, arom.), 7.47 (d, $J = 8.6$ Hz, 2H, arom.), 7.65 (s, 1H, H-5), 7.85 (s, 1H, H-8), 8.19 (d, $J = 7.7$ Hz, 2H, α-pyrid.), 10.52 (s, 1H, NH), 13.20 (br, s, 1H, NH^+) ppm. ^{13}C-NMR (DMSO-d_6) δ 19.26, 93.12, 106.87, 125.55, 127.15, 128.39, 130.53, 131.52, 131.99, 134.42, 135.31, 136.18, 139.05, 150.02, 156.87, 161.78, 161.98, 170,19 ppm. Anal. Calc. ($C_{25}H_{21}Cl_2N_5O_5S_2$): C, 49.51; H, 3.49; N, 11.55. Found: C, 49.63; H, 3.61; N, 11.50%.

4.15. 4-Dimethylaminopyridinium 5-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodi-thiazin-3-yl)-4,6-dioxo-2-thioxoperhydro-5-pyrimidinide (**18**)

Starting from 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide (**2**) (4.41 g) and thiobarbituric acid (2.3 g), the title compound **18** was obtained (3.9 g, 51%); m.p. 271–272 °C dec. IR (KBr) 3440, 3350, 3180 (NH), 2870, 2600, 2145, 1990 (NH^+), 1680, 1645 (C=O), 1615 (C=N), 1340, 1145, 1130 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3-7), 3.17(s, 6H, CH_3–N–CH_3), 6.96 (d, $J = 6.7$ Hz, 2H, β-pyrid.), 7.69 (s, 1H, H-5), 7.87 (s, 1H, H-8), 8.19 (d, $J = 6.7$ Hz, 2H, α-pyrid.), 11.32 (s, 1H, NH), 11.37 (s, 1H, NH), 12.8–13.2 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{19}H_{18}ClN_5O_4S_3$): C, 44.57; H, 3.54; N, 13.68. Found: C, 44.51; H, 3.43; N, 14.00%.

4.16. Preparation of 4-dimethylaminopyridinium 2-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)benzofuran-3-olate (**19**) and 6-chloro-3-(3-hydroxybenzofuran-2-yl)-7-methyl-1,4,2-benzodithiazine 1,1-dioxide (**20**)

A solution of methylthiobenzodithiazine (**2**) (4.41 g, 0.015 mol), DMAP (1.83 g, 0.015 mol) and 2,3-dihydrobenzofuran-3-one in dry toluene (45 mL) was

refluxed with stirring until the evolution of CH_3SH had ceased (60 h). After cooling to r.t. the suspension was left overnight. The precipitate was collected by filtration, washed successively with toluene(6×3 mL) and methanol (10×3 mL), and dried. The title pyridinium salt **19** was obtained (5.2 g, 69%); m.p. 230–232 °C. IR (KBr) 3195 (NH), 2680, 2660, 1945 (NH^+), 1645 (C=N), 1345, 1165 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 3.16 (s, 6H, CH_3N–CH_3), 6.95 (d, $J = 7.6$ Hz, 2H, β-pyrid.), 7.09–7.71 (m, 5H, arom), 8.17 (s, 1H, benzodithiazine), 8.19 (d, $J = 7.6$ Hz, 2H, α-pyrid.), 13.15 (br, s, 1H, NH^+) ppm. Anal. Calc. ($C_{23}H_{20}ClN_3O_4S_2$): C, 55.03; H, 4.01; N, 8.37. Found: C, 55.22; H, 4.17; N, 8.40%.

A solution of the pyridinium salt **19** (3 g, 0.006 mol) in acetic acid (80 mL) was refluxed for 1 min and then cooled to r.t. After 2 h the product **20** that precipitated was collected by filtration, washed successively with acetic acid (2×2 mL) and toluene (4×2 mL), and dried (2g, 87%); m.p. 287–288 °C. IR (KBr) 3090 (OH), 1615 (C=N), 1595 (C=C), 1340, 1160 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.47 (s, 3H, CH_3), 7.3–7.4 (br, s, 1H, OH), 7.64 (d, $J = 3.6$ Hz, 2H, arom.), 7.99 (s, 1H, H-5, benzodithiazine), 8.04 (s, 1H, H-8, benzodithiazine), 8.08 (d, $J = 6.7$ Hz, 2H, arom.) ppm. ^{13}C-NMR (DMSO-d_6) δ 19.71, 107.38, 112.90, 121.67, 122.62 123.70, 126.93, 128.43, 128.87, 129.61, 131.74, 137.93, 138.89, 155.01, 155.34, 159.75 ppm. Anal. Calc. ($C_{16}H_{10}ClNO_4S_2$): C, 50.59; H, 2.65; N, 3.69. Found: C, 50.42; H, 2.61; N, 3.76%.

4.17. X-ray structure analysis of **9**

Crystal data for $(C_{14}H_9Cl_2N_2O_3S_2)^-(C_7H_{11}N_2)^+$: monoclinic, space group $P2_1/c$, $a = 9.6127(3)$, $b = 28.706(1)$, $c = 9.2904(3)$ Å, $\beta = 101.505(3)^\circ$, $V = 2512.1(1)$ Å^3, $Z = 4$, $d_x = 1.448$ g cm^{-3}, $T = 130$ K. Data were collected for a crystal with dimensions $0.4 \times 0.15 \times 0.02$ mm^3 on a KumaCCD diffractometer using graphite monochromated Mo $K\alpha$ radiation. Final R indices for 3881 reflections with $I > 2\sigma(I)$ and 336 refined parameters are: $R_1 = 0.0481$, $wR_2 = 0.0859$ ($R_1 = 0.0652$, $wR_2 = 0.0928$ for all 4588 data). Atom labeling is shown in Fig. 2.

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