Full Paper

Synthesis, Cytotoxicity Testing, and Structure–Activity Relationships of Novel 6-Chloro-7-(4-phenylimino-4H-3,1-benzoxazin-2-yl)-3-(substituted)-1,4,2-benzodithiazine 1,1-dioxides

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A new series of 16 6-chloro-1,1-dioxo-7-{4-[4-R1-phenyl]imino-4H-3,1-benzoxazin-2-yl}-3-(substituted amino)-1,4,2-benzodithiazines 7–22 was prepared in order to evaluate the cytotoxic activity against six human cancer cell lines. The structures of the new compounds were confirmed by IR, 1H-, and 13C-NMR, elemental analysis and in the cases of 11 and 31 by X-ray crystal structure analysis. This analysis showed that contrary to our earlier report the structures contain a benzoxazine ring instead of the proposed quinazolinone ring. The bioassay indicated that the benzodithiazine derivatives 7–22 possess cancer cell growth-inhibitory properties. Some compounds showed a high level of selectivity for certain cell lines. The most active compounds 11, 12, 16, 19, 21, and 22 exhibited potency higher or comparable to cisplatin. The compounds were particularly effective in LCLC-103H and MCF-7 cell lines with IC50 values of 0.49–1.60 μM. Quantitative structure activity relationships (QSAR) revealed that a chloro substituent R1 in the phenyl ring as well as the shape of the substituted amino group at R2 (e.g., unsaturation is beneficial) are important for potency.

Keywords: 6-Chloro-7-(4-phenylimino-4H-3,1-benzoxazin-2-yl)-3-(substituted)-1,4,2-benzodithiazine 1,1-dioxides / Cytotoxic activity / QSAR

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Introduction

Aryl and heteroarylsulfonyl sulfonamides are attracting attention as anticancer agents. Our systematic studies on the synthesis of 1,4,2-benzodithiazine-1,1-dioxides and their subsequent transformation into N-(azolyl or azinyl)-2-mercaptobenzenesulfonamides A (Fig. 1) have resulted in promising anticancer agents [1–5] and potent inhibitors of HIV-1 integrase (MBSAs) [6]. We also found that cyclic sulfonamide derivatives of 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-b][1,4,2]benzodithiazine (B, Fig. 1) [7, 8] or of type C–D (Fig. 1) possess interesting in-vitro anticancer properties [9–11]. In addition, various 1,3-benzoxazine derivatives have been found to show versatile bioactivities such as antimicrobial, antiviral, antifungal [12], anti-human coronavirus [13], and anticancer activity [14].

In the search for more potent and selective agents against cancer, we report the synthesis of 16 new compounds of type E and the results of their in-vitro evaluation for cytotoxic activity. The scaffold of this class of compounds consists of...
two moieties: 1,1-Dioxo-1,4,2-benzodithiazine and 1,3-benzoxazine. The objective of this drug-design approach was to merge these two moieties to enhance their activity against cancer cells. Our previous studies with this class of compounds showed that electronic character of the benzodithiazine ring system substituent at position-3 was an important factor influencing cytotoxicity [15]. However, in this previous publication we reported that the compounds were quinazolinone derivatives. Based on X-ray crystal structure analysis of two representative compounds we now correct the structures to be benzoxazine derivatives. Herein, the effects of further structural modifications on antitumor activity were explored within two structural domains: The benzodithiazine ring (substituent \( R^2 \)) and substituents of benzoxazine moiety (\( R^1 \)-phenyl). A correlation between the structures of these derivatives and the potency to inhibit the growth of cancer cells was investigated by using quantitative structure activity relationship (QSAR) methods.

Results and discussion

Chemistry

The previously described methods were employed for the synthesis of 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazin-7-carbonyl chloride 1 [16, 17]. The reaction of 1 with the appropriate 2-aminobenzanilide was carried out in boiling toluene in the presence of pyridine and afforded the appropriate 2-aminobenzanilide was carried out in boiling methanol proceeded with elimination of methyl mercaptane, thiol group of benzodithiazin-2-yl-3-R\(^1\)-1,4,2-benzodithiazine dioxides (Figs. 2–4), and not the quinazolinone structure we previously reported [15].

The mechanism of the reaction pathway was not investigated but it can be postulated as follows. First, the reaction of carboxamide 2 or 3 with thionyl chloride involves the initial formation of unstable intermediate A, which with evolution of the hydrogen chloride giving rise to formation intermediate B. In turn, the latter intermediate subsequently undergoes an intramolecular cyclocondensation with evolution molecule of the sulfur dioxide to afford the final products 4–6. Carboxamides 2 or 3 did not undergo the intramolecular cyclization (Scheme 1) in the presence of dehydrating agents such as phosphorous oxychloride or thionyl chloride to obtain quinazolinone C [18–20].

Furthermore, nucleophilic displacement of the 3-methylthiol group of 4–6 by the appropriate amine in boiling methanol proceeded with elimination of methyl mercaptan, leading to the target benzodithiazines 7–22 in 27-78% yields (Scheme 2). All final products were characterized by IR and NMR spectroscopy as detailed in the experimental section. Elemental analyses were in accordance with the proposed structures.

Molecules 11 and 31 [15] with their labeling scheme are shown in Figures 2 and 3. Bonding geometries of the benzoxazine-4-imine and 1,4,2-benzodithiazin-3-amino 1,1-dioxide units are similar to those found in previously reported structures [10, 11], [21–24]. Bond lengths indicate a double bond character at C14-N19 [1.274(2) and 1.254(6) Å] and C16-N24 [1.262(3) and 1.257(6) Å], a relevant degree of single bond for C25-N24 [1.417(3) and 1.426(6) Å] and a strong conjugation in the amidine N3-C3-N31 fragment with the C–N bond lengths in the range 1.299(7) to 1.320(8) Å.

The analogous parts for the two molecules have very similar conformations as shown in Fig. 4, where superposition of the molecular structures of 11 and 31 is presented. A 1,1-dioxo-1,4,2-dithiazine ring with the lone pairs of electrons on the S atom is known to prefer the boat conformation [25] and this conformation is adopted by this ring in both molecules, leading to their butterfly shape. The central part that comprises a 3,1-benzoxazine system and the fragment of 1,4,2-benzodithiazine unit, consisting of the benzene ring and S substituent atoms, is virtually planar with dihedral angles between the two parts being 3.6 and 6.1° in 11 and 31, respectively.

Biology

A microtiter based assay based on the staining of adherent cells with crystal violet was used to quantify the antiprolifer-
Primary screening of compounds 7–22 for antiproliferative activity took place on three human cancer cell lines: A-427, DAN-G and LCLC-103. All compounds showed inhibition of cell growth by more than 50% at 20 μM in one or more of the cell lines. Secondary screening to determine potency was performed on a panel of 6 human cancer cell lines: RT-4 (urinary bladder transitional cell cancer), 5637 (urinary bladder cancer), DAN-G (pancreas cancer), LCLC-103H (large cell lung cancer), A-427 (lung cancer), and MCF-7 (breast cancer). Table 1 lists the average IC₅₀ values calculated from the dose-response data obtained from three independent experiments. The IC₅₀ is the concentration required to inhibit cell growth by 50% compared to the untreated control over a 96 h treatment period [26].

The present results show that further structural modification of earlier tested benzodithiazine derivatives [15] can lead to further increases in potency. In the series of derivatives 7–22, the compounds differ in a substituent at the position 3 of the benzodithiazine scaffold and in substitution in the position 4 of the phenyl attached to the benzoxazine moiety (chlorine, hydrogen or methyl group).

Based on the substituent at position 3 (R₂), compounds can be divided into three groups: 9–11 possessing an aliphatic chain, 12–22 with a pyridine moiety and 7–8 with the morpholine entity. The cytotoxicity of compounds with the morpholine moiety is much lower than of the other benzodithiazines, although their selectivity towards the 5637 cell line is apparent. The most active group appears to be those with pyridine substituent. However, the mean IC₅₀ value of the compound 11 with allylamino group is lower comparable to pyridine substituted compounds 12, 16, and 21.

A comparison with activity of anticancer-agent cisplatin indicates that benzodithiazines 7–22 possess very good cell
growth inhibitory properties. The values of the IC$_{50}$ were taken from a previously published study [26] conducted in analogous conditions to our assay. In general these results show the greatest similarities in activity of tested benzodithiazines to the alkylating agents like cisplatin and DACH-Pt. The LCLC-103H cell line is the most sensitive of the six cell lines. In this cell line some of the compounds showed similar (16, 19, and 21) or even greater (10–13 and 22) potency compared to cisplatin. Moreover, the RT-4 cell line was susceptible to cell growth inhibition by $12 < 19 < 22 < 11 < 14 < 16$, which was similar or greater than cisplatin in the case of compounds 13, 21, and 18.

### Quantitative structure activity relationship studies

**QSAR** are frequently used in medicinal chemistry to establish a predictive relationship between structure and potency [28]. In search of possible QSAR with our data, multiple regression analysis was performed with 18 quantitative descriptors for the amines at R$_2$ and two indicator variables for either a chlorine atom or a methyl group at R$_1$ (see Table S1, Supplementary Material). The dependent variable was the –log of the IC$_{50}$ values. The compounds used in the QSAR analysis included the 16 compounds described in this work as well as 8 compounds, 23–30 (Scheme 2) reported in an earlier study [15]. The range in the IC$_{50}$ values for the...
Figure 2. View of the molecular structure of 11 with the DMSO solvent molecule. Displacement ellipsoids are drawn at the 50% probability level. Disorder of the allyl group is not shown.

Figure 3. View of the molecular structure of 13 [15] with the isopropanol solvent molecule. Displacement ellipsoids are drawn at the 50% probability level. Disorder of the phenylethylene group is not shown.

Figure 4. Superposition of the molecules 1 and 13 [15] (only the fitted atoms are labeled; r.m.s. 0.054 Å).

| Table 1. IC₅₀ values (µM) for the inhibition of in-vitro cell growth of human cancer cell lines by compounds 7-22. | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human cell line | | | | | | | | | | | | | | | | | |
| K14 | 4.5 | 2.4 | 2.2 | 1.1 | 2.8 | 8.4 | 1.6 | 3.6 | 6.0 | 1.0 | 2.9 | 6.0 | 2.8 | 1.6 | 2.0 | 3.6 | 6.0 |
| IC50 μM | 4.5 | 2.4 | 2.2 | 1.1 | 2.8 | 8.4 | 1.6 | 3.6 | 6.0 | 1.0 | 2.9 | 6.0 | 2.8 | 1.6 | 2.0 | 3.6 | 6.0 |
| Average | 4.5 | 2.4 | 2.2 | 1.1 | 2.8 | 8.4 | 1.6 | 3.6 | 6.0 | 1.0 | 2.9 | 6.0 | 2.8 | 1.6 | 2.0 | 3.6 | 6.0 |
| RSDe (%) | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 | 1.5 | 0.58 |
| a Values are averages of three independent determinations. | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |
| b Values were from [21]. | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |
| " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |
| c Nd – not determined. | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |
| d Averaged IC₅₀ values over all tested cancer cell lines. | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |
| e Relative standard deviation. | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " | " |

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24 compounds was ca. 20-fold in the RT-4, DAN-G and the MCF-7 lines, 12-fold in the LCLC-103H line and less than 6-fold in the remaining two lines. Both the IC\textsubscript{50} values for the individual cell lines as well as the average IC\textsubscript{50} values over all six lines where included in the analysis. The single most important parameter equation the kappa shape index, order 1 (KSI-1) [29] has a positive effect while too large a molar volume (MV) is detrimental.

\begin{align*}
-\log(\text{IC}_{50})_{\text{all lines}} &= 0.024(\pm 0.006) \text{USA} + 0.306(\pm 0.091) \text{IC}_1 + 4.336(\pm 0.249) \\
& n = 24 \quad rCv^2 = 0.400 \quad r = 0.768 \quad s = 0.252 \quad F = 15.139
\end{align*}

(1)

\begin{align*}
-\log(\text{IC}_{50})_{\text{all lines}} &= 0.442(\pm 0.094) \text{KSI}_1 - 0.024(\pm 0.008) \text{MV} + 0.367(\pm 0.074) \text{IC}_1 + 4.811(\pm 0.175) \\
& n = 24 \quad rCv^2 = 0.585 \quad r = 0.861 \quad s = 0.282 \quad F = 19.082
\end{align*}

(2)

In the cases of three individual cell lines where significant correlations where found, the unsaturated surface area of the group at R\textsuperscript{2} and the presence of a chlorine atom at R\textsuperscript{1} were again important determinates for activity. This was particularly apparent when the data was fitted to the two best parameters, as in the cases of Eqs. (3), (5), and (7). In the cases of the cell lines LCLC-104H and RT-4, inclusion of a second indicator variable for the methyl group at R\textsuperscript{1} improved the correlation considerably (see Eqs. (4) and (6)). This was not unexpected because the methyl is bioisotopic with Cl.

\begin{align*}
-\log(\text{IC}_{50})_{\text{LCLC}} &= 0.011(\pm 0.003) \text{USA} + 0.568(\pm 0.135) \text{IC}_1 + 5.048(\pm 0.131) \\
& n = 24 \quad rCv^2 = 0.419 \quad r = 0.747 \quad s = 0.350 \quad F = 13.23
\end{align*}

(3)

\begin{align*}
-\log(\text{IC}_{50})_{\text{LCLC}} &= 0.010(\pm 0.003) \text{USA} + 0.718(\pm 0.123) \text{IC}_1 + 0.462(\pm 0.148) \text{IC}_3 + 4.925(\pm 0.117) \\
& n = 24 \quad rCv^2 = 0.597 \quad r = 0.838 \quad s = 0.393 \quad F = 15.72
\end{align*}

(4)

\begin{align*}
-\log(\text{IC}_{50})_{\text{RT4}} &= 0.013(\pm 0.003) \text{USA} + 0.4544(\pm 0.119) \text{IC}_3 + 4.912(\pm 0.115) \\
& n = 24 \quad rCv^2 = 0.502 \quad r = 0.767 \quad s = 0.327 \quad F = 15.01
\end{align*}

(5)

\begin{align*}
-\log(\text{IC}_{50})_{\text{RT4}} &= 0.012(\pm 0.003) \text{USA} + 0.575(\pm 0.112) \text{IC}_3 + 0.371(\pm 0.135) \text{IC}_3 + 4.813(\pm 0.106) \\
& n = 24 \quad rCv^2 = 0.757 \quad r = 0.838 \quad s = 0.357 \quad F = 15.67
\end{align*}

(6)

In the case of the MCF-7 cell line, the inclusion of the three best parameters in the regression equations still indicated that the shape of the substituent at R\textsuperscript{2} is important but now the saturated surface area (SSA) is detrimental while the water accessible surface area (WASA) is beneficial to activity (Eq. (8)). The result that the saturated surface area is detrimental is consistent with the regression equations that showed the unsaturated surface area to be beneficial because the one parameter is the opposite of the other.

\begin{align*}
-\log(\text{IC}_{50})_{\text{MCF7}} &= 0.011(\pm 0.003) \text{USA} + 0.370(\pm 0.108) \text{IC}_1 + 5.011(\pm 0.105) \\
& n = 24 \quad rCv^2 = 0.370 \quad r = 0.755 \quad s = 0.287 \quad F = 13.90
\end{align*}

(7)

\begin{align*}
-\log(\text{IC}_{50})_{\text{MCF7}} &= -0.013(\pm 0.003) \text{SSA} + 0.012(\pm 0.002) \text{WASA} + 0.316(\pm 0.088) \text{IC}_1 + 4.925(\pm 0.244) \\
& n = 24 \quad rCv^2 = 0.610 \quad r = 0.855 \quad s = 0.325 \quad F = 18.10
\end{align*}

(8)
Conclusion

The above data show the usefulness of uniting benzoxazine and benzodithiazine moieties to build a scaffold with good cytotoxic activity. It can be concluded that the shape (e.g., unsaturation) of the substituents R² of the benzodithiazine ring system as well as a chlorine or methyl group in the phenyl ring at R¹ positively influence the cytotoxicity potency of the compounds. Further structural modification along the lines of the QSAR may help to further increase the potency of this interesting new class of cytotoxic compounds.

Experimental

Chemistry

General

Melting points are uncorrected and were determined on a Büchi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland). The IR spectra were recorded on 1600 FTIR Perkin Elmer (Perkin Elmer, Norwalk, CT, USA) spectrometer by using potassium bromide pellets and frequencies were expressed in cm⁻¹. The ¹³C- and ¹H-NMR spectra were obtained on a Varian Gemini (50 MHz and 200 MHz) or Varian Unity Plus (125 MHz and 500 MHz) spectrometers (Varian Inc., Palo Alto, CA, USA). The chemical shift values δ were expressed in ppm relative to the residual solvent signal at 2.50 ppm and 39.5 ppm, respectively. The analytical results for C, H, and N were within ±0.4% of the theoretical values and results are reported in Table S2 (Supplementary Material). The starting 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazin-7-carboxamide [16], 6-chloro-1,1-dioxo-7-{4-[phenylimino]-4H-3,1-benzoxazin-2-yl}-3-methylthio-1,4,2-benzodithiazine [17], N-[2-(phenylcarbamoyl)phenyl]-6-chloro-3-methylthio-1,4,2-benzodithiazin-7-carboxamide [15], and 6-chloro-1,1-dioxo-3-methylthio-7-[4-(phenylimino)-4H-3,1-benzoxazin-2-yl]-1,4-benzodithiazine 6 [15] were obtained by the previously described methods.

7-N[2-(R¹-Phenylcarbamoyl)phenyl]-6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-carboxamides 2, 3

To a suspension of compound 1 (3.42 g, 10 mmol) and 2-amino-N,N-toly or 4-chlorophenyl)benzamide (11 mmol) in anhydrous toluene (120 mL), pyridine (0.8 mL, 10 mmol) in anhydrous toluene (50 mL) was added. The reaction mixture was stirred at room temperature for 10 h and then refluxed until the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h)] until the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h)] until the evolution of MeSH had ceased (20–40 h) until the evolution of MeSH had ceased (20–40 h)]. The precipitate was filtered off, washed with toluene (5 mL), or with methanol (20 mL), and dried. The mixture was stirred for 1 h, filtered off, and dried successively with water (2 × 50 mL), methanol (20 mL), and dried. In this manner, the following compounds were obtained.

N-[2-(4-Methylphenylcarbamoyl)phenyl]-6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-carboxamide 2

Starting from 2-amino-N-(4-chlorophenyl)benzamide (2.71 g); yield: 4.4 g (80%); mp 242–244°C. IR (KBr, cm⁻¹) 3276 (NH), 1666 (CO), 1628, 1603 (C=N), 1350, 1165 (SO₂). ¹H-NMR (200 MHz, DMSO-d₆): δ 2.72 (s, 3H, SCH₃), 7.28–7.45 (m, 3H, aromat.), 7.54–7.82 (m, 4H, aromat.), 7.29 (d, J = 7.7 Hz, 1H, aromat.), 8.15 (s, 1H, H-5 benzodit.), 8.32 (s, 1H, H-8 benzodit.), 10.58 (s, 1H, NH), 10.94 (s, 1H, NH) ppm.

6-Chloro-7-[4-[(4-R¹-Phenylcarbamoyl)phenyl]-4H-3,1-benzoxazin-2-yl]-3-methylthio-1,4,2-benzodithiazine 1,1-dioxides 4–5

A mixture of carboxamide 2 or 3 (10 mmol) and triethyl chloride (50 mL) was refluxed for 30 h. The thionyl chloride was distilled off (80°C) then to the residue toluene was added (2 × 40 mL) and distilled off (111°C). The dry residue was recrystallized from anhydrous toluene (100 mL) to give 4 or 5.

6-Chloro-1,1-dioxo-7-[4-[(4-Methylphenylcarbamoyl)phenyl]-4H-3,1-benzoxazin-2-yl]-3-methylthio-1,4,2-benzodithiazine 4

Starting from 2 (5.32 g); yield: 1.3 g (25.3%); mp 256–258°C. IR (KBr, cm⁻¹) 1670 (N––C), 1630, 1605 (C––N), 1345, 1170 (SO₂). ¹H-NMR (500 MHz, CDCl₃): δ 2.36 (s, 3H, PhCH₃), 2.74 (s, 3H, SCH₃), 7.03 (d, J = 8 Hz, 2H, PhCH₃), 7.09 (d, J = 8 Hz, 2H, PhCH₃), 7.48 (s, 1H, H-5 benzodit.), 7.59 (t, J = 7.3 Hz, 1H, aromat.), 7.64 (d, J = 8.3 Hz, 1H, aromat.), 7.76 (t, J = 7.3 Hz, 1H, aromat.), 8.46 (d, J = 7.8 Hz, 1H, aromat.), 8.66 (s, 1H, H-8 benzodit.) ppm; ¹³C-NMR (500 MHz, CDCl₃); δ 16.69 (SCH₃), 20.97 (CH₃), 119.0, 122.58, 125.86, 127.28, 127.56, 127.64, 128.28, 129.13, 129.47, 129.90 (two overlapping signals), 129.95, 130.51, 131.91, 134.71, 135.45, 138.04, 142.35 (aromat.), 146.97, 152.22 (C=N), 179.91 (C=N) ppm.

6-Chloro-1,1-dioxo-7-[4-[(4-Chlorophenylcarbamoyl)phenyl]-4H-3,1-benzoxazin-2-yl]-3-methylthio-1,4,2-benzodithiazine 5

Starting from 3 (5.52 g); yield: 3.8 g (70%); mp 228–230°C. IR (KBr, cm⁻¹) 1670 (N=C), 1630, 1605 (C=N), 1345, 1170 (SO₂). ¹H-NMR (200 MHz, CDCl₃): δ 2.72 (s, 3H, SCH₃), 7.09 (d, J = 8.7 Hz, 2H, PhCl), 7.32 (d, J = 8.7 Hz, 2H, PhCl), 7.48 (s, 1H, H-5 benzodit.), 7.52–7.63 (m, 2H, aromat.), 7.72 (d, J = 1.5 Hz, J = 8.2 Hz, J = 8.5 Hz, 1H, aromat.), 8.31 (ddd, J = 7.7 Hz, J = 8.8 Hz, J = 12 Hz, 1H, aromat.), 8.64 (s, 1H, H-8 benzodit.) ppm; ¹³C-NMR (200 MHz, CDCl₃); δ 16.94 (SCH₃), 119.66, 124.14 (two overlapping signals), 127.28, 127.56, 127.88, 129.25, 129.47 (two overlapping signals), 129.95, 130.51, 131.91, 134.71, 135.45, 138.04, 142.35 (aromat.), 146.97, 152.22 (C=N), 179.91 (C=N) ppm.

General procedure for preparation of 6-chloro-1,1-dioxo-7-[4-[(4-R¹-Phenylcarbamoyl)phenyl]-4H-3,1-benzoxazin-2-yl]-3-R²-1,4,2-benzodithiazines 7–22

A mixture of the corresponding benzodithiazine 4–6 (1 mmol) and the appropriate amine (1.1 mmol) in dry methanol (20 mL) was stirred at room temperature for 10 h and then refluxed until the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h) [Caution: because of the evolution of MeSH had ceased (20–40 h)] until the evolution of MeSH had ceased (20–40 h) until the evolution of MeSH had ceased (20–40 h) until the evolution of MeSH had ceased (20–40 h)] until the evolution of MeSH had ceased (20–40 h)].
solution. The precipitate was filtered off, washed successively with methanol (5 ml), chloroform (5 ml), and dried. In this manner, the following compounds were obtained:

6-Chloro-1,1-dioxo-7-[4-[(4-methylphenyl)imino]-4H-3,1-benzoaxazin-2-yl]-3-morpholinol-1,4,2-benzodithiazine
Starting from 4 (0.51 g) and morphology (0.096 g); yield: 0.29 g (53%); mp 276–278 °C. IR (KBr, cm\(^{-1}\)) 1673 (N=C), 1630 (C=N), 1320, 1160 (SO\(_2\)). \(^1\)H-NMR (200 MHz, CDCl\(_3\)): \(\delta\) 2.23 (s, 3H, CH\(_3\)), 3.62–4.05 (m, 8H, 4 \& CH\(_3\)), 6.95–7.10 (2H, pyH), 7.18–7.31 (m, 2H, PhCH\(_2\)), 7.49 (s, 1H, H-5 benzodit.), 7.50–7.80 (m, 3H, aromat.), 8.39 (d, \(J = 7.7\) Hz, 1H, aromat.), 8.62 (s, 1H, H-8 benzodit.) ppm.

6-Chloro-1,1-dioxo-7-[4-[(4-chlorophenyl)imino]-4H-3,1-benzoaxazin-2-yl]-3-morpholinol-1,4,2-benzodithiazine
Starting from 5 (0.53 g) and morphology (0.096 g); yield: 0.3 g (52.6%); mp 245–247 °C. IR (KBr, cm\(^{-1}\)) 1665 (N=C), 1625 (C=N), 1310, 1160 (SO\(_2\)). \(^1\)H-NMR (200 MHz, CDCl\(_3\)): \(\delta\) 3.53–4.1 (m, 4H, 4 \& CH\(_3\)), 7.11 (d, \(J = 8.5\) Hz, 2H, PhCl), 7.31 (d, \(J = 8.5\) Hz, 2H, PhCl), 7.52 (s, 1H, H-5 benzodit.), 7.47–7.62 (2H, aromat.), 7.67–7.76 (m, 1H, aromat.), 8.33 (d, \(J = 7.7\) Hz, 1H, aromat.), 8.61 (s, 1H, H-8 benzodit.) ppm.

6-Chloro-1,1-dioxo-7-[4-[(4-chlorophenyl)imino]-4H-3,1-benzoaxazin-2-yl]-3-isopropylamino-1,4,2-benzodithiazine
Starting from 5 (0.53 g) and morpholine (0.096 g); yield: 0.3 g (52.6%); mp 165–167 °C. IR (KBr, cm\(^{-1}\)) 1665 (N=C), 1625 (C=N), 1310, 1160 (SO\(_2\)). IR (KBr, cm\(^{-1}\)) 1665 (N=C), 1625 (C=N), 1310, 1160 (SO\(_2\)). \(^1\)H-NMR (200 MHz, CDCl\(_3\)): \(\delta\) 3.53–4.1 (m, 4H, 4 \& CH\(_3\)), 7.11 (d, \(J = 8.5\) Hz, 2H, PhCl), 7.31 (d, \(J = 8.5\) Hz, 2H, PhCl), 7.52 (s, 1H, H-5 benzodit.), 7.47–7.62 (2H, aromat.), 7.67–7.76 (m, 1H, aromat.), 8.33 (d, \(J = 7.7\) Hz, 1H, aromat.), 8.61 (s, 1H, H-8 benzodit.) ppm.

6-Chloro-1,1-dioxo-7-[4-[(4-chlorophenyl)imino]-4H-3,1-benzoaxazin-2-yl]-3-isopropylamino-1,4,2-benzodithiazine
Starting from 5 (0.53 g) and 2-aminopropene (0.11 g, 18 mmol); yield: 0.22 g (40%); mp 284–285 °C. IR (KBr, cm\(^{-1}\)) 1320 (NH), 1675 (N=C), 1625 (C=N), 1303, 1150 (SO\(_2\)). IR (KBr, cm\(^{-1}\)) 1675 (N=C), 1625 (C=N), 1303, 1150 (SO\(_2\)). IR (KBr, cm\(^{-1}\)) 1675 (N=C), 1625 (C=N), 1303, 1150 (SO\(_2\)). \(^1\)H-NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 1.18 (d, \(J = 6.6\) Hz, 6H, 2CH\(_3\)), 4.04–4.21 (m, 1H, CH\(_3\)), 7.23 (d, \(J = 8.5\) Hz, 2H, PhC\(_2\)), 7.36 (d, \(J = 8.5\) Hz, 2H, PhC\(_2\)), 7.56–7.68 (m, 2H, aromat.), 7.76–7.86 (m, 1H, aromat.), 8.07 (s, 1H, H-5 benzodit.), 8.23 (d, \(J = 7.9\) Hz, 1H, aromat.), 8.39 (s, 1H, H-8 benzodit.), 9.77 (s, 1H, NH) ppm. \(^13\)C-NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 12.25 (C, 2 \& CH\(_3\)), 46.45 (CH\(_3\)), 119.26, 124.29 (two overlapping signals), 126.43, 127.23, 127.51, 128.32, 128.94 (two overlapping signals), 129.80, 130.09, 130.52, 131.78, 133.87, 134.75, 135.29, 142.14, 144.33 (18C, aromat.), 146.19, 152.51 (C=N), 160.67 (N=C) ppm.
6-Chloro-1,1-dioxo-7-[(4-methylphenyl)limino]-4H,3,1-benzoxazin-2-yl]-3-[2-(2-pyridyl)ethylamino]-1,4,2-benzodithiazine 15

Starting from 4 (0.51 g) and 3-(aminomethyl)pyridine (0.13 g); yield: 0.30 g (53%); mp 213–214°C. IR (KBr, cm⁻¹) 3424 (NH), 1674 (C=N), 1603 (C=N), 1312, 1160 (SO₂). 1H-NMR (500 MHz, DMSO-d₆) ð 2.28 (3 H, CH₃); 3.04 (t, J = 7.2 Hz, 2H, CH₂); 3.77 (t, J = 7.3 Hz, 2H, NCH₂); 7.10–7.32 (m, 6H, H-3 and H-5 pyrid.), 4H aromat.), 7.63–7.68 (m, 2H, H-4 pyrid. and aromat.), 7.72 (t, J = 7.8 Hz, 1H, aromat.), 8.74 (t, J = 7.8 Hz, 1H, aromat.), 8.06 (s, 1H, H-5 benzodit.), 8.29 (d, J = 7.8 Hz, 1H, aromat.), 8.39 (s, 1H, H-8 benzodit.), 8.51 (d, J = 3.9 Hz, 1H, H-6 pyrid.), 9.96 (s, 1H, NH) ppm. 13C-NMR (DMSO-d₆) ð 30.9 (CH₂), 44.51 (NCH₂), 119.47, 124.3 (two overlapping signals), 130.98, 132.14, 134.35, 135.40, 135.78, 140.75, 142.56 (aromat.), 122.52, 124.47 (C-3 and 5 pyrid.), 137.34 (C-4 pyrid.), 147.09, 149.87 (C-2 and 6 pyrid.), 152.94, 165.58 (C=N), 162.24 (C=N) ppm.

6-Chloro-7-[(4-chlorophenyl)limino]-4H,3,1-benzoxazin-2-yl]-3-[2-(3-pyridyl)ethylamino]-1,4-dioxo-1,2-benzodithiazine 16

Starting from 4 (0.53 g) and 2-(aminomethyl)pyridine (0.15 g, 12 mmol); yield: 0.48 g (78%); mp 288–290°C. IR (KBr, cm⁻¹) 3210 (NH), 1688 (C=N), 1630 (C=N), 1320, 1160 (SO₂). 1H-NMR (200 MHz, DMSO-d₆) ð 2.27 (3 H, CH₃); 2.91 (t, J = 6.1 Hz, 2H, CH₂); 3.64 (t, J = 6.0 Hz, 2H, CH₂); 7.05–7.39 (m, 5H, 4H, PhCl and H-5 pyrid.), 7.54–7.88 (m, 4H, H-4 pyrid. and aromat.), 8.06 (s, 1H, H-5 benzodit.), 8.27 (d, J = 7.8 Hz, 1H, aromat.), 8.34–8.50 (m, 3H, H-2,6 pyrid. and H-8 benzodit.), 9.94 (s, 1H, NH) ppm. 13C-NMR (DMSO-d₆) ð 20.45 (CH₃), 30.99 (CH₂), 44.99 (NH₂), 119.82, 123.79 (two overlapping signals), 125.27, 126.59, 127.29, 127.45, 128.44, 129.48, 129.93, 130.56, 131.62, 133.81, 134.22, 134.97, 135.37, 140.32, 142.10 (aromat.), 122.59, 123.02 (C-3 and 5 pyrid.), 136.52 (C-4 pyrid.), 148.03, 150.16 (C-2 and 6 pyrid.), 146.63, 152.49 (C=N), 161.91 (C=N) ppm.

6-Chloro-1,1-dioxo-7-[(4-chlorophenyl)limino]-4H,3,1-benzoxazin-2-yl]-3-[6-chloro-3-pyridylmethyl)amino]-1,4,2-benzodithiazine 18

Starting from 5 (0.53 g) and 2-(aminomethyl)-2-chloropyridine (0.18 g, 13 mmol); yield: 0.40g (63%); mp 277–278°C. IR (KBr, cm⁻¹) 3165 (NH), 1665 (C=N), 1630, 1160 (SO₂). 1H-NMR (200 MHz, DMSO-d₆) ð 6.46 (s, 2H, NH₂); 7.22 (d, J = 8.6 Hz, 2H, PhCl), 7.36 (d, J = 8.6 Hz, 2H, PhCl), 7.46–7.67 (m, 3H, aromat. and H-5 pyrid.), 7.71–7.87 (m, 2H, H-4 pyrid. and aromat.), 8.1 (s, 1H, H-5 benzodit.), 8.22 (d, J = 7.8 Hz, 1H, aromat.), 8.4 (s, 2H, H-8 benzodit. and H-2 pyrid.), 10.31 (s, 1H, NH) ppm. 13C-NMR (DMSO-d₆) ð 43.76 (NCH₂), 119.26, 124.27 (two overlapping signals), 127.23, 126.73, 128.32, 128.92 (two overlapping signals), 129.81, 130.23, 130.59, 131.47, 132.17, 133.66, 134.74, 139.7, 142.14, 143.34 (aromat.), 142.48, 146.23 (C-3 and 5 pyrid.), 135.43 (C-4 pyrid.), 149.68 (two overlapping signals of C-2 and 6 pyrid.), 146.17, 152.46 (C=N), 162.43 (C=N) ppm.

6-Chloro-1,1-dioxo-7-[(4-phenylmethyl)limino]-4H,3,1-benzoxazin-2-yl]-3-[6-chloro-3-pyridylmethyl)amino]-1,4,2-benzodithiazine 19

Starting from 6 (0.50 g) and 2-(aminomethyl)-2-chloropyridine (0.18 g, 13 mmol); yield: 0.45 g (76%); mp 251–253°C. IR (KBr, cm⁻¹) 3210 (NH), 1680 (C=N), 1625 (C=N), 1315, 1160 (SO₂). 1H-NMR (200 MHz, DMSO-d₆) ð 4.62 (s, 2H, NCH₂), 7.02–7.4 (m, 5H, Ph), 7.43–7.69 (m, 3H, aromat. and H-5 pyrid.), 7.71–7.89 (m, 2H, H-4 pyrid. and aromat.), 8.07 (s, 1H, H-5 benzodit.), 8.23 (d, J = 7.2 Hz, 1H, aromat.), 8.4 (s, 2H, H-8 benzodit. and H-2 pyrid.), 10.20 (brs, 1H, NH) ppm. 13C-NMR (DMSO-d₆) ð 43.9 (NCH₂), 119.46, 122.41 (two overlapping signals), 124.50, 126.39, 127.18, 125.75, 129.0 (two overlapping signals), 129.75, 130.33, 130.45, 131.58, 132.31,
133.76, 139.50, 142.10 (aromat.), 124.35, 124.47 (C3 and 5 pyrid.), 135.38 (C4 (pyrid.), 149.67 (two overlapping signals of C2 and 6 pyrid.), 145.28, 152.76 (C=N), 162.27 (N–C ppm).

6-Chloro-7-[(4-[(4-methylphenyl)limino]-4H-3,1-benzoxazin-2-yl)-3-[(6-chloro-3-pyridylimethy)limino]-1,1-dioxo-1,2-benzothiazine 21

Starting from 4 (0.51 g) and 5(aminomethyl)-2-chloropyridine (0.18 g, 13 mmol); yield: 0.25 g (42%); mp 221–223 °C. IR (KBr, cm–1): 3225 (NH), 1672 (N–C), 1630, 1602 (C–N), 1320, 1160 (SO2) ppm. 

Crystallographic data for compounds 11 and 31 have been deposited with the Cambridge Crystallographic Data Centre, with the deposition Nos CCDC 768913 & 768914.

In-vitro cytotoxicity studies

The microtiter plate method used for cytotoxicity testing is based on crystal violet staining of cells and has been described in detail elsewhere [26, 27]. All the cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Braunschweig, FRG). Stock solutions of compounds were prepared in DMSO and diluted 1000-fold with cell culture medium (RPMI medium + 10% fetal calf serum) for testing. For IC50 determinations, all substances were tested at 5 serially diluted concentrations. The corrected IC values (T/Ccorr) for each concentration were calculated with the following equation:

\[
\frac{(T/C)_{corr}}{C0} = \frac{(T/D)_{corr}}{C0} \times 100
\]

where \( T \) is the optical density at \( \lambda = 570 \text{ nm} \) (OD570) of the treated cells at after a 96 h treatment time, \( C \) is the OD570 of the untreated cells after 96 h of growth without substance, \( C0 \) is the OD570 of the cells on the at time of treatment (i.e. 96 h before \( T \) and \( C \)). Linear regression analysis of the log concentration versus the T/Ccorr plots was used to estimate the concentration of substance that caused a T/Ccorr = 50% (IC50). At least three independent experiments were done to determine the IC50 values.

Descriptor calculations

Molecular models of the amines R2 were constructed and minimized with the software PCModel (Serena Software, Bloomington, IN, USA, ver 8.50). The MMX force field of PCModel was used in the calculation of the following descriptors: the saturated surface area (SSA), the unsaturated surface area (USA), the polar surface area (PSA), the molecular volume (molvol) and the molar volume (Mvol) of each fragment. The structures were imported into the program CACHé (Fujitsu Biosystems Group, Beaverton, OR, USA, ver 7.5.0.85) and the following descriptors were calculated with the PM5 semi-empirical method at the PM5 minimum: Dipole moment (DM), HOMO and LUMO energies, molecular refractivity (MR), polarizability (P), heat of formation (HF), water assessable surface area (WASA) and ionization potential (IP). Further descriptors calculated by CACHé were: Molecular weight (MW), logP, and the kappa shape indexes, orders 1, 2 and 3 (KSI1, KSI2, KSI3).

Correlation analysis

Initial multiple regression analysis was performed with the CACHé ProjectLeader software. The dependent variable used in

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References

[1] E. Pomarnacka, Z. Brzozowski, Acta Polon. Pharm. Drug Res. 1997, 54, 215–221.
[2] E. Pomarnacka, A. Kornicka, Farmaco 2001, 56, 571–577.
[3] E. Pomarnacka, I. Kozlarska-Kędra, Farmaco 2003, 58, 423–429.
[4] E. Pomarnacka, A. Kornicka, F. Sączewski, Heterocycles 2001, 55, 753–760.
[5] E. Pomarnacka, A. Kornicka, P. J. Bednarski, A. Charkiewicz, Polish J. Chem. 2009, 83, 63–73.
[6] L. C. Kuo, H. Assefa, Z. Brzozowski, J. Sławinski, F. Sączewski, J. K. Buolamwini, N. Nemati, J. Med. Chem. 2004, 47, 385–399.
[7] E. Pomarnacka, M. Gdaniec, Bioorg. Med. Chem. 2003, 11, 1259–1267.
[8] E. Pomarnacka, P. J. Bednarski, P. Reszka, E. Dziemidowicz-Borys, A. Bieniczak, W. Werel, R. Halasa, Eur. J. Med. Chem. 2006, 41, 633–639.
[9] Z. Brzozowski, F. Sączewski, J. Med. Chem. 2002, 45, 430–437.
[10] Z. Brzozowski, F. Sączewski, M. Gdaniec, Eur. J. Med. Chem. 2003, 38, 991–999.
[11] Z. Brzozowski, F. Sączewski, J. Sławinski, P. J. Bednarski, R. Grünert, M. Gdaniec, Bioorg. Med. Chem. 2007, 15, 2560–2572.
[12] K. M. Pritchard, J. Al-Rawi, C. Bradley, Eur. J. Med. Chem. 2007, 42, 1200–1210.
[13] Y. Tabuchi, Y. Ando, H. Kanemura, I. Kawasati, T. Ohishi, et al. Bioorg. Med. Chem. 2009, 17, 3959–3967.
[14] S. E. Rokita, K. D. Karlin, J. J. Humphreys, L. Li, N. N. Murthy, Pat. US 7365060, April 29, 2008.
[15] E. Pomarnacka, M. Maruszak, K. Langowska, P. Reszka, P. J. Bednarski, Arch. Pharm. Life Sci. 2008, 341, 485–490.
[16] Z. Brzozowski, J. Sławinski, Acta Polon. Pharm. 1984, 41, 5–13.
[17] Z. Brzozowski, F. Gajewski, J. Sławinski, E. Pomarnacka, Acta Polon. Pharm. Drug Res. 1993, 50, 199–203.
[18] D. J. Connolly, D. Cusack, T. P. O’Sullivan, P. J. Guiry, Tetrahedron 2005, 61, 10153–10172.
[19] A. Witt, J. Bergman, Tetrahedron 2000, 56, 7245–7253.
[20] H. Fuwa, T. Kobayashi, T. Tokitoh, Y. Torii, H. Natsugari, Tetrahedron 2005, 61, 4297–4312.
[21] P. Molina, M. Alajarin, A. Vidal, M. C. de la Foces-Foces, F. H. Cano, Tetrahedron 1989, 45, 4263–4286.
[22] S. Canestrari, A. Mar’In, P. Sgarabotto, L. Righi, L. Greci, J. Chem. Soc, Perkin Trans. 2000, 2, 833–838.
[23] L. Dupont, F. Somers, P. De Tuilio, B. Pirotte, Acta Cryst. 2003, E59, o1509–o1511.
[24] Z. Brzozowski, F. Sączewski, M. Gdaniec, Bioorg. Med. Chem. 2003, 11, 3673–3681.
[25] M. R. Bryce, S. Yoshida, A. S. Batsanov, J. A. K. Howard, J. Chem. Soc, Perkin Trans. 1997, 1, 1157–1161.
[26] K. Bracht, Boubakari, R. Grünert, P. J. Bednarski, Anti-Cancer Drugs 2006, 17, 41–51.
[27] K. Rinke, R. Grünert, P. J. Bednarski, Pharmazie 2001, 56, 763–769.
[28] C. Hansch, A. Leo, Exploring QSAR, ACS Press, Washington, DC 1995.
[29] L. H. Hall, L. B. Kier, K. B. Lipkowitz, D. B. Boyd, in Reviews of Computational Chemistry, Vol. 2, Wiley VCH, Weinheim 1991, 367–422.
[30] CrysAlis Pro Software. vers. 1.171.33, Oxford Diffraction Ltd, Abingdon, Oxfordshire, UK 2008.
[31] G. M. Sheldrick, Acta Cryst. 2008, A64, 112–122.