New Pyrazole-Annulated Azathioxanthenes as Potential Intercalators: Synthesis and Structure Elucidation

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In memory of Professor Nicholas Alexandrou

As part of our program aiming at developing efficacious intercalating agents, a new series of pyrazole-annulated azathioxanthenes **15a-e** has been synthesized. Structure elucidation of **15a-e** was based on their spectral data and especially the NOESY nmr spectrum of analog **15a**.

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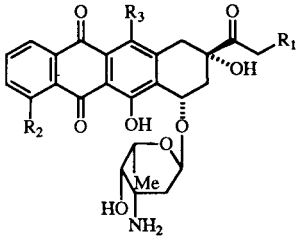
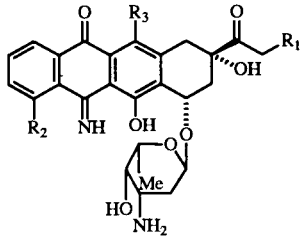
Introduction.

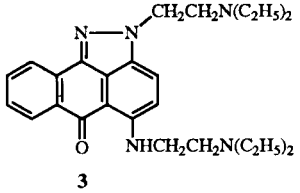
One of the most important classes of antitumor drugs in clinical use today is that of intercalators [1-3]. Although the exact mode of action of these agents is not yet fully understood, the current view is that they bind to DNA producing a distortion to its structure. This plays an important role in blocking RNA [4,5] and DNA [6] syntheses and in the cleavage and damage of DNA strands [7,8]. However, the study of the mechanism of action of intercalators is hampered by toxic effects, especially their severe cumulative cardiotoxicity [9-13]. Even the most potent intercalators in use today, the anthracycline antibiotics daunorubicin (DR) and adriamycin (ADR) (Figure 1), suffer from these side effects. The anthracycline cardiac toxicity has in part been attributed to the formation of reactive oxygen species and subsequent intracellular lipid peroxidation from enzymatic reduction of the quinone chromophore to a semiquinone radical species [14].



R_1 = OH, R_2 = MeO, R_3 = OH: Doxorobucin or Adriamycin (ADR)
R_1 = H, R_2 = MeO, R_3 = OH: Daunorubicin (DR)



R_1 = OH, R_2 = MeO, R_3 = OH: 5-Iminodoxorobucin **1**
R_1 = H, R_2 = MeO, R_3 = OH: 5-Imidaunorobucin **2**



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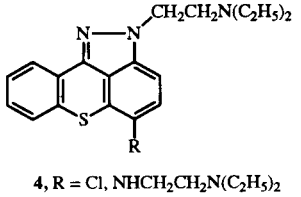

4, R = Cl, $NHCH_2CH_2N(C_2H_5)_2$

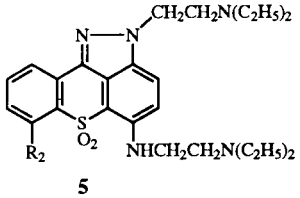

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Figure 1.

The demand for non toxic antitumor drugs with the broad-spectrum activity of daunorubicin and adriamycin has spurred the search for new analogs. The initial attempts were focused on the quinone derivatization in the anthracycline series. 5-Iminodoxorubicin **1** [15] and 5-iminodaunorubicin **2** [16] (Figure 1) provided the first examples of this approach. This concept was later further developed with the synthesis of the anthrapyrazoles **3** [17], benzothiopyranoindazoles **4** [18] and benzothiopyranoindazole dioxides **5** [18] (Figure 1), which resulted in reduced toxicity. Selected compounds in these series were chosen for clinical trials.

Encouraged by these results we designed a new series of potential intercalators, the pyrazole annulated azathioxanthenes **15a-e** (Scheme 1), the synthesis of which we report here.

Results and Discussion.

The synthetic pathway followed for the preparation of **15a-e** is delineated in Scheme 1. Thus, commercially available 2-chloronicotinic acid **6** reacted with excess 2,5-dichlorobenzenethiol **7** to give the hitherto unknown thioether **8** [19]. The latter was converted to the acid chloride **9** upon treatment with thionyl chloride [19]. Friedel-Crafts intramolecular ring closure of **9** in the presence of aluminum trichloride [19] gave ketone **10**. The incorporation of the fourth ring D to the three nucleus (A-B-C) skeleton of **10** was effected upon condensation of the latter

with 2-hydroxyethylhydrazine. Although two regioisomers, **11** and **12**, could be expected from this type of reaction [20] only the isomer **12** was obtained. The structure of **12** was fully elucidated from the nmr spectral data obtained for this compound as well as the NOESY nmr spectrum obtained for its closely related derivative **15a**.

In detail, the ^{1}H nmr spectrum of **12** revealed the presence of only one regioisomer. The signals at 8.27 ppm (dd, J = 1.8, 8.0 Hz) and at 8.16 ppm (dd, J = 1.8, 8.0 Hz) correspond to H-8 and H-10, while the doublets at 6.92 ppm and 7.18 ppm are attributed to protons H-3(4), H-4(3) and the coupling constant J = 8.8 Hz is indicative of their *ortho* coupling. The structure of **12** was unequivocally elucidated from the NOESY nmr experiment (Figure 2) of its structurally related analog **15a**. The observed nOe between H-3 and N-CH_2 is consistent only with the desired regioisomer **12**. Otherwise we should have observed nOe between H-10 and N-CH_2. In addition, the NOESY nmr spectrum revealed correlations in the aromatic region between protons H-8 and H-9, H-9 and H-10 and H-8 and H-10.

The synthesis of chloride **14**, precursor of the final products **15a-e**, was effected upon treatment of alcohol **12** in pyridine with *p*-toluenesulfonyl chloride. In this reaction, the initially formed tosylate **13** was converted to the desired primary chloride **14** *via* nucleophilic displacement by the excess chloride ion present in the reaction mixture [21,22]. The target molecules **15a-e** were obtained from

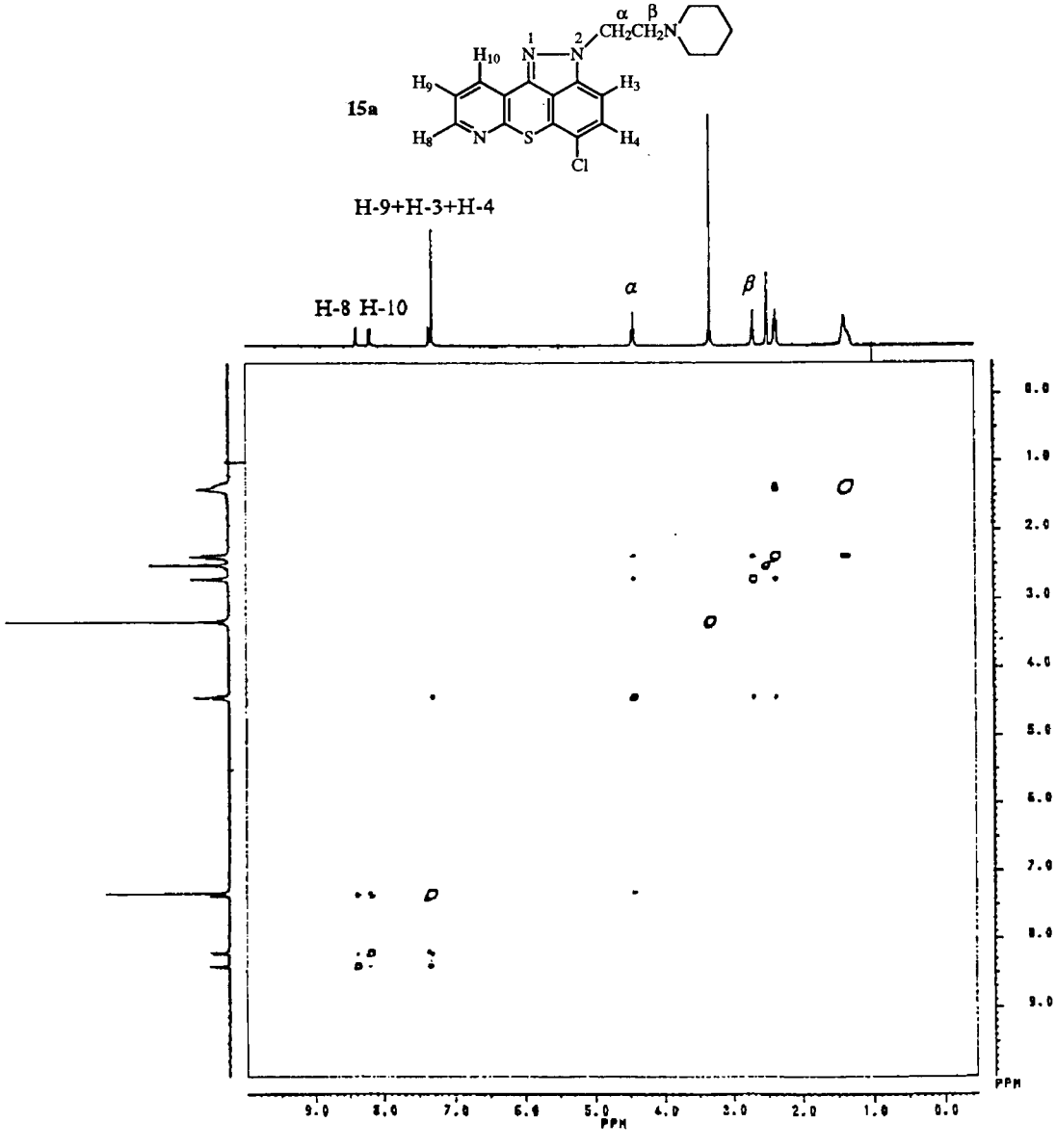
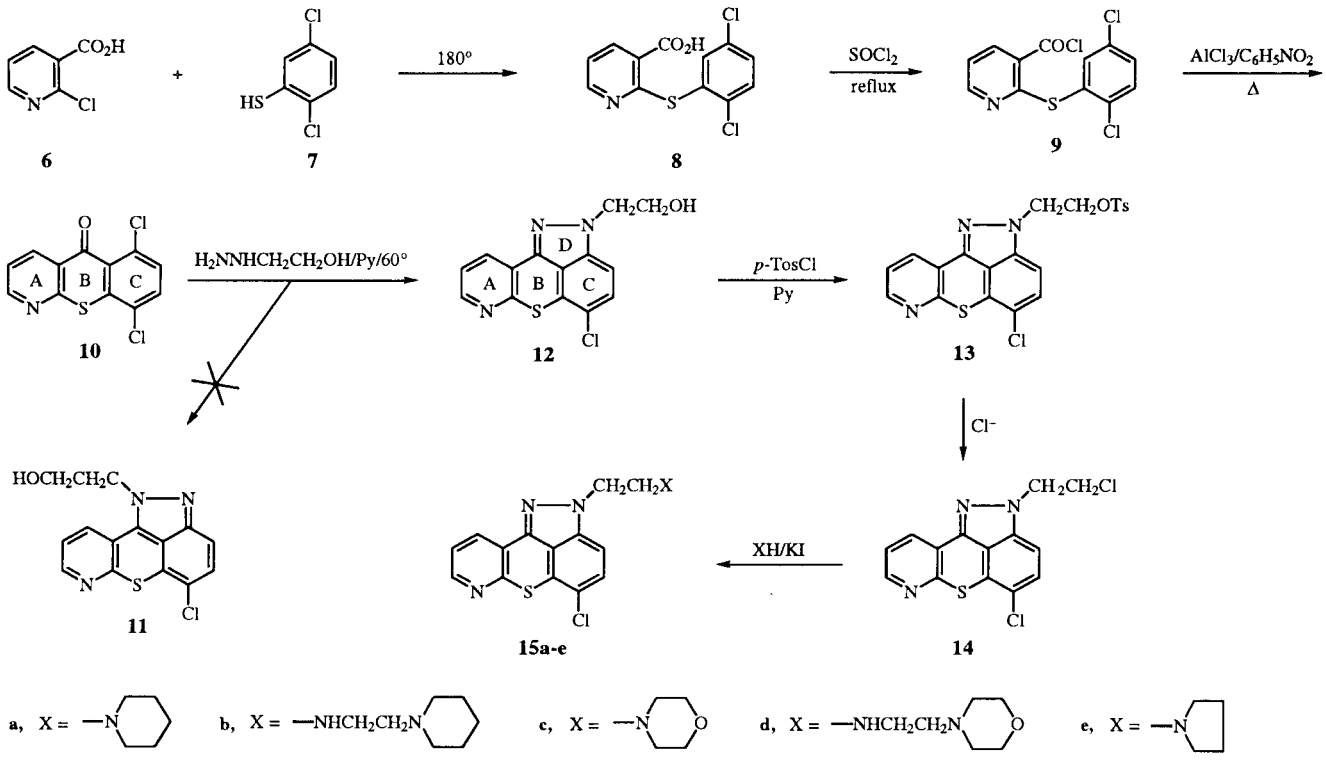


Figure 2. NOESY spectrum of compound **15a**.

Scheme 1



the reaction of **14** in pyridine with the appropriate primary or secondary amine.

The new compounds **15a-e** are currently evaluated for their cytotoxicity against a broad spectrum of tumor cell lines.

EXPERIMENTAL

Melting points were determined on a Buchi-530 melting point apparatus and are uncorrected. Infrared spectra were run on a Perkin Elmer 883 spectrophotometer. The ^1H and ^{13}C nmr spectra were recorded on a Bruker AC 200 or AC 300 MHz spectrometer using tetramethylsilane as internal standard. Molecular weights were determined by DCI mass spectrometry on a VG Trio 1000 mass spectrometer. Silica gel plates (Merck F_{254}) were used for thin layer chromatography. Elemental analyses were performed by Service Central de Microanalyses of CNRS in Vernaison, France.

2-(2,5-Dichlorophenylthio)nicotinic Acid (**8**).

A mixture of 2-chloronicotinic acid (**6**) (1.01 g, 6.35 mmoles) and 2,5-dichlorobenzenethiol (**7**) (2.27 g, 12.7 mmoles) was heated at 180° for 2.5 hours. The mixture was cooled to room temperature and the precipitate formed was filtered and dissolved in saturated aqueous sodium bicarbonate solution. The mixture was extracted with diethyl ether and the aqueous layer was acidified with acetic acid (*p*H 4). The deposited material was filtered and recrystallized from ethanol to give 1.8 g (95%) of **8**, mp 238-240° (ethanol); ^1H nmr (dimethyl sulfoxide-d_6, 300 MHz): δ 7.24-7.28 (m, 1H), 7.56-7.57 (m, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.4 Hz, 1H), 8.25-8.27 (m, 1H), 8.40-8.41 (m, 1H).

Anal. Calcd. for $C_{12}H_7Cl_2NO_2S$: C, 48.02; H, 2.35; N, 4.67. Found: C, 47.97; H, 2.20; N, 4.80.

5,8-Dichloro-9*H*-4-azathioxanth-9-one (**10**).

A solution consisting of thionyl chloride (6.54 ml) and sulfide **8** (1.57 g, 5.23 mmoles) was heated at reflux for 3 hours. The excess thionyl chloride was then removed under reduced pressure and the solid formed was filtered and washed with dry benzene (3 x 15 ml). The crude acid chloride **9** was dissolved in nitrobenzene (13 ml) and aluminum trichloride (3.92 g, 29.64 mmoles) was added. The mixture was heated at 100-120° for 4 hours, cooled to room temperature and poured slowly into crushed ice. The precipitate formed was filtered, washed with saturated aqueous sodium carbonate solution, air-dried and after recrystallization from benzene gave 1.06 g (72%) of the ketone **10**, mp 209-210° (benzene); ^1H nmr (dimethyl sulfoxide-d_6, 300 MHz): δ 7.64-7.68 (m, 1H), 7.68 (d, J = 8.6 Hz, 1H), 7.96 (d, J = 8.5 Hz, 1H), 8.60 (dd, J = 1.8, 8.1 Hz, 1H), 8.89 (dd, J = 1.8, 4.6 Hz, 1H).

Anal. Calcd. for $C_{12}H_5Cl_2NOS$: C, 51.08; H, 1.79; N, 4.97. Found: C, 51.18; H, 1.67; N, 4.97.

5-Chloro-2-(2-hydroxyethyl)]pyrido[2',3':2,3]thiopyrano-[4,3,2-*cd*]indazole (**12**).

A solution of 2-hydrazinoethanol (2.90 g, 43 mmoles) in pyridine (3 ml) was added over 3.5 hours under a nitrogen atmosphere to a stirred mixture of ketone **10** (4.07 g, 14.0 mmoles) in pyridine (28 ml) at 60°. After stirring at this temperature for 60 hours, the solution was slowly poured to 85 g of

ice. The precipitated yellow solid was filtered, washed with water, air-dried and after recrystallization from ethanol:chloroform (50:50, v/v) gave 3.59 g (74%) of **12**, mp 188-190° (ethanol-chloroform); ^{1}H nmr (deuteriochloroform, 200 MHz): δ 4.12-4.14 (m, 2H, NCH_2CH_2OH), 4.37 (t, J = 4.2 Hz, 2H, NCH$_2$CH_2OH), 6.92 (d, J = 8.8 Hz, 1H, H-(3)4), 7.06-7.12 (m, 1H), 7.18 (d, J = 8.8 Hz, 1H, H-(4)3), 8.16 (dd, J = 1.8, 8.0 Hz, 1H, H-10), 8.27 (dd, J = 1.8, 4.8 Hz, 1H, H-8).

Anal. Calcd. for $C_{14}H_{10}ClN_3OS$: C, 55.36; H, 3.32; N, 13.83. Found: C, 55.70; H, 3.15; N, 13.74.

5-Chloro-2-(2-chloroethyl)pyrido[2',3':2,3]thiopyrano-[4,3,2-*cd*]indazole (**14**).

A mixture consisting of alcohol **12** (0.375 g, 1.34 mmoles), *p*-toluenesulfonylchloride (1.16 g, 6.09 mmoles) and pyridine (3.5 ml) was stirred under a nitrogen atmosphere at room temperature for 72 hours. The resulting mixture was poured slowly to crushed ice and the solid formed was filtered, washed with water and air-dried. Recrystallization from benzene-hexane (80:20, v/v) gave 0.191 g (48%) of the chloride **14**, mp 180-182° (benzene-hexane); ^{1}H nmr (deuteriochloroform, 200 MHz): δ 3.96 (t, J = 6.1 Hz, 2H, NCH$_2$CH_2Cl), 4.57 (t, J = 6.1 Hz, 2H, NCH_2CH$_2$Cl), 6.95 (d, J = 8.8 Hz, 1H), 7.15-7.20 (m, 2H), 8.22 (dd, J = 2.1, 7.9 Hz, 1H), 8.38 (dd, J = 1.8, 4.7 Hz, 1H).

Anal. Calcd. for $C_{14}H_9Cl_2N_3S$: C, 52.18; H, 2.82; N, 13.04. Found: C, 52.53; H, 2.94; N, 12.66.

General Procedure for the Preparation of the **15 a,c,e**.

A mixture of the chloride **13** (0.109 g, 0.338 mmole), the appropriate secondary amine (3.38 mmoles), a catalytic amount of potassium iodide and dimethyl sulfoxide (2 ml) was heated at 60°, under nitrogen for 10-24 hours depending on the amine. The reaction mixture was cooled to room temperature and water (10 ml) was added. The solid formed was filtered, washed with saturared aqueous ammonium chloride solution, water and air-dried. Recrystallization from benzene gave the desired products in 41-56% yield.

5-Chloro-2-(2-piperidinoethyl)pyrido[2',3':2,3]thiopyrano[4,3,2-*cd*]indazole (**15a**).

This compound was obtained in a 56% yield as an orange powder (benzene), mp 119-120°; ir (nujol): ν C = N 1700, ν C-Cl 1122 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d$_6$, 200 MHz): δ 1.22 (broad s, 6H, N(CH$_2$CH_2)$_2$CH_2), 2.36 (broad s, 4H, N(CH_2CH$_2$)$_2$CH$_2$), 2.65 (t, J = 6.0 Hz, 2H, β-CH$_2$), 4.42 (t, J = 6.0 Hz, 2H, α-CH$_2$), 7.30-7.36 (m, 3H), 8.18 (dd, J = 1.8, 8.2 Hz, 1H, H-10), 8.37 (dd, J = 1.7, 4.7 Hz, 1H, H-8); ^{13}C nmr (dimethyl sulfoxide-d$_6$): δ 23.4, 25.1, 46.6, 53.6, 57.4, 107.6, 115.0, 119.6, 122.4, 125.7, 128.3, 130.2, 138.3, 148.7, 154.8; ms: m/z 370 (M$^+$).

Anal. Calcd. for $C_{19}H_{19}ClN_4S\cdot H_2O$: C, 58.68; H, 5.44; N, 14.41. Found: C, 58.44; H, 4.80; N, 14.45.

5-Chloro-2-(2-morpholinoethyl)pyrido[2',3':2,3]thiopyrano-[4,3,2-*cd*]indazole (**15c**).

This compound was obtained in a 41% yield as an orange powder (benzene), mp 129°; ir (nujol): ν C = N 1700, ν C-Cl 1122 cm^{-1}; ^{1}H nmr (deuteriochloroform, 200 MHz): δ 2.48 (t, J = 4.6 Hz, 4H, N(CH_2CH$_2$)$_2$O), 2.84 (t, J = 6.7 Hz, 2H, β-CH$_2$), 3.63 (t, J = 5.1 Hz, 4H, N(CH$_2$CH_2)$_2$O), 4.37 (t, J = 6.6 Hz, 2H, α-CH$_2$), 6.88 (d, J = 8.8 Hz, 1H), 7.12-7.18 (m, 2H), 8.18 (dd, J = 1.7, 7.8 Hz, 1H), 8.35 (dd, J = 1.8, 4.7 Hz, 1H).

Anal. Calcd. for $C_{18}H_{17}N_4OSCl$: C, 57.98; H, 4.60; N, 15.03. Found: C, 57.95; H, 4.57; N, 14.78.

5-Chloro-2-(2-pyrrolidinoethyl)pyrido[2',3':2,3]thiopyrano-[4,3,2-*cd*]indazole (**15e**).

This compound was obtained in a 52% yield as an orange powder (benzene), mp 139-140°; ir (nujol): ν C = N 1690, ν C-Cl 1120 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d$_6$, 200 MHz): δ 1.61 (broad s, 4H, N(CH$_2$CH_2)$_2$), 2.88 (broad s, 2H, β-CH$_2$), 4.48 (broad s, 2H, α-CH$_2$), 7.36 (m, 3H), 8.25 (m, 1H), 8.41 (m, 1H); ^{13}C nmr (dimethyl sulfoxide-d$_6$): δ 22.7, 48.8, 54.4, 56.5, 107.6, 116.6, 117.2, 119.7, 122.5, 125.8, 127.9, 128.5, 130.3, 138.2, 148.8, 154.8; ms: m/z 356 (M$^+$).

Anal. Calcd. for $C_{18}H_{17}N_4SCl\cdot HCl\cdot 0.5H_2O$: C, 53.73; H, 4.76; N, 13.93. Found: C, 53.55; H, 4.47; N, 13.11. We and other workers have encountered problems with nitrogen analyses from compounds with several nitrogen atoms [23a-c].

General Procedure for the preparation of **15b,d**

A mixture of the chloride **13** (0.109 g, 0.338 mmole) and the appropriate primary amine (3.38 mmoles) in dimethyl sulfoxide (2 ml) was heated at 60-80°, under nitrogen for 10-24 hours depending on the amine. The reaction mixture was cooled to room temperature and water (10 ml) was added. The resulting solution was extracted with dichloromethane. The organic layer was washed with saturated ammonium chloride solution, water, dried (sodium sulfate) and concentrated *in vacuo*. The oily residue was converted to its picrate salt upon addition of a saturated methanolic solution of picric acid and recrystallized from acetone-ether (90:10, v/v) to afford **15b** and **15d** in 51% and 64% yield respectively.

5-Chloro-2-[(2-[(2-piperidinoethyl)amino]ethyl]-pyrido[2',3':2,3]thiopyrano[4,3,2-*cd*]indazole (**15b**).

This compound was obtained as a yellow powder (acetone-ether), mp 209-210°; ir (nujol): ν C = N 1700, ν C-Cl 1122 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d$_6$, 200 MHz): δ 1.39-1.62 (m, 6H, N(CH$_2$CH_2)$_2$CH_2), 1.62-1.80 (m, 4H, N(CH_2CH$_2$)$_2$CH$_2$), 2.97 (m, 2H) 4.70 (broad s, 2H, NCH_2CH$_2$NH), 7.30-7.43 (m, 3H), 8.15 (dd, J = 1.7, 7.9 Hz, 1H), 8.43 (dd, J = 1.7, 4.7 Hz, 1H), 8.54 (s, 4H, phenyl, picric acid), 8.90 (broad s, 3H, NCH$_2$CH$_2$NH_2^+ and NH^+CH$_2$CH$_2$NH); ^{13}C nmr (dimethyl sulfoxide-d$_6$): δ 20.5, 22.2, 45.0, 45.9, 50.6, 52.3, 107.3, 115.8, 120.0, 122.0, 122.4, 124.0, 124.9, 125.9, 128.9, 130.4, 138.2, 139.3, 141.1, 149.2, 155.0, 160.4; ms: m/z 413 (M$^+$-2x$C_6H_3N_3O_7$).

Anal. Calcd. for $C_{21}H_{24}N_5ClS\cdot 2C_6H_3N_3O_7\cdot 2.75H_2O$: C, 43.00; H, 3.88; N, 16.72. Found: C, 42.67; H, 3.26; N, 17.47.

5-Chloro-2-[(2-[(2-morpholinoethyl)amino]ethyl]pyrido-[2',3':2,3]thiopyrano[4,3,2-*cd*]indazole (**15d**).

This compound was obtained as a yellow powder (acetone-ether), mp 153-155°; ir (nujol): ν C = N 1700 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d$_6$, 200 MHz): δ 3.19-3.44 (m, 6H, N(CH_2CH$_2$)$_2$O+CH_2), 3.98-4.05 (m, 4H, N(CH$_2$CH_2)$_2$O), 4.71 (t, 2H, NCH_2CH$_2$NH), 7.34-7.44 (m, 3H), 8.20-8.24 (m, 1H), 8.45-8.47 (m, 1H), 8.56 (4H, phenyl, picric acid), 9.60 (broad s, 3H, NCH$_2$CH$_2$NH_2^+ and NH^+CH$_2$CH$_2$NH); ^{13}C nmr (dimethyl sulfoxide-d$_6$): δ 44.9, 45.9, 50.9, 51.4, 63.2, 107.2, 115.8, 119.9, 122.0, 122.5, 124.1, 124.9, 125.9, 128.9, 130.5, 138.2, 139.3, 141.1, 149.2, 155.0, 160.3; ms: m/z 415 (M$^+$-2x$C_6H_3N_3O_7$).

Anal. Calcd. for $C_{20}H_{22}N_5OSCl\cdot 2C_6H_3N_3O_7\cdot 1.5H_2O$: C, 42.65; H, 3.47; N, 17.10. Found: C, 42.91; H, 3.57; N, 16.43.

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