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Synthesis and Antitumor Activities of 5-Methyl-1- and 2-[[2-Dimethylaminoethyl]amino]-aza-thiopyranoindazoles

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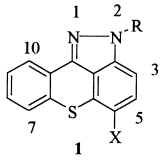
Abstract—The synthesis of 1- and 2-substituted aza-benzothiopyranoindazoles has been accomplished. The comparisons of the in vitro antitumor activities of the 2-substituted analogues with the benzothiopyranoindazole chemotypes indicate that the positioning of the nitrogen atom at C-9 (9-aza analogue **4d**) leads to a substrate with potent antitumor activity. The 1-substituted aza-benzothiopyranoindazoles, in comparison with the corresponding 2-substituted analogues, exhibit a much lower potency.

The benzothiopyrano[4,3,2-*cd*]indazole system has been known for more than 80 years.[1] In 1963, it was reported that analogue **1a** showed no antitumor activity against



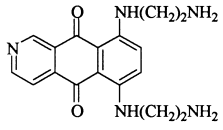
a, R = X = CH_3
b, R = $(CH_2)_2NR_1R_2$, X = $NH(CH_2)_2NR_3R_4$
c, R = $(CH_2)_2NEt_2$, X = CH_2NHSO_2Me; CH_2NHCO_2Me
d, R = $(CH_2)_2NEt_2$, X = CH_3
e, R = $(CH_2)_2NEt_2$, X = CH_2OH

1

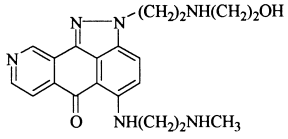
transplanted mouse tumors L1210 leukemia, sarcoma 180 and adenocarcinoma 755.[2] Subsequently it was found that benzothiopyranoindazoles such as **1b** exhibited excellent antitumor activity against a variety of cell lines.[3–5] These molecules bind intercalatively to DNA with high affinities, produce single- and double-stranded breaks and are inhibitors of nucleic acid synthesis. Several 5-aminomethylthiopyranoindazole analogues related to **1c** (holding hydroxy substitution at C-9) have demonstrated in vitro and in vivo cytotoxicities[6] and were found to intercalate into DNA and to inhibit the function of topoisomerase II.

Of particular note, however, was the reported excellent in vitro and in vivo antitumor activity of **1d**[7] which holds a methyl substituent at C-5 (compare with **1a** which is devoid of antitumor activity). Chemotype **1d** and its related analogue **1e** show a selectivity of binding of AT sequences of DNA and promote enhanced DNAase I cleavages at GC rich sequences and homo-oligomeric runs of adenines or thymines.[8] This sequence selectivity is of considerable interest since many intercalators favor GC rich sites. The sequence specificity could clearly have an effect on the interactions with an enzyme such as topoisomerase II[9] since this enzyme cleaves DNA within its binding or recognition sites.

Our previous research dealing with aza-bioisoteric modifications of antitumor carbocyclic pharmacophores has led to the development of several potent antitumor agents such as **2**[10] (BBR2778 Phase II candidate) and **3a**,**b**[11,12] (Phase I candidates). The position of the nitrogen has been found to exert a profound influence on the expressed antitumor activity.[10–12]



2 (dimaleate salt)



3 a, R = H (dihydrochloride)
b, R = CH_3 (dihydrochloride)

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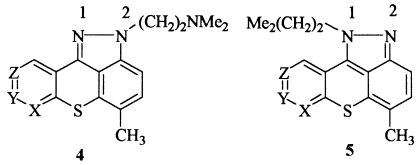
With the goal of developing new drugs able to overcome multiple drug resistance (MDR) and to prepare analogues with possible AT sequence binding in DNA for mechanistic studies, we wish to report the synthesis and antitumor comparisons of the 2-substituted benzothiopyranoindazole **4a** and the chromophore modified aza-benzothiopyranoindazoles **4b–d**. In addition, the 1-substituted chemotypes **5a**, **b** and **c** have been prepared and their antitumor activities will be discussed.

Chemistry

Recently we have reported that treatment of 1-chloro-4-methylthioxanthene-9-one (**6a**) with *N*-2-[2-(dimethylamino)ethyl]hydrazine (**7**) led to **4a** as the major product along with **5a** as a minor product.[13] Following a similar procedure, the aza analogues **6b–d** on treatment with hydrazine **7** afforded the 2-substituted chemotypes **4b–d** and **5b–d**, respectively, with the 1-substituted analogues being formed in minor amounts. Since only small amounts of **5d** were formed in the reaction of **6d** with hydrazine **7**, it could not be isolated in a pure state.

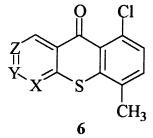


4

a, X = Y = Z = CH
b, X = N, Y = Z = CH

5

c, Y = N, X = Z = CH
d, Z = N, X = Y = CH



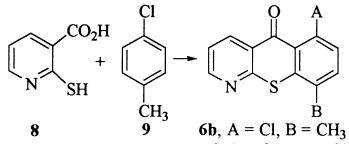
6

a, X = Y = Z = CH
b, X = N, Y = Z = CH
c, Y = N, X = Z = CH
d, Z= N, X = Y = CH

$H_2NNH(CH_2)_2N(CH_3)_2$

7

Benzothiopyranopyridine **6b** was prepared by the pathway shown in Scheme 1.

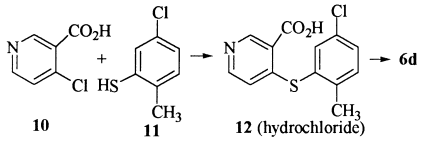


8 **9** **6b**, A = Cl, B = CH_3 **b'**, A = CH_3, B = Cl

Scheme 1.

Treatment of 2-mercaptonicotinic acid (**8**) with 4-chlorotoluene (**9**) in concentrated sulfuric acid led to a regioisomeric mixture of **6b** and **6b′** (1:1). Treatment of this mixture with **7** led only to the S_NAr displacement of the chloride in **6b** to afford regioisomers **4b** and **5b**, which are separated by tedious chromatography.

Analogue **6c** was prepared by a reported procedure[2] and **6d** was obtained as shown in Scheme 2.



10 **11** **12** (hydrochloride)

Scheme 2.

Treatment of **10**[14] with **11**[15] led to **12** (hydrochloride salt) which on treatment with thionyl chloride followed by aluminum chloride led to **6d**.

Cytotoxicities

The in vitro cytotoxicities were evaluated in five different cell lines which included a murine leukemia L1210, a murine sarcoma S-180, a murine sarcoma resistant to doxorubicin (S180/Dx), a human colon adenocarcinoma (LoVo) and a human colon adenocarcinoma resistant to doxorubicin (LoVo/Dx). The in vitro cytotoxic activities are tabulated in Table 1 along with comparative data for mitroxantrone and doxorubicin.

The tabulated data clearly shows a pronounced effect of the position of the nitrogen atom on the observed cytotoxicities. The cytotoxicity comparisons of **4a** (carbocyclic chromophore) with **4b** (7-aza analogue) and **4c** (8-aza analogue) show little change in potency in the five cell lines. On the other hand, the analogue **4d** (9-aza) exhibits a dramatic increase in potency in comparison to **4a–c** in all the cell lines studied. It might also be noted that **4d** is able to overcome the induced resistant in the S180/Dx and LoVo/Dx cell lines. In the LoVo and LoVo/Dx cell lines, the 9-aza analogue **4d** is more potent than doxorubicin or mitoxantrone. The data indicates that the N-1 substituted derivatives are generally less potent than their N-2 substituted congeners.

Studies to evaluate the in vivo antitumor activity of **4d** along with topoisomerase II inhibitions are contemplated.

Experimental[16]

Synthesis

6-Chloro-9-methyl-5*H*-[1]benzothiopyrano[2,3-*b*]pyridine-5-one (6b) and 6-methyl-9-chloro-5H-[1]benzothiopyrano-[2,3-*b*]pyridin-5-one (6b′). A mixture of **8** (1.0 g, 6.5 mmols) and **9** (1.64 g, 12.96 mmols) in concentrated sulfuric acid (18 mL) was stirred for 23 h at room temperature and then poured over ice (200 mL). The solid was collected by filtration, suspended in 7% ammonium hydroxide and steam distilled. The resultant solid was collected by filtration and dried to afford **6b** and **6b′** (1.40 g, 83%) in a 1:1 ratio (NMR analysis).

***N*,*N*,5-Trimethyl-2*H*-pyridol[3′,3′:5,6]thiopyrano[4,3,2-*cd*]-indazole-2-ethanamine (4b) and *N*,*N*,5-trimethyl-1*H*-pyrido-[3′,2′:5,6]thiopyrano[4,3,2-*cd*]indazole-1-ethanamine (5b).** A mixture of **6b** and **6b′** (0.50 g, 0.96 mmols of each regioisomer) and **7** (1.18 g, 11.5 mmols) was heated

Table 1. Cytotoxic activity of **4a–d**, **5a–c** and reference compounds mitoxantrone and doxorubicin

	IC_{50} μg/mL (SD)[a]				IC_{50} μg/mL (SD)[a]		
Compd	L1210	S180	S180/Dx	RI[b]	LoVo	LoVo/Dx	RI[b]
4a	0.07 (0.01)	0.035 (0.09)	0.50 (0.12)	1.4	0.70[c]	1.7[c]	2.4
4b	0.08 (0.01)	0.46 (0.07)	0.62 (0.27)	1.4	3.1[c]	3.3[c]	1.1
4c	0.045 (0.01)	0.08 (0.02)	0.70 (0.18)	8.8	2.3[c]	2.3[c]	1.0
4d	0.0006 (0.0002)	0.003 (0.006)	0.009 (0.006)	3.0	0.004[d]	0.018[d]	4.5
5a	0.25[d]	0.50[d]	0.75[d]	1.5	—	—	—
5b	0.26[d]	0.30[d]	0.33[d]	1.1	—	—	—
5c	0.25[d]	0.35[d]	0.40[d]	1.1	—	—	—
Mit	—	—	—	—	0.023 (0.016)[e]	0.62 (0.28)	27
Dx	—	—	—	—	0.52 (0.40)[f]	48.9 (41.7)[f]	97

[a]IC_{50}, drug (DMSO as solvent) concentration inhibiting 50% of cellular growth; SD, standard deviation.
[b]RI, resistance index = IC_{50} (cell line/Dx)/IC_{50} (cell line).
[c]Mean of two experiments.
[d]Single experiment.
[e]Mean of 97 experiments.
[f]Mean of 171 experiments.

at 170 °C for 4 h. The cooled mixture was diluted with crushed ice (25 mL) containing potassiium hydroxide (50%, 2 mL). The yellow solid was collected by filtration and air-dried (0.51 g). The crude product was flash chromatographed using gradient elution with methanol:dichloromethane 0.5:99.5 and increasing gradually to 3:97. The resultant mixture was separated using a chromatotron with a silica gel plate of 2 mm thickness. Unreacted **6b′** eluted first followed by **4b**, mp 113–115 °C; ^{1}H NMR ($CDCl_3$) δ 8.35 (dd, J = 1.5, 4.7 Hz), 8.24 (dd, J = 1.5, 7.7 Hz, 1H), 7.15 (dd, J = 4.7, 7.7 Hz), 7.10 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz), 4.40 (t, J = 7.0 Hz, 2H), 2.85 (t, J = 7.0 Hz, 2H), 2.33 (s, 6H), 2.26 (s, 3H). The last eluents led to **5b**, mp 131–134 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, J = 3.2 Hz, 1H), 7.91 (d, J = 6.8 Hz, 1H), 7.11 (m, 2H), 6.97 (d, J = 8.7 Hz, 1H), 4.70 (t, J = 7.6 Hz, 2H), 2.35 (s, 6H), 2.16 (s, 3H).

6-Chloro-9-methyl-5*H*-[1]benzothiopyrano[2,3-*c*]pyridin-5-one (6c).[2] ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.70 (d, J = 5.2 Hz, 1H), 8.21 (d, J = 5.2 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 2.55 (s, 3H).

***N*,*N*,5-Trimethyl-2*H*-pyrido[4′3′:5,6]thiopyrano[4,3,2-*cd*]-indazole-2-ethanamine (4c) and *N*,*N*,5-trimethyl-1*H*-pyrido-[4′,3′;5,6]thiopyrano[4,3,2-*cd*]indazole-1-ethanamine (5c).** Thioxanthenone **6c** was treated with **7** following the procedure used for the conversion of **6b** to **4b** and **5b**. Initial flash chromatography over silica gel led to a 4:1 mixture of **4c**:**5c**. Chromatotron separation led initially to **4c**, mp 99–100 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 8.30 (d, J = 5 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 4.32 (t, J = 7.0 Hz, 2H), 2.78 (t, J = 7.0 Hz, 2H), 2.27 (s, 6H), 3.13 (s, 3H). The last eluents led to **5c**, mp 130–132 °C; ^{1}H NMR ($CDCl_3$) δ 8.47 (s, 1H), 8.34 (d, J = 5.4 Hz, 1H), 7.48 (d, J = 5.4 Hz, 1H), 7.11 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 8.7 Hz, 1H), 4.74 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 2.35 (s, 6H), 2.13 (s, 3H).

4-[(2-Methyl-5-chlorophenyl)thio]pyridine-3-carboxylic acid hydrochloride (12). A solution of **11** (0.76 g, 4.76 mmols) in dry acetone was added to **10** (0.50 g, 3.17 mmols). The suspension was refluxed for 3 h, cooled, the solid collected by filtration and washed with cold acetone to afford **12** (0.95 g, 95%), mp 168–170 °C, ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H), 8.43 (d, J = 5.8 Hz, 1H), 7.69 (s, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 5.8 Hz, 1H), 2.26 (s, 3H).

9-Chloro-6-methyl-5*H*-[1]benzothiiopyrano[3,2-*c*]pyridin-5-one (6d). Acid **12** (0.25 g, 0.79 mmol) and thionyl chloride (1 mL) were refluxed for 1.5 h. The excess thionyl chloride was removed under vacuum, the residue was dissolved in nitrobenzene and aluminum chloride (0.60 g, 4.49 mmols) was added. The mixture was heated at 100 °C for 3 h, poured over ice and the solid collected by filtration. The residue was suspended in ammonium hydroxide (7%, 25 mL) and the mixture steam distilled. The solid was collected by filtration, air dried and recrystallized from dimethylformamide to afford **6d** (0.21 g, 98%, mp 219–220 °C; ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1H), 8.72 (d, J = 5.4 Hz, 1H), 7.87 (d, J = 5.4 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 2.54 (s, 3H).

***N*,*N*,5-Trimethyl-2*H*-pyrido[3′,4′:5,6]thiopyrano[4,3,2-*cd*]-indazole-2-ethanamine (4d).** Following the procedure for the preparation of **4b** and **4c**, **6d** was converted into **4d**. Flash chromatography of the crude material led to a yellow solid (45%) which showed about 3% of regioisomer **5d**. Purification on a chromatotron led to **4d** (43%), mp 112–113 °C; ^{1}H NMR ($CDCl_3$) δ 9.16 (s, 1H), 9.29 (d, J = 5.4 Hz, 1H), 7.17 (d, J = 5.4 Hz, 1H), 7.08 (d, J = 7.0 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 4.41 (t, J = 7.0 Hz, 2H), 2.87 (t, J = 7.0 Hz, 2H), 2.33 (s, 6H), 2.21 (s, 3H).

Biological assays

The methodology used for the in vitro evaluations against human colon adenocarcinoma (LoVo) and human colon adenocarcinoma resistant to doxorubicin (LoVo/Dx) has been previously described.[11] The

protocols for the L1210, S180 and S180/Dx followed a published procedure,[17] except the drugs were dissolved in DMSO.

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