Synthesis of new tetrazolyldienylphenothiazines as potential multidrug resistance inhibitory compounds

Ildikó Nagy[a], Zsuzsanna Riedl[a], György Hajós[a], András Messmer[a], Nóra Gyémánt[b], and József Molnár[b]

[a] Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1525 Budapest, POBox 17, Hungary **,**
[b] Department of Medical Microbiology, University of Szeged, H-6720, Dóm tér 10, Szeged, Hungary
E-mail: ghajos@chemres.hu

Dedicated to Professor Sándor Antus on his 60th birthday
(received 20 Dec 03; accepted 28 Feb 04; published on the web 05 Apr 04)

Abstract
Ring opening of tetrazolopyridinium salts with phenothiazines yielded stable *cis-trans* tetrazolyldienamines. One of the new derivatives proved to inhibit significantly the multidrug resistant efflux pump mechanism and, thus, these new phenothiazine derivatives are regarded as promising candidates for further drug research in the MDR area.

Keywords: Ring opening, dienamine, phenothiazine, multidrug resistance

Introduction

Cancer is the third or second most important cause of death worldwide. Multidrug resistant (MDR) efflux pumps extrude anticancer drugs from the tumor cells and, therefore, multidrug resistance leads to treatment difficulties in the majority of cancer patients. The effectiveness of chemotherapy could be increased by the combination of cytostatic drugs with resistance modifiers. Compounds that inhibit the activity of ATP-binding cassette (ABC) transporters can enhance drug accumulation in cancer cells and, consequently, the antiproliferative or cytotoxic action for tumor cells could be enhanced. Several years ago some amphiphilic compounds such as traditional phenothiazines were found to be effective inhibitors of MDR1 encoded phosphoglycoprotein 170 KD (pgp) efflux protein in a relatively high concentration in vitro. 1-3

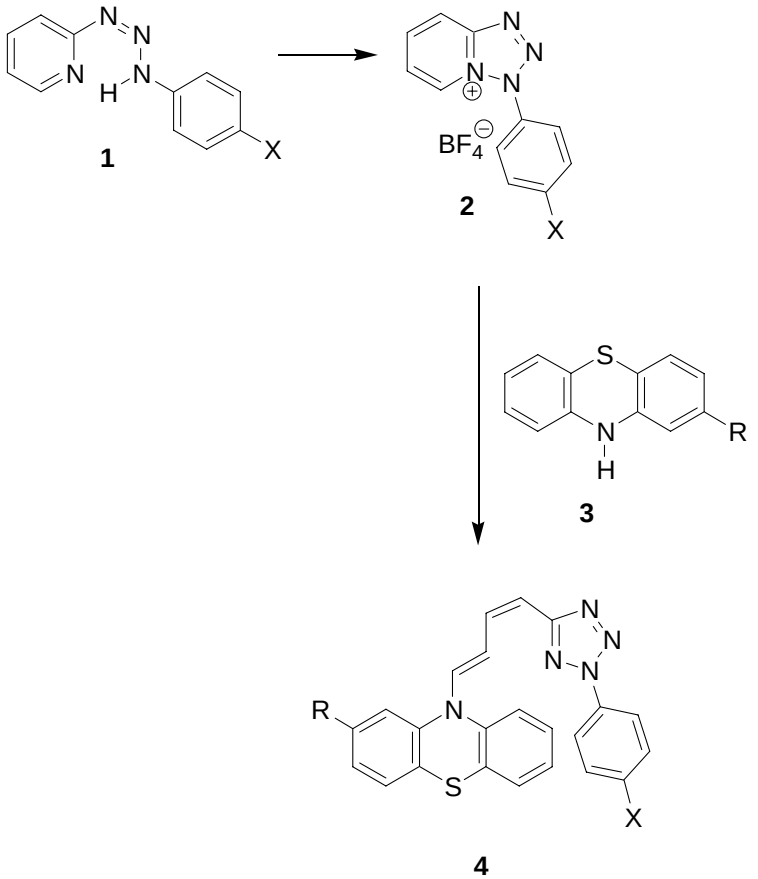
Based on the former results the main aim of our study was to develop new and more effective phenothiazines with MDR reversal action.

Results and Discussion

In the course of our earlier results on fused azolium salts we elaborated a facile synthetic route to various azolyl dienamines[4-7] and we have found that the phenothiazine moiety also can be introduced into these compounds.[8] These derivatives can be obtained in a single step by ring opening of azolopyridinium salts (**2**) by secondary amines. Thus, a series of tetrazolyldienamines has been obtained.

Based on some recent biological results in the area of efflux pump inhibitory compounds,[1] an enhanced interest has been focused to phenothiazine derivatives, particularly to those having a carbon chain substituent on the nitrogen atom. This recognition prompted us to apply our preparative technique for the synthesis of novel phenothiazines having a dienyl chain attached to a substituted tetrazole ring.

To this end, we decided to extend our well established ring opening methodology for the synthesis of phenothiazinyldienes and, in light of the variation possibilities in the structure, introduction of different functional groups seemed to be straightforward. In this paper, we describe the synthesis of seven differently substituted phenothiazinyldienes (**4a-g**) by ring opening of the tetrazolopyridinium salts (**2a-c**), easily accessible by cyclization of triazenes **1a-c**.



Transformation of **2** to **4** was carried out by treatment of the starting material with the sodium salt of the appropriate phenothiazine (**3**) at room temperature in tetrahydrofuran. Comparison of

the coupling constants in the ^{1}H-NMR data of the dienyl products to those observed with analogous compounds prepared earlier[8] revealed that the double bond C3-C4 retained its *cis* geometry, whereas the C1-C2 double bond had a *trans* substitution pattern.

In order to test the biological activity of the new compounds, one representative derivative: **4e** was subjected to investigations and its reversal of MDR was studied in human MDR1 gene transfected mouse lymphoma cells by measuring rhodamine 123 accumulation in the model experiments.

Samples	μg/ml	Fluorescence Activity Ratio
Verapamil	10	12.23
4e	4	2.50
	40	10.01
DMSO Control		1.01

The efficiency of this new phenothiazine (**4e**) seems to be promising because 4 μg/ml of **4e** increased the rhodamine accumulation by 2.5 fold in our in vitro flow cytometric studies.

Further structural modifications of phenothiazines of this type and tests of the new derivatives are in progress.

Conclusions

These results indicate that the preparative pathway elaborated by us to the new dienylphenothiazines may open a relatively facile route to a new group of efflux pump inhibitory compounds. Further investigations in this area are in progress.

Experimental Section

General Procedures. Melting points were determined using a Büchi apparatus and are uncorrected. The IR spectra were recorded with an Avatar 320 FT-IR spectrophotometer; the ^{1}H and ^{13}C NMR spectra were recorded using Varian UNITY-INOVA (400 MHz) and Varian Gemini 2000 (200 MHz) spectrometers. Starting materials **1a**,**b** and **2a**,**b** were prepared according to the literature procedure.[9]

Reversal of MDR of tumor cells

The L5178 mouse T-cell lymphoma cell line was infected with pHa MDR1/A retrovirus as previously described.[10] MDR-1-expressing cell lines were selected by culturing the infected cells

with 60 ng/mL colchicine. The MDR and PAR (parent) cell lines were grown in McCoy's 5A medium with 10%heat-inactivated horse serum, L-glutamine and antibiotics. The cells were adjusted to a density of 2 x 10^6/mL resuspended in serum-free McCoy's 5A medium and distributed in 0.5 mL aliquots into Eppendorf centrifuge tubes. Various volumes (2-20□L) of the 1.0 mg/mL stock solutions of the tested compounds were then added and the cells were incubated for 10 min at room temperature. 10 μL (5.2 μM final concentration) rhodamine 123 indicator was then added and the cells were incubated for a further 20 min at 37 ºC, washed twice, and resuspended in 0.5 mL phosphate-buffered saline (PBS) for analysis. The fluorescence of the cell population was measured by flow cytometry, using a Beckton-Dickinson FACScan instrument. Verapamil was used as a positive control. The percentages of the control mean fluorescence intensity were calculated for MDR and parental cells as compared with untreated cells.[10] The activity ratio was calculated from the measured fluorescence values using the following formula:

$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\,treated \,/\, parental\,control}$$

Ethyl 4-[(2*E/Z*)-3-pyridin-2-yltriaz-2-en-1-yl]benzoate (1c). This compound was prepared from 2-aminopyridine (1.9 g, 20 mmol) and 4-(ethoxycarbonyl)benzenediazonium tetrafluoroborate (22 mmol) according to the procedure described for the parent derivatives[9], yield: yellow crystals, 3.24 g (12 mmol, 60 %), mp 178-180 ^oC (toluene). IR (KBr): 1717, 1599, 1589, 1534, 1444, 1432, 1403, 1277, 1198, 1150, 999, 772 cm^{-1}; ^1H NMR δ ($CDCl_3$): 1.42 (t, 3H, *J* = 7.0 Hz), 4.40 (q, 2H, *J* = 7, 0 Hz), 7.03 (dd, 1H, *J* = 7.0, 2.0 Hz), 7.56-7.76 (m, 4H), 8.08-8.12 (AA'), 8.56 (d, 1H, *J* = 7.0 Hz); ^{13}C NMR δ ($CDCl_3$): 14.7, 61.3, 109.3, 118.8, 121.5, 129.5, 130.9, 139.0, 148.0, 153.2, 155.2, 166.6. Anal. Calcd. for $C_{14}H_{14}N_4O_2$ (270.29): C, 62.21; H, 5.22; N, 20.73. Found: C, 62.08; H, 5.24; N, 20.85.

3-(4-Ethoxycarbonyl-phenyl)-3H-tetrazolo[1,5-a]pyridin-4-ylium tetrafluoroborate (2c). This compound was prepared by the reaction of **1c** (2.7 g, 10 mmol) and tribromophenol bromine (13.3 g, 31 mmol) according to the procedure described for the parent derivatives[9], yield: colorless crystals, 1.63 g (4.6 mmol, 46 %), mp 159-160 ^oC (iPrOH). IR (KBr): 1717, 1497, 1293, 1055, 763 cm^{-1}; ^1H NMR δ (DMSO-d_6): 1.44 (t, 3H, *J* = 7.0 Hz), 4.48 (q, 2H, *J* = 7.0 Hz), 8.21-8.33 (m, 3H), 8.48-8.52 (AA'), 8.70 (dd, 1H, *J* = 7.3, 8.8 Hz), 9.14 (d, 1H, *J* = 8.8 Hz), 9.64 (d, 1H, *J* = 6.5 Hz); ^{13}C NMR δ (DMSO-d_6): 15.0, 62.7, 119.3, 125.8, 128.4, 132.6, 134.3, 135.5, 140.6, 150.1, 165.3. Anal. Calcd. for $C_{14}H_{13}BF_4N_4O_2$ (356.08): C, 47.22; H, 3.68; N, 15.73. Found: C, 47.08; H, 3.54; N, 15.68.

General procedure for the synthesis of butadienylphenothiazines

Sodium hydride (60% suspension in paraffin, 0.046 g, 1.21 mmol) was added to freshly distilled tetrahydrofuran (10 ml) under water-free conditions and the appropriate phenothiazine (1.1 mmol) was added. The mixture was stirred for 10 min at room temperature. Then, the tetrazolopyridinium salt (**2**, 1 mmol) was added to the reaction mixture in small portions in 5 min, approximately, and stirring was continued for an additional 60 min. The yellow colored suspension turned dark. The reaction mixture was poured onto ice-water and the product was

extracted with dichloromethane (3 x 20 ml), the combined organic layer was washed with water and was dried over sodium sulfate. After evaporation the residue was triturated with diethyl ether – unless otherwise mentioned - to yield the pure product. Samples for analysis were either recrystallized from the given solvent or – if decomposition took place upon heating - were further purified by chromatography.

10-{(1E,3Z)-4-[2-(4-Chlorophenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-10*H*-phenothiazine (4a). Yield: yellow crystals, 0.35 g (0.81 mmol, 81 %), mp 176-177 °C (EtOAc). IR (KBr): 1622, 1583, 1500, 1463, 1305, 1257, 1201, 994, 828, 766 cm^{-1}; ^1H NMR δ (DMSO-d_6): 6.15 (d, 1H, *J* = 11.0 Hz), 6.85 (pseudo t, 1H, *J* = 11.0 Hz), 7.51 (dd, 1H, *J* = 11.0, 13.5 Hz), 7.59 (d, 1H, *J* = 13.5 Hz), 7.33 (2H), 7.45 (2H), 7.50 (2H), 7.67 (2H), 7.76 (BB'), 8.00 (AA'). Anal. Calcd. for $C_{23}H_{16}ClN_5S$ (429.92): C, 64.25; H, 3.75; N, 16.29; S 7.46. Found: C, 64.13; H, 3.64; N, 15.98; S, 7.31.

2-Chloro-10-{(1E,3Z)-4-[2-(4-chlorophenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-10*H*-phenothiazine (4b). Yield: yellow crystals, 0.40 g (0.86 mmol, 86 %), mp 129-131 °C. IR (KBr): 1621, 1582, 1501, 1462, 1299, 1239, 1200, 1098, 992, 827 cm^{-1}; ^1H NMR δ ($CDCl_3$): 6.23 (d, 1H, *J* = 11.0 Hz), 6.62 (dd, 1H, *J* = 11.0, 11.4 Hz), 7.13-7.38 (m, 6H), 7.47-7.69 (m, 5H), 7.99-8.03 (AA'); ^{13}C NMR δ ($CDCl_3$): 104.5, 106.9, 121.1, 122.7, 122.9, 126.0, 126.1, 128.0, 128.5, 129.1, 129.5, 130.0, 130.5, 133.6, 135.4, 135.6, 136.6, 140.8, 141.5, 142.9, 165.0. Anal. Calcd. for $C_{23}H_{15}Cl_2N_5S$ (464.37): C, 59.49; H, 3.26; N, 15.08; S 6.91. Found: C, 59.13; H, 3.24; N, 14.95; S, 6.71.

10-{(1E,3Z)-4-[2-(4-Chlorophenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-2-(trifluoromethyl)-10*H*-phenothiazine (4c). Yield: pale yellow crystals, 0.33 g (0.66 mmol, 66 %), mp 130-133 °C. IR (KBr): 1621, 1585, 1501, 1326, 1248, 1200, 1107, 992, 821 cm^{-1}; ^1H NMR δ ($CDCl_3$): 6.24 (d, 1H, *J* = 11.4 Hz), 6.64 (pseudo t, 1H, *J* = 11.4 Hz), 7.15-7.26 (m, 2H), 7.33-7.49 (m, 6H), 7.56-7.63 (m, 2H), 7.72 (s, 1H), 7.93-7.97 (AA'); ^{13}C NMR δ ($CDCl_3$): 104.7, 107.2, 120.5, 120.8 (q, *J* = 250 Hz), 121.1, 121.5, 122.5, 123.1, 126.4, 127.1, 128.1, 128.7, 128.8, 130.0, 131.0, 135.4, 135.6, 136.5, 140.7, 141.0, 142.5, 164.9. Anal. Calcd. for $C_{24}H_{15}ClF_3N_5S$ (497.92): C, 57.89; H, 3.04; N, 14.07; S, 6.44. Found: C, 57.64; H, 2.91; N, 13.70; S, 6.10.

10-{(1E,3Z)-4-[2-(4-Methoxyphenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-10*H*-phenothiazine (4d). Yield: pale yellow crystals, 0.31 g (0.74 mmol, 74 %), mp 134-136 °C (CH_3CN). IR (KBr): 1618, 1582, 1513, 1462, 1302, 1254, 1200, 1032, 995, 830, 758 cm^{-1}; ^1H NMR δ ($CDCl_3$): 3.90 (s, 3H), 6.17 (d, 1H, *J* = 11.0 Hz), 6.60 (dd, 1H, *J* = 11.0, 11.5 Hz), 6.98 (BB'), 7.15 (dd, 2H), 7.16 (dd, 1H, *J* = 14.0 Hz), 7.31 (dd, 2H), 7.32 (d, 2H), 7.53 (d, 2H), 7.67 (dd, 1H, J = 11.5, 14.0 Hz), 7.93 (AA'); ^{13}C NMR δ ($CDCl_3$): 55.8, 103.9, 106.4, 114.7, 121.2, 122.6, 125.7, 127.6, 128.2, 130.6, 130.7, 136.2, 141.0, 141.8, 160.4, 164.6; Anal. Calcd. for $C_{24}H_{19}N_5OS$ (425.51): C, 67.74; H, 4.50; N, 16.46; S, 7.54. Found: C, 67.54; H, 4.47; N, 16.71; S, 7.36.

2-Chloro-10-{(1E,3Z)-4-[2-(4-methoxyphenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-10*H*-phenothiazine (4e). Yield: yellow crystals, 0.27 g (0.59 mmol, 59 %), mp 114-116 °C. IR (KBr): 1624, 1582, 1513, 1462, 1302, 1251, 998, 830, 746 cm^{-1}; ^1H NMR δ ($CDCl_3$): 3.89 (s, 3H), 6.23 (d, 1H, *J* = 11.4 Hz), 6.59 (pseudo t, 1H, *J* = 11.4 Hz), 6.98-7.02 (BB'), 7.10-7.38 (m, 6H), 7.50-7.55 (m, 2H), 7.65 (dd, 1H, *J* = 11.4, 13.5 Hz), 7.94-7.99 (AA'); ^{13}C NMR δ ($CDCl_3$): 56.0,

104.7, 107.4, 114.9, 121.5, 122.7, 125.9, 126.1, 128.0, 128.5, 129.1, 129.3, 130.4, 130.8, 133.7, 135.4, 135.9, 140.3, 141.5, 143.1, 160.6, 164.6. Anal. Calcd. for $C_{24}H_{18}ClN_5OS$ (459.95): C, 62.67; H, 3.94; N, 15.23; S, 6.97. Found: C, 62.76; H, 3.91; N, 14.96; S, 6.77.

10-{(1E,3Z)-4-[2-(4-Methoxyphenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl}-2-(trifluoromethyl) -10*H*-phenothiazine (4f). Yield: yellow crystals, 0.19 g (0.39 mmol, 39 %), mp 110-112 ^{o}C. IR (KBr): 1624, 1585, 1513, 1426, 1332, 1245, 1152, 1104, 998, 881, 830, 746 cm^{-1}; ^{1}H NMR δ ($CDCl_3$): 3.89 (s, 3H), 6.25 (d, 1H, *J* = 11.4 Hz), 6.61 (dd, 1H, *J* = 11.4, 11.7 Hz), 6.97-7.02 (BB'), 7.14-7.26 (m, 2H), 7.33-7.41 (m, 4H), 7.58-7.67 (m, 2H), 7.71 (s, 1H), 7.90-7.95 (AA'); ^{13}C NMR δ ($CDCl_3$): 56.0, 104.9, 107.7, 114.8, 120.7 (q, *J* = 270 Hz), 121.5, 122.4, 123.1, 126.3, 127.1, 128.2, 128.6, 129.8, 130.5, 130.8, 135.5, 135.8, 140.2, 141.0, 141.3, 142.7, 160.6, 164.6. Anal. Calcd. for $C_{25}H_{18}F_3N_5OS$ (493.50): C, 60.84; H, 3.68; N, 14.19; S, 6.50. Found: C, 60.84; H, 3.69; N, 14.19; S, 6.33.

Ethyl 4-{5-[(1E,3Z)-4-(10*H*-phenothiazin-10-yl)buta-1,3-dien-1-yl]-2*H*-tetrazol-2-yl}benzoate (4g). Yield: yellow crystals, 0.29 g (0.63 mmol, 63 %), mp 166 ^{o}C (CH_3CN). IR (KBr): 1711, 1618, 1582, 1462, 1281, 1257, 1200, 1128, 1110, 986, 761 cm^{-1}; ^{1}H NMR δ ($CDCl_3$): 1.45 (t, 3H, *J* = 7.0 Hz), 4.45 (q, 2H, *J* = 7.0 Hz), 6.19 (d, 1H, *J* = 11.0 Hz), 6.66 (dd, 1H, *J* = 11.0, 11.4 Hz), 7.17-7.26 (m, 3H), 7.30-7.38 (m, 4H), 7.52-7.57 (m, 2H), 7. 66 (dd, 1H, *J* = 11.4, 13.5 Hz), 8.08-8.22 (m, 4H); ^{13}C NMR δ ($CDCl_3$): 14.7, 61.8, 103.8, 105.8, 119.4, 122.7, 126.0, 127.7, 128.4, 130.9, 131.3, 137.4, 139.9, 141.8, 165.3, 165.8. Anal. Calcd. for $C_{26}H_{21}N_5O_2S$ (467.54): C, 66.79; H 4.53; N, 14.98; S, 6.86. Found: C, 66.87; H, 4.65; N, 15.05; S, 6.70.

Acknowledgments

Thanks are due to financial support provided by OTKA T 33105, T 35176, 1/047 NKFP MediChem, COST B16 as well as the QLK2-CT-2002-90436 project funded by the European Union for Center of Excellence in Biomolecular Chemistry.

References

1. Viveiros, M.; Amaral, L. *Int. J. Antimicrob. Agents* **2001**, *17*, 225
2. Motohashi, N. *Anticancer Res.* **1991**, 11, 1125
3. Kolaczkowski, M.; Michalak, K.; Motohashi, N. *Int. J. Antimicrob. Agents* **2003**, *22*, 279
4. Hajós, Gy.; Messmer, A.; Koritsánszky, T. *J. Org. Chem*. **1987**, *52*, 2015
5. Messmer, A.; Hajós, G.; Timári, G. *Tetrahedron* **1992**, *48*, 8451
6. Kotschy, A.; Hajós, G.; Timári, G.; Messmer, A. *J. Org. Chem*. **1996**, *61*, 4423
7. Béres, M.; Hajós, G.; Riedl, Z.; Timári, G.; Messmer, A.; Holly, S.; Schantl, J. G. *Tetrahedron* **1997**, *53*, 9393
8. Gelléri, A.; Messmer, A.; Nagy, S.; Radics, L. *Tetrahedron Lett*. **1980**, *21*, 663
9. Messmer A.; Gelléri, A.; Hajós, Gy. *Tetrahedron* **1986**, *42*, 4827.
10. Cornwell M. M.; Pastan, I.; Gottesmann, M. M. *J. Biol. Chem.* **1987**, *262*, 2166