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Synthesis of Polycarpine, a Cytotoxic Sulfur-Containing Alkaloid from the Ascidian *Polycarpa Aurata*, and Related Compounds

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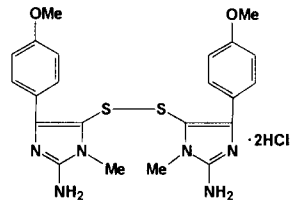
Abstract: Polycarpine **1**, a highly cytotoxic marine natural product, has been synthesized in three steps from p-methoxyphenacyl bromide **4** in 57% overall yield. The key reaction for construction of the symmetrically substituted disulfide linkage of polycarpine is the treatment of 2-amino-4-(4-methoxyphenyl)-1-methylimidazole **17** with S_2Cl_2 in acetic acid. In a similar way ten related compounds, including three thiazole analogues, have been prepared. Most of them exhibit high cytotoxic activities against an array of human cancer cell lines. © 1997 Elsevier Science Ltd.

Bis[2-amino-4-(4-methoxyphenyl)-1-methyl-5-imidazolyl]disulfide dihydrochloride (polycarpine) is a novel cytotoxic alkaloid recently isolated by Fedoreyev et al.[1] from the Pacific Ocean ascidian (or tunicate) *Polycarpa aurata* which was collected at Flinders Reef (Coral Sea). Polycarpine was subsequently reported from the Indian Ocean ascidian *Polycarpa clavata* (Western Australia) by Kang and Fenical[2] and from the ascidian *Polycarpa aurata* (Chuuk atoll, Federated States of Micronesia) by Schmitz et al.[3].



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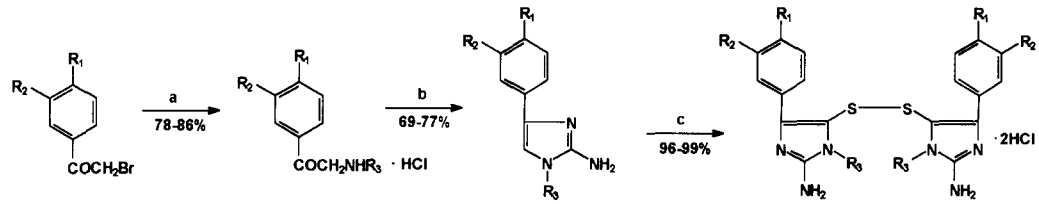
Polycarpine is attracting considerable interest, not only due to its high biological activity, but also as the first representative of a new structural type of alkaloid from an ascidian. In spite of a wide variety of metabolites isolated earlier from different ascidians, compounds containing 2-aminoimidazole cycles linked by a disulfide bridge[4-8] were not among the sulfur-containing metabolites.

In order to investigate the structure-activity relationships of this structurally simple but biologically very interesting molecule and other compounds of this type, we needed a simple and general synthesis of polycarpine and related compounds. Here we report the first synthesis of the title compound and several of its analogues. Our synthetic plan was to prepare initially the key intermediate **17**. We considered that two molecules of **17** could be linked by a disulfide bridge with the help of a suitable reagent. Polycarpine **1** was prepared in three steps from the commercially available p-methoxyphenacyl bromide **4** in 57% overall yield as outlined in Scheme 1 (**4**→**10**→**17**→**1**).

Scheme 1.



2	R_1=R_2=H	**8**	R_1=R_2=H, R_3=Me	**15**	R_1=R_2=H, R_3=Me	**22**	R_1=R_2=H, R_3=Me
3	R_1=Me, R_2=H	**9**	R_1=R_3=Me, R_2=H	**16**	R_1=R_3=Me, R_2=H	**23**	R_1=R_3=Me, R_2=H
4	R_1=OMe, R_2=H	**10**	R_1=OMe, R_2=H, R_3=Me	**17**	R_1=OMe, R_2=H, R_3=Me	**1**	R_1=OMe, R_2=H, R_3=Me
5	R_1=OEt, R_2=H	**11**	R_1=OMe, R_2=H, R_3=Et	**18**	R_1=OMe, R_2=H, R_3=Et	**24**	R_1=OMe, R_2=H, R_3=Et
6	R_1=Cl, R_2=H	**12**	R_1=OEt, R_2=H, R_3=Me	**19**	R_1=OEt, R_2=H, R_3=Me	**25**	R_1=OEt, R_2=H, R_3=Me
7	R_1=R_2=OMe	**13**	R_1=Cl, R_2=H, R_3=Me	**20**	R_1=Cl, R_2=H, R_3=Me	**26**	R_1=Cl, R_2=H, R_3=Me
		14	R_1=R_2=OMe, R_3=Me	**21**	R_1=R_2=OMe, R_3=Me	**27**	R_1=R_2=OMe, R_3=Me

a: $MeNH_2$ (or $EtNH_2$), MeOH, r.t., 30 min, then workup with dry HCl; b: S-ethylthiourea, NaOH, H_2O, r.t., 24 h; c: S_2Cl_2, AcOH, r.t., 6 h.

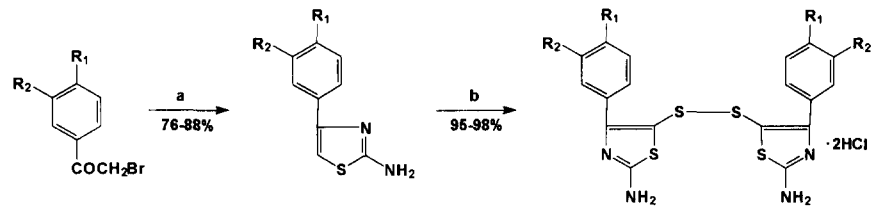
Nucleophilic substitution of a bromine atom by a NHMe group in substrate **4** gave satisfactory results only with the use of a large excess of $MeNH_2$. Due to low stability of the free base, the corresponding α-amino ketone has been isolated in the hydrochloride form **10**. Conversion of intermediate **10** into the 2-aminoimidazole **17** was performed through reaction with S-ethylthiourea in water in the presence of NaOH. The final step in the sequence was realized by the use of S_2Cl_2 in acetic acid which appeared to be an ideal reagent for coupling of two molecules of **17** via a disulfide bridge. Spectral characteristics and m.p. of synthetic polycarpine are identical to those of natural material[9].

Similarly, six related compounds **22**-**27** were synthesized. In the case of analogues **25** and **27** starting compounds **5** and **7** were obtained by bromination of commercially available acetophenones (Br_2, $CHCl_3$, reflux, 30 min, 65-78%). Total yields of analogues **22**-**27** were 40, 54, 62, 39 (calculated on four steps), 59, and 32% (on four steps), respectively.

The thiazole analogue of polycarpine **32** and two related compounds **33** and **34** were prepared in accordance with Scheme 2. The starting bromide **28** was prepared in the same fashion as for **5** and **7**. Conversion of phenacyl bromides **4**, **7**, and **28** into the corresponding 2-aminothiazoles **29**-**31** was readily accomplished by the action of thiourea in EtOH under reflux. Formation of disulfide linked compounds **32**-**34** was carried out in the same manner as for **22**-**27** but the 2-aminothiazoles **29**-**31** reacted with S_2Cl_2 more rapidly than the 2-aminoimidazoles **15**-**21**. Total yields of compounds **32**-**34** were 74, 47 (on three steps), and 58% (on four steps), respectively.

Lastly, the 2-desamino analogue of polycarpine **38** was synthesized as outlined in Scheme 3. Methylation of imidazole **35** using MeI in acetone in the presence of a large excess of dry KOH yielded a mixture of isomers **36** and **37** (ca. 3:1). The major product **36** was easily separated by crystallization from EtOAc in 62% yield. Treatment of **36** with S_2Cl_2 in AcOH afforded analogue **38** in 34% overall yield. It is interesting to note that the other structural isomer **37** did not react with S_2Cl_2 under the same conditions.

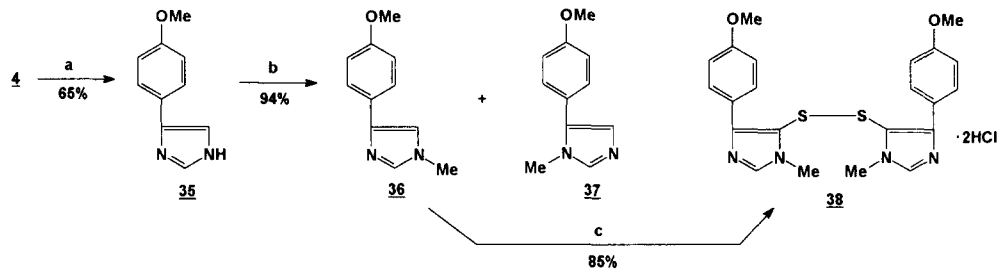
Scheme 2.



4	R_1=OMe, R_2=H	29	R_1=OMe, R_2=H	32	R_1=OMe, R_2=H
7	R_1=R_2=OMe	30	R_1=R_2=OMe	33	R_1=R_2=OMe
28	R_1=F, R_2=H	31	R_1=F, R_2=H	34	R_1=F, R_2=H

a: thiourea, EtOH, reflux, 3 h, then workup with NH_3, H_2O, pH~9; b: S_2Cl_2, AcOH, r.t., 30 min.

Scheme 3.



a: $HCONH_2$, NH_3, 160°C, 5 h; b: MeI, Me_2CO, KOH, r.t., 7 min; c: S_2Cl_2, AcOH, r.t., 5 h.

All disulfides prepared were tested in the *in vitro* disease-oriented primary antitumor screen against 60-75 lines of cancer cells (National Cancer Institute, Bethesda, Maryland, USA). The panel consisted of leukemia, non-small and small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines.

Synthetic polycarpine **1** was particularly active against cell lines of CNS cancer (lines SNB-75, GI_{50}=0.02 μg/ml, LC_{50}=20.79 μg/ml; XF 498, GI_{50}=1.12 μg/ml, LC_{50}=6.01 μg/ml; SNB-78, GI_{50}=1.43 μg/ml), colon cancer (HCT-116, GI_{50}=0.80 μg/ml, LC_{50}=4.32 μg/ml; SW-620, GI_{50}=0.97 μg/ml, LC_{50}=3.74 μg/ml), non-small cell lung cancer (NCI-H522, GI_{50}=0.87 μg/ml, LC_{50}=2.94 μg/ml; LXFL 529, GI_{50}=0.92 μg/ml, LC_{50}=3.82 μg/ml; HOP-92, GI_{50}=0.96 μg/ml, LC_{50}=4.57 μg/ml), leukemia (6 lines, GI_{50}=0.99-1.69 μg/ml), melanoma (SK-MEL-5, GI_{50}=1.04 μg/ml, LC_{50}=4.41 μg/ml; LOX IMVI, GI_{50}=1.11 μg/ml, LC_{50}=9.00 μg/ml), ovarian cancer (OVCAR-5, GI_{50}=1.21 μg/ml, LC_{50}=7.46 μg/ml), renal cancer (RXF-393, GI_{50}=1.07 μg/ml, LC_{50}=3.60 μg/ml), and breast cancer (MDA-N, GI_{50}=1.11 μg/ml, LC_{50}=3.77 μg/ml).

Among analogues the highest cytostatic and cytotoxic activities were shown by the following compounds: against CNS cancer - **22** (SNB-78, GI_{50}=0.81 μg/ml, LC_{50}=4.40 μg/ml) and **23** (XF 498, GI_{50}=0.80 μg/ml, LC_{50}=2.79 μg/ml); against colon cancer - **23** (SW-620, GI_{50}=0.74 μg/ml, LC_{50}=3.06 μg/ml); against non-small cell lung cancer - **24** (HOP-92, GI_{50}=0.03 μg/ml, LC_{50}=10.76 μg/ml), **33** (HOP-92,

GI_{50}=0.22 μg/ml, LC_{50}=5.89 μg/ml), **22** (NCI-H522, GI_{50}=0.62 μg/ml, LC_{50}=2.68 μg/ml; LXFL 529, GI_{50}=0.62 μg/ml, LC_{50}=3.56 μg/ml), and **23** (NCI-H522, GI_{50}=0.62 μg/ml, LC_{50}=2.53 μg/ml; LXFL 529, GI_{50}=0.79 μg/ml, LC_{50}=7.61 μg/ml); against leukemia - **27** (CCRF-CEM, GI_{50}=0.21 μg/ml), **26** (HL-60(TB), GI_{50}=0.58 μg/ml, LC_{50}=4.45 μg/ml; RPMI-8226, GI_{50}=0.72 μg/ml, LC_{50}=4.56 μg/ml), **25** (CCRF-CEM, GI_{50}=0.75 μg/ml), and **24** (CCRF-CEM, GI_{50}=0.86 μg/ml); against melanoma - **27** (UACC-257, GI_{50}=0.40 μg/ml, LC_{50}=2.64 μg/ml), **22** (LOX IMVI, GI_{50}=0.80 μg/ml, LC_{50}=2.92 μg/ml; SK-MEL-5, GI_{50}=0.86 μg/ml, LC_{50}=2.71 μg/ml); against breast cancer - **26** (MDA-MB-231/ATOC, GI_{50}=0.77 μg/ml, LC_{50}=3.26 μg/ml) and **22** (MDA-MB-435, GI_{50}=0.88 μg/ml, LC_{50}=2.88 μg/ml), against ovarian cancer - **27** (OVCAR-8, GI_{50}=0.81 μg/ml, LC_{50}=3.27 μg/ml), and against prostate cancer -**33** (RB, GI_{50}=0.48 μg/ml; DHM, GI_{50}=0.55 μg/ml; WMF, GI_{50}=0.74 μg/ml).

Polycarpine **1** also showed significant antitumor activity *in vivo* (white mice) against P_{388} murine leukemia, L_{1210} leukemia, carcinoma Ehrlich cells and high inhibitory activity against reverse transcriptases from Raus sarcoma and avian myeloblastosis viruses *in vitro* ($IC_{50}=3.5\times10^{-6}$ M) and Na^+,K^+-ATPase isolated from brain of the rats ($IC_{50}=5.0\times10^{-7}$ M).

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9. Polycarpine **1**: yellow-orange powder, m.p. 209-211°C (EtOH-$CHCl_3$, 4:1) (cf. 201-203°C[2], 201-204°C[3]). IR ($CHCl_3$) ν_{max}: 1558, 1611, 1650, 3100-3413 cm^{-1}. UV (EtOH) λ_{max}, ε: 210(17900), 263(23500), 362(5000) nm. ^1H NMR (CD_3OD) δ: 3.25 (s, 6H, 2×NMe), 3.96 (s, 6H, 2×OMe), 7.07 (m, $4H_{arom.}$), 7.49 (m, $4H_{arom.}$). ^{13}C NMR (CD_3OD): 30.0 (q), 56.7 (q), 112.0 (s), 115.0 (d), 119.0 (s), 129.6 (d), 139.2 (s), 149.1 (s), 163.3 (s). EIMS (70 eV, 150°C) m/z (rel. int.): 468(M^+, 0.1), 235(13), 234(20), 233(100), 232(4), 218(12), 204(15), 203(55), 202(21), 194(2), 193(6), 192(28), 188(21), 152(29).

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