TLC purification (silica gel, chloroform) gave 36 mg (34%) of **23** as an unstable solid, which decomposed on heating: ^{1}H NMR δ 8.17 (s, 1 H, H-11), 6.93 (s, 1 H, H-8), 6.63 (s, 1 H, H-3), 6.49 (d, 1 H, J = 1.9 Hz, 7-$CHCl_2$), 5.77 (d, 1 H, J = 5.1 Hz, 5-$CHCl_2$), 4.64 (d, 1 H, J = 1.9 Hz, H-7α), 3.97 (s, 3 H, 9-CH_3O), 3.94 (s, 3 H, 2-CH_3O), 3.91 (s, 3 H, 10-CH_3O), 3.91 (dd, 1 H, J = 16.0, 8.0 Hz, H-4β), 3.81 (dd, 1 H, J = 8.0, 5.1 Hz, H-5α), 3.66 (s, 3 H, 1-CH_3O), 3.20 (d, 1 H, J = 16.0 Hz, H-4α), 3.11 (s, 3 H, NCH_3); ^{13}C NMR δ 154.61 (s), 149.84 (s), 148.33 (s), 144.31 (s), 131.93 (s), 129.65 (s), 129.01 (s), 121.77 (s), 120.40 (s), 114.83 (d), 111.78 (s), 111.02 (d), 110.51 (d), 77.44 (s), 75.52 (d), 75.32 (d), 67.63 (d) 59.89 (q), 55.97 (q), 55.86 (q), 55.76 (q), 55.40 (d), 39.37 (q), 29.49 (t); IR (KBr) 2940, 2820, 1600, 1575, 1515, 1460, 1395, 1320, 1255, 1215, 1110, 1080, 1025, 980, 880, 870, 800, 780, 770, 735 cm^{-1}.

5-(Dichloromethyl)-7-formyl-6a,7-didehydroglaucine (24). A solution of **23** (15 mg) in absolute ethanol (6 mL) and water (0.1 mL) was refluxed for 30 min. The solvent was removed, and the residue was purified by preparative TLC (silica gel, chloroform) to give the aldehyde **24** (10 mg, 69%), which crystallized from absolute ethanol: mp 192-194 °C; ^{1}H NMR δ 10.48 (s, 1 H, CHO), 9.15 (s, 1 H, H-11), 8.95 (s, 1 H, H-8), 7.03 (s, 1 H, H-3), 5.49 (d, 1 H, J = 9.2 Hz, 5-$CHCl_2$), 4.10 (s, 3 H, 9-CH_3O), 4.07 (s, 3 H, 2-CH_3O), 4.04 (s, 3 H, 10-CH_3O)8 3.87 (s, 3 H, 1-CH_3O)8 3.76-3.62 (m, 2 H, H-4β, H-5α), 3.50 (d, 1 H, J = 15.5 Hz, H-4α), 3.49 (s, 3 H, NCH_3); IR (KBr) 2920, 1660, 1590, 1580, 1500, 1460, 1390, 1240, 1120, 1050, 990, 775, 755 cm^{-1}; MS, m/e 465 (M^+ + 2, 23), 463 (M^+ 35), 437 (8), 435 (13), 381 (26), 380 (100), 364 (20), 352 (60), 336 (17), 334 (10), 322 (12), 306 (9); HR-MS calcd for $C_{23}H_{23}Cl_2NO_5$ 463.0948, found 463.0952.

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Registry No. **1**, 49715-56-2; **2**, 96839-01-9; **4**, 96839-04-2; **5**, 108510-88-9; **6**, 96838-54-9; **7**, 96838-53-8; **7**·MeI, 96838-55-0; **8**, 475-81-0; **9**, 103769-56-8; **10**, 96839-03-1; **12**, 96838-49-2; **13**, 96838-45-8; **13**·MeI, 96838-52-7; **14**, 108510-92-5; **14**·MeI, 108511-06-4; **15**, 103769-55-7; **16**, 108510-93-6; **17a**, 22212-26-6; **17b**, 72498-26-1; **17c**, 98931-17-0; **17d**, 108510-97-0; **18a**, 96839-02-0; **19a**, 96838-46-9; **19a**·MeI, 108511-03-1; **19b**, 96838-47-0; **20a**, 96838-50-5; **20a**·MeI, 96838-51-6; **20b**, 96838-48-1; **21b**, 108510-98-1; **23**, 108510-99-2; **24**, 108511-00-8.

Brominated Tyrosine Metabolites from an Unidentified Sponge

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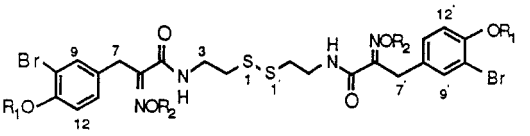
Two new isomeric brominated tyrosine metabolites containing disulfide linkages have been isolated from an unidentified sponge from Guam. Their structures were determined primarily from ^{1}H and ^{13}C NMR data. (3-Bromo-4-hydroxyphenyl)acetonitrile was also isolated.

Bromotyrosine-derived metabolites are typical of the sponge family Verongidae.[2] Especially novel among these are the bastadins, some of which are macrocycles comprised of several bromotyrosine units.[3] We wish to report novel additions to the bromotyrosine metabolite group, namely, symmetrical compounds with cystamine as the central unit. Among the many brominated tyrosine derivatives, the new compounds **1** and **5** are the first to contain a disulfide moiety. Although the sponge from which these compounds were isolated is unidentified, it is probably of the Verongidae family judging from the nature of its metabolites. Examination of this sponge was prompted by the fact that its extracts showed cytotoxicity, but the compounds described herein are not the cytotoxic components.

The metabolites were obtained from methanol and methanol/chloroform extracts and are quite polar but could be purified by silica gel chromatography followed by reverse-phase HPLC. For the predominant metabolite **1** the molecular formula $C_{22}H_{24}Br_2N_4O_6S_2$, implying 12 degrees of unsaturation, was established by combustion analysis, high-resolution fast atom bombardment (FAB) mass spectrometry, and ^{1}H and ^{13}C NMR (Table I). Since



	R_1	R_2
1 (E,E)	H	H
2 (E,E)	Ac	Ac
3 (E,E)	Me	Me
5 (E,Z)	H	H

only 11 carbon resonances were observed in the ^{13}C NMR spectrum, it could be concluded that **1** was symmetrically dimeric. The presence of amide and possibly oxime or imine groups was indicated by distinct infrared absorptions at 1636 and 1657 cm^{-1}, respectively. ^{13}C NMR signals at 167.5 and 154.7 ppm provided further evidence for these functionalities. Exchangeable proton signals were observed in the ^{1}H NMR spectrum in pyridine-d_5 at 14 (s), 12 (s), and 8.56 (t) ppm, consistent with the presence of oxime and phenolic protons and the proton of an amide flanked by a methylene group. The amide proton (8.56 ppm) was identified as part of the limited spin system shown in partial structure A by decoupling and deuterium exchange (3.48 ppm, q → t). Decoupling confirmed that the three aromatic proton signals were part of a 1,2,4-trisubstituted benzene unit and NOE difference data confirmed that the two-proton singlet at 4.01 ppm was due to a benzylic methylene group situated as shown in partial structure B.

(1) Taken in part from the Ph.D. Dissertation of Lili Arabshahi, University of Oklahoma, 1986.

(2) Faulkner, D. J. *Nat. Prod. Rep.* **1986**, *3*, 1 and references cited therein. *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic: New York, 1978-1982; Vol. I-V.

(3) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, *34*, 765. Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1980**, *21*, 2277.

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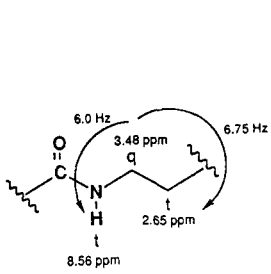
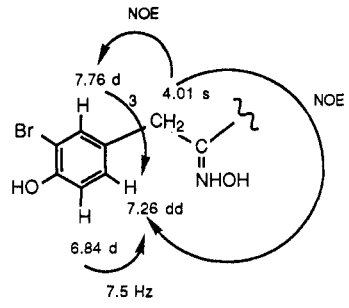


A

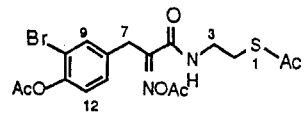


B

A hydroxyl substituent was required ortho to the proton absorbing at 6.84 ppm to account for this upfield aromatic proton absorption.[4a] The remaining aromatic substituent was identified as bromine on the basis of mass spectral fragment ions at m/z 185, 187 and 211, 213, corresponding to a benzylic carbocation derived from partial structure B and the (3-bromo-4-hydroxyphenyl)acetonitrile cation, respectively. Also, the chemical shifts of partial structure B are similar to those of the related portions of several of the bastadins.[3]

The ^{13}C NMR multiplicities for 1 were determined by an APT experiment,[5] and chemical shift assignments of protonated carbons were determined by selective single-frequency on-resonance decouplings. These data are in agreement with partial structures A and B. Long-range couplings determined by additional selective $^1H/^{13}C$ decoupling experiments (low decoupling power) confirmed the locations of the 155.4 and 112.1 ppm carbons in the benzene rings; these were in agreement with the assigned OH and Br substituents. Irradiation of the benzylic protons confirmed long-range couplings to aromatic carbons C-9 and C-13 but also collapsed the signal at 154.7 (t, J = 7 Hz) ppm to a singlet and reduced the quintet at 167.5 (J = 3 Hz) ppm to a triplet. Irradiation of the quartet due to the methylene protons next to the amide nitrogen of partial structure A also simplified the 167.5 ppm multiplet. Thus this carbon absorption could be assigned to the amide carbonyl and the 154.7 ppm signal to the oxime carbon in partial structure B. Since the benzylic protons are coupled to both the oxime and amide carbonyl carbons, partial structure B must be joined to the carbonyl group of A as shown in 1. The only site remaining at which to connect sulfur is the methylene carbon of partial structure A, an assignment consistent with the proton and carbon shifts observed. Structure 1 then follows for this metabolite to account for the symmetry revealed by the spectral data.

Confirmatory evidence for structure 1 was obtained by acetylation to give 2 and methylation (CH_3I, K_2CO_3,DMF)[3] to give 3. Both of these were stereoisomerically pure as indicated by their ^1H NMR spectra which were also consistent with completely symmetrical structures. Thus 2 and 3 each have identical stereochemistries at both oxime centers. Reaction of 1 with triphenylphosphine effected the expected reductive cleavage[6] of the disulfide link to give a monomeric product, which was immediately acetylated and then isolated and characterized as the triacetate 4. If the monomeric reduction product was not acetylated soon after completion of the reaction, it slowly underwent oxidative dimerization to regenerate 1.



4

Compound 5, a minor component isolated from the sponge extract, exhibited an ^1H NMR spectrum very similar to that of 1 (see Table I), except that nearly every signal was doubled, suggesting that the compounds had the same gross structure, but that 5 was not symmetrical. However, compound 5 isomerized over a period of a couple of weeks to 1 as evidenced by the ^1H NMR spectrum. During this time the sample was transferred from $CDCl_3/CD_3OD$ to DMSO-d_6 and back again with attendant warming to evaporate solvents. When ^1H NMR spectra revealed a change in the relative heights of members of the doubled peaks, the isomers were repurified by HPLC, but ultimately all of 5 isomerized to 1. In view of this interconversion, 5 was assigned the structure shown which is stereoisomeric with 1 at one of the oxime centers.

The stereochemistry of the oxime groups is assigned as E,E in 1 and E,Z in 5 on the basis of ^{13}C and ^1H NMR data. In the ^{13}C spectrum of 1 in Me_2SO-d_6, the methylene carbon signals appear at 27.7, 37.0, and 38.1 ppm, whereas in 5 the corresponding signals occur at 27.5, 35.7, 36.6, 36.8, 37.6, and 38.0 ppm. Clearly, it is one of the methylene carbons at 27.7 ppm in 1 that has shifted downfield to 35.7 ppm in the spectrum of 5. Such a shift is only consistent with an E to Z oxime configuration change.[4b] Hence, 1 must have the E,E geometry. Also, the benzylic protons of 1 occur at the lower (3.87 ppm) of the two benzylic signal positions noted for 5 (3.68 and 3.87 ppm) in $CDCl_3/CD_3OD$. This follows the pattern observed for other oximes; i.e., a methylene group α to an oxime resonates at lower field when it is oriented cis to the oxime OH than when trans.[7] The E geometry assigned to 1 is the same as that confirmed by X-ray analysis for one of the bastadins.[3]

Since the E,Z isomer 5 isomerizes to the E,E form 1, it is possible that 5 (or the Z,Z isomer thereof) is the natural metabolite and that isomerization during extraction, extract storage, and/or chromatography results in 1 being isolated as the predominant product.

A third, minor metabolite was identified as (3-bromo-4-hydroxyphenyl)acetonitrile (6)[8] on the basis of IR, ^1H NMR, and mass spectral data.

Experimental Section[9]

Extraction and Isolation Procedures. The sponge was collected at Western Shoals in Apra Harbor in Guam in April of 1983 at −3 to −5 m and shipped frozen to Oklahoma. Freshly thawed specimens (382 g wet weight) were soaked in methanol, then the mixture was filtered, and the filtrate was evaporated to

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(5) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535.
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(8) Goo, Y. M.; Rinehart, K. L. Jr. In *Drugs from the Sea: Myth or Reality*; Kaul, P. N., Sindermann, C. J., Eds.; University of Oklahoma: Norman, OK, 1978; p 107.
(9) Melting points, taken on a Kofler hot stage or Hoover melting point apparatus, are uncorrected. IR spectra were taken on a Perkin-Elmer 298 or Nicolet 8000 FT-IR (200 SXV vacuum bench) instrument, and the UV spectrum was taken on a Perkin-Elmer Lambda 3 spectrophotometer. ^1H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.4 MHz on a Varian XL-300 spectrometer; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Mass spectra were taken on Hewlett-Packard 5985 (low resolution) and Kratos MS-50 triple analyzer (high resolution) mass spectrometers. LiChrosorb Si60 5-μm silica gel and 5-μm C_{18} preparative (10 mm × 25 cm) columns were used for HPLC separations with a differential refractometer detector. Merck silica gel 60 (230–240 mesh) was used for column chromatography).

Table I. NMR Data for the Compounds 1 and 5

	1				5	
C	δ_{13C}[a]	δ_{13C}[b]	δ_{1H}[c]	δ_{1H}[d]	δ_{13C}[b]	δ_{1H}[c]
2,2′	40.1 (t, 140)	37.0[e]	2.87 (t, 7)	2.65 (t, 7)	36.6,[e] 36.7	2.95 (m)
3,3′	41.2 (t, 140)	38.1[e]	3.63 (t, 7)	3.48 (q, 7)	37.6,[e] 38.0[e]	3.50, 3.65 (m)
N-4,4′				8.56 (t, 7)		
5,5′	167.5 (m, 3)				161.9, 163.1	
6,6′	154.7 (t, 7)	151.8[f]			151.1,[f] 151.6[f]	
7,7′	30.3 (tt, 129, 3)	26.7	3.87 (s)	4.01 (s)	27.5, 35.7	3.68 (s), 3.87 (s)
8,8′	132.1 (m)	128.8			128.2	
9,9′	136.0 (dm, 163)	132.9	7.62 (d, 2)	7.76 (d, 2)	132.5, 139.7	7.40 (d, 2), 7.48 (d, 2)
10,10′	112.1 (dm, 8, 2)	108.8			108.8, 108.9	
11,11′	155.4 (dm, 8)	152.3[f]			152.6,[f] 152.9[f]	
12,12′	118.7 (d, 159)	116.2	6.85 (d, 8)	6.84 (d, 8)	115.9	6.83 (d, 8), 6.87 (d, 8)
13,13′	131.9 (dm, 159)	129.1	7.15 (dd, 8, 2)	7.26 (dd, 8, 2)	128.9, 129.1	7.10 (dd, 8, 2), 7.17 (dd, 8, 2)
OH				12 (br s)		
OH				14 (br s)		

[a] In CD_3OD. [b] In Me_2SO-d_6. [c] In $CDCl_3/CD_3OD$. [d] In Py-d_5. [e,f] Assignments with identical letters within a column may be interchanged.

dryness to give 10.9 g of crude extract (ED_{50} = 0.7 μg/mL against PS).[10] The sponge residue was soaked two more times in methanol–chloroform (1:1) to give, after evaporation of the solvents, additional extracts, 9.4 g (ED_{50} against PS = 2.1 μg/mL) and 4.4 g.

The methanol extracts (10.9 g) were chromatographed over silica gel (44 g) using a step gradient beginning with hexane–chloroform mixtures and progressing to chloroform and chloroform–methanol combinations. Fractions containing **6**, which eluted just before those containing a mixture of **1** and **5**, were resolved further by elution through a C_{18} Sep-Pak using methanol followed by HPLC on a C_{18} column using methanol–water (60:40) to give 1 mg of pure **6**. The column chromatographic fraction containing a mixture of **1** and **5** was processed in an identical manner to give (in order of elution) pure **1** (0.39 g) and impure **5**. Metabolite **5** was purified by repetition of the chromatography on the C_{18} reversed-phase column using methanol–water (1:1).

The two crude methanol–chloroform extracts were processed in like manner to give additional quantities of **1** and **5**; totals: **1**, 1.18 g; **5**, 19 mg; **6**, 1 mg. Pure **1** was not cytotoxic and **5** isomerized before any testing could be done.

(*E*,*E*)-*N*,*N*′-Bis[3-(3′-bromo-4′-hydroxyphenyl)-2-oximidopropionyl]cystamine (1): 1.18 g, white powder; attempted crystallization from hexane/ethanol gave an amorphous powder; mp 172–174 °C; UV (100% ethanol) λ_{max} 277 nm (ε 4875); IR (KBr) 3348, 1657, 1637, 1536, 1025, 679 cm^{-1}; ^1H and ^{13}C NMR data, see Table I; FAB MS, *m*/*z* (relative intensity) 689 (5), 687 (15), 685 (7), M^+ + Na), 667 (62), 665 (100), 663 (50), M^+ + H); FAB HRMS, obsd (M^+ + H) 664.9560, calcd for $C_{22}H_{24}{}^{79}Br^{81}BrN_4O_6S_2$ 664.9560.

Anal. Calcd for $C_{22}H_{24}Br_2N_4O_6S_2$: 39.76; H, 3.61; Br, 24.0; N, 8.43; S, 9.64. Found: C, 39.84; H, 3.69; Br, 21.23; N, 8.03; S, 8.78.

Acetylation of 1. Acetic anhydride (20 μL, 0.2 mmol) was added to a stirred solution of **1** (5 mg, 0.007 mmol) in pyridine (150 μL) at room temperature. TLC analysis during the early stages of the reaction indicated formation of four reaction products, but after the mixture was stirred overnight only a single product was detected. The reaction mixture was then quenched with water and 5% sodium bicarbonate and extracted with diethyl ether. The dried (magnesium sulfate) organic layer was evaporated to give 4.5 mg of **2**: IR (KBr) 3373, 2926, 1773, 1678, 1624 cm^{-1}; ^1H NMR spectrum ($CDCl_3$) 2.25, 2.35 (each 3 H, s, AcO), 2.84 (2 H, t, *J* = 7 Hz), 3.63 (2 H, q, *J* = 5 Hz), 4.0 (2 H, s, benzylic CH_2), 7.04 (1 H, d, *J* = 9 Hz), 7.05 (1 H, dd, *J* = 9, 2 Hz), 7.51 (1 H, t, *J* = 6 Hz), 7.55 ppm (1 H, d, *J* = 2 Hz).

Reduction of 1 and Acetylation of the Product. Triphenylphosphine (6.3 mg, 0.024 mmol) was added to a solution of disulfide **1** (13.2 mg, 0.020 mmol) in a mixture of dioxane (400 μL) and water (200 μL),[6] and the mixture was stirred for 42 h at room temperature, at which time TLC analysis indicated absence of starting material. Most of the solvent was removed with a stream of dry N_2 at 35–40 °C, and the residue was extracted with diethyl ether and dried over anhydrous magnesium sulfate. Shortly after workup the product was acetylated (100 μL of Ac_2O/350 μL Py) as described above for **1**. The product **4** was purified by using SiO_2 HPLC (30% acetone/hexane): ^1H NMR (Py-d_5) 2.18, 2.27, 2.31 (each 3 H, s, AcO), 3.26 (2 H, t, H-2), 3.71 (2 H, q, H-3), 4.14 (2 H, s, H-7) 7.30 (1 H, d, H-12), 7.51 (1 H, br d, H-13), 7.91 (1 H, d, H-9), 9.53 ppm (1 H, t, H-4); low-resolution mass spectrum (12 eV), *m*/*z* (relative intensity) 460 [M + 2^+ (2.0)], 458 [M^+ (2.0)], 386 (7.2), 384 (7.5), 273 (16.0), 271 (16.0).

Methylation of 1. Following the procedure of Kazlauskas et al.,[3] 15.6 mg (0.025 mmol) of **1** was methylated over 18 h at room temperature with methyl iodide (0.15 ml, 4 mmol) in dry dimethylformamide (5 mL) containing anhydrous potassium carbonate (0.244 g, 1.77 mmol) to give **3**: 16.9 mg, 96% yield; ^1H NMR ($CDCl_3$) 2.81 (2 H, t, H-2), 3.62 (2 H, q, H-3), 3.80 (2 H, H-7), 3.83, 3.99 (each 3 H, s, OCH_3), 6.78 (1 H, d, H-12), 7.08 (1 H, t, H-4), 7.18 (1 H, dd, H-13), 7.45 ppm (1 H, d, H-9); DCI (NH_3) mass spectrum, *m*/*z* (relative intensity) 739 (27), 737 (42), 735 [M^+ + NH_3 (17)], 722 (10), 720 (20), 718 [M^+ (7)].

(*E*,*Z*)-*N*,*N*′-Bis[3-(3′-bromo-4′-hydroxyphenyl)-2-oximidopropionyl]cystamine (5). A total of 19.0 mg of **5** was obtained as a white powder. The following spectroscopic data were obtained shortly after isolation. (After standing in NMR solvents for 1–2 weeks this metabolite isomerized to **1**.) IR (KBr) 3300 (br d), 1670, 1540; ^1H NMR data, Table I; ^{13}C NMR (Me_2SO-d_6), see Table I.

(3-Bromo-4-hydroxyphenyl)acetonitrile (6): total, 1.0 mg; IR (KBr) 3255 (OH), 2271 (C≡N), 1620, 1580 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) 3.38 (2 H, s), 3.65 (3 H, s), 6.88 (1 H, d, *J* = 7 Hz), 7.08 (1 H, dd, *J* = 7.5, 1 Hz), 7.40 (1 H, *J* = 1 Hz) ppm; low-resolution mass spectrum (12 eV), *m*/*z* (relative intensity) 213 (73), 211 (71), 132 (100).

Acknowledgment. This research was supported by Grant NA83AA-D-00011 and NA86AA-D-SG074 from the Office of Sea Grant, NOAA, U.S. Department of Commerce. The high-resolution mass spectrum was obtained at the Midwest Center for Mass Spectrometry at the University of Nebraska supported under the National Science Foundation Regional Instrumentation Facilities Program. We gratefully acknowledge the use of the University of Guam Marine Laboratory facilities, Mangilao, Guam.

(10) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3* (2), 1. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in micrograms/milliliter of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an ED_{50} ≤ 20 μg/mL. PS (P388) refers to in vitro lymphocytic leukemia.