Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 581–583

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Organic Phenyl Arsonic Acid Compounds with Potent Antileukemic Activity

Xing-Ping Liu, Rama Krishna Narla and Fatih M. Uckun*

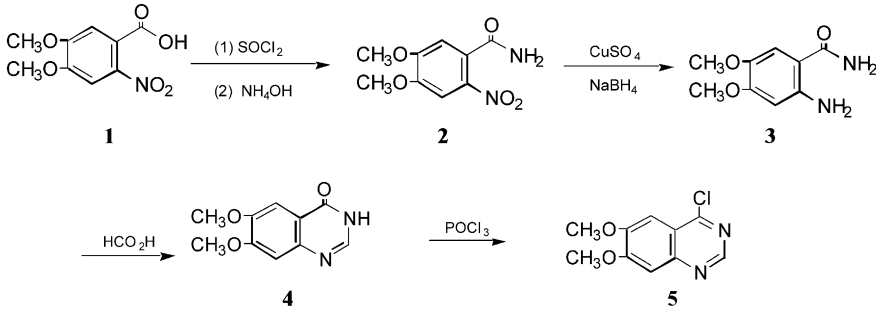
Parker Hughes Cancer Center, Parker Hughes Institute, 2699 Patton Road, St. Paul, MN 55113, USA

Received 14 December 2001; accepted 23 July 2002

Abstract—A series of 12 organic arsonic acid compounds has been synthesized and evaluated against human B-lineage (NALM-6) and T-lineage (MOLT-3) acute lymphoblastic leukemia (ALL) cell lines. The lead compounds 2-trichloromethyl-4-[4′-(4″-phenylazo)phenylarsonic acid]aminoquinazoline (compound **19**, **PHI-P518**; $IC_{50} = 1.1 \pm 0.5$ μM against NALM-6 and 2.0 ± 0.8 μM against MOLT-3) and 2-methylthio-4-(2′-phenylarsonic acid)aminopyrimidine (compound **15**, **PHI-P381**; $IC_{50} = 1.5 \pm 0.3$ μM against NALM-6 and 2.3 ± 0.5 μM against MOLT-3) exhibited potent antileukemic activity at low micromolar concentrations.
© 2002 Elsevier Science Ltd. All rights reserved.

Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. The outcome of ALL patients who experience a relapse after frontline contemporary chemotherapy is dismal.[1–3] Therefore, there is an urgent need for novel antileukemic agents. The inorganic arsenic compound arsenic trioxide (As_2O_3) is a chemotherapeutic drug that has been used in the treatment of hematologic malignancies.[4–9] Daily intravenous infusion of 10 mg As_2O_3 induced complete remission in acute promyelocytic leukemia patients who are refractory to conventional chemotherapy.[5,6] In an effort aimed at identifying arsenic compounds with more favorable toxicity profile and more broad-spectrum anticancer activity, we decided to synthesize a new class of organic arsenic compounds by derivatizing the less toxic pentavalent organic arsenical, phenylarsonic acid (PAA).[10] In the present study, we synthesized a series of 12 phenlarsonic acids and they were tested for their antileukemic activity using two leukemia cell lines (NALM-6 And MOLT-3).

4-Chloro-6, 7-dimethoxyquinazoline, as one of the key starting materials (**5**) was prepared as outlined in Scheme 1. In this procedure, 4,5-dimethoxy-2-nitrobenzoic acid (**1**) was treated with thionyl chloride $SOCl_2$ first, then the reaction mixture was directly treated with ammonia NH_4OH to give the 4, 5-dimethoxy-2-nitrobenzamide (**2**).[11] Compound **2** was reduced with sodium borohydride $NaBH_4$ and catalyzed by copper sulfate $CuSO_4$,[12,13] to give 2-amino-4,5-dimethoxy-benzamide (**3**), which was directly refluxed with formic acid HCO_2H to give 6,7-dimethoxy-4-(3*H*)-quinazolinone (**4**).



1 **2** **3** **4** **5**

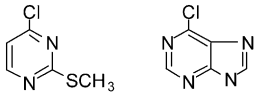
Scheme 1.

*Corresponding author. Tel.: +1-651-796-5450; fax: +1-651-796-5493; e-mail: fatih_uckun@ih.org

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00928-9

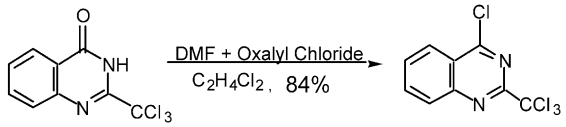
Compound **4** was refluxed with phosphorus oxychloride $POCl_3$ to provide the key starting material 4-chloro-6, 7-dimethoxyquinazoline (**5**) with good yield.[11]

Another two starting materials 4-chloro-2-methylthiopyrimidine (**6**) and 6-chloropurine (**7**) were commercially available.



6 **7**

4-Chloro-2-trichloromethylquinazoline as the fourth key starting material (**9**) was prepared as outlined in Scheme 2.

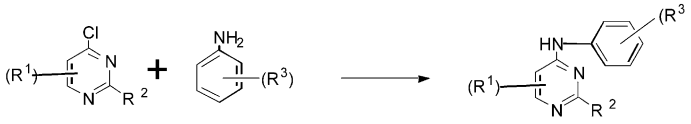


8 **9**

Scheme 2.

The arsonic acid compounds for this study were prepared by the condensation of 4-chloro-6,7-dimethoxyquinazoline (**5**), 4-chloro-2-methylthiopyrimidine (**6**), 6-chloropurine (**7**) or 4-chloro-2-trichloromethylquinazoline (**9**) and phenylarsonic acid (Table 1). In brief, a mixture of 2 mmol of compounds **5**, **6**, **7**, or **9,** and 2.2 mmol of 4-(4-aminophenylazo)phenylarsonic acid (to give **10**, **13**, **16**, and **19**), 4-aminophenylarsonic acid (to give **11**,

Table 1. Structure and antileukemic activities of phenylarsonic acid derivatives against human leukemia NALM-6 (B-lineage ALL) and MOLT-3 (T-lineage ALL) cell lines



Compd	PHI-P	R^1	R^2	R^3	IC_{50} (μM)	
					NALM-6	MOLT-3
10	273	5,6-H_3CO	H	4′-N=N-As(O)(OH)	16.9 ±3.4	13.9±2.6
11	379	5,6-H_3CO	H	4′-As(O)$(OH)_2$	>100	>100
12	378	5,6-H_3CO	H	2′-As(O)$(OH)_2$	46.6±12.4	55.6±12.4
13	370	H	SCH_3	4′-N=N-As(O)(OH)	6.1±1.6	4.1±1.7
14	380	H	SCH_3	4′-As(O)$(OH)_2$	5.6±2.1	9.1±3.4
15	381	H	SCH_3	2′-As(O)$(OH)_2$	1.5±0.3	2.3±0.5
16	371	5,6-	H	4′-N=N-As(O)(OH)	22.5±6.7	21.6±4.3
17	385	5,6-	H	4′-As(O)$(OH)_2$	>100	>100
18	386	5,6-	H	2′-As(O)$(OH)_2$	>100	>100
19	518	5,6-	CCl_3	4′-N=N-As(O)(OH)	1.1±0.5	2.0±0.8
20	517	5,6-	CCl_3	4′-As(O)$(OH)_2$	>100	>100
21	516	5,6-	CCl_3	2′-As(O)$(OH)_2$	>100	>100
4-(4′-Aminophenylazo)phenylarsonic acid					>100	
4-Aminophenylarsonic acid					>100	
2-Aminophenylarsonic acid					>100	

Cells were treated with compounds in Table 1 in 96-well plates and incubated for 48 h at 37 °C. The cytotoxic activity was determined using MTT assays[17] and the IC_{50} values were calculated using Graphpad Prism software. The starting materials 4-(4′-aminophenylazo)phenylarsonic acid, 4-aminophenylarsonic acid and 2-aminophenylarsonic acid were not active (IC_{50} values > 100 μM).

14, **17**, and **20**) or 2-aminophenylarsonic acid (to give **12**, **15**, **18**, and **21**) in 2-propanol (40–80 mL) was heated to reflux for 8–24 h in the presence of concentrated HCl (2–4 drops) as a catalys,[14,15] to give the expected compounds. All of compounds gave satisfactory analytical and spectroscopic data. Selected data were listed.[16]

The cytotoxic activities of the compounds were evaluated against two ALL cell lines, NALM-6 (B-lineage ALL) and MOLT-3 (T-lineage ALL) using MTT assay.[17] The IC_{50} values were determined using Graphpad Prism software, version 2.0 (San Diego, CA, USA). The comparison of the IC_{50} values (Table 1) revealed significant information regarding the structure–activity relationships affecting the antileukemic activity of this series of compounds. The antileukemic activity of the phenylarsonic acid-containing compounds was strongly dependent on the type of ligand (quinazoline, pyrimidine, and purine) and nature of the arsonic acid substituent present. Three compounds containing 2-methylthiopyrimidine (**13**, **14**, and **15**) showed superior cytotoxic activity than the compounds containing 6,7-dimethoxyquinazoline (**10**, **11**, and **12**), purine (**16**, **17**, and **18**) or 2-trichloromethylquinazoline (**19**, **20**, and **21**). The compounds containing 4-(4-phenylazo)phenylarsonic acid (**10**, **13**, **16**, and **19**) also showed excellent cytotoxic activities whose IC_{50} values ranged from 1.1 to 22.5 μM against NALM-6 cells and from 2.0 to 21.6 μM against MOLT-3 cells. The presence of arsonic acid at *ortho*-position (**12**, **15**, **18**, and **21**) rather than *para*-position (**11**, **14**, **17**, and **20**) was associated with significantly better cytotoxic activity. Although **15** containing 2-methylthiopyrimidine and *ortho*-position asonic acid showed excellent cytotoxic activity, other compounds with *ortho*-position asonic acid were surprisingly inactive. Combination of 2-trichloromethylquinazoline and 4-phenylazophenylarsonic acid (**19**) yielded the best compound with an IC_{50} value of 1.1 ± 0.5 μM against NALM-6 cells and 2.0 ± 0.8 μM against MOLT-3 cells (Table 1).

The p53 deficient NALM-6 cell line was previously shown to be resistant to multiple chemotherapeutic agents, including alkylating agents, steroids, topoisomerase I inhibitors, topoisomerase II inhibitors, vincristine and taxol.[18,19] Therefore, the exquisite sensitivity of NALM-6 cells to arsonic acid derivatives is quite encouraging. Thus, further development of these lead compounds (**15** and **19**) may provide the foundation for more effective treatment programs for therapy-refractory or recurrent ALL patients.

References and Notes

1. Uckun, F. M.; Sather, H. N.; Gaynon, P. S.; Arthur, D. C.; Trigg, M. E.; Tubergen, D. G.; Nachman, J.; Steinherz, P. G.; Sensel, M. G.; Reaman, G. H. *Blood* **1997**, *90*, 28.
2. Uckun, F. M.; Herman-Hatten, K.; Crotty, M. L.; Sensel, M. G.; Sather, H. N.; Tuel-Ahlgren, L.; Sarquis, M. B.; Bostrom, B.; Nachman, J. B.; Steinherz, P. G.; Gaynon, P. S.; Heerema, N. *Blood* **1998**, *92*, 810.
3. Uckun, F. M.; Kersey, J. H.; Haake, R.; Weisdorf, D.; Nesbit, M. E.; Ramsay, N. K. *N. Engl. J. Med.* **1993**, *329*, 1296.
4. Chen, G. Q.; Zhu, J.; Shi, X. G.; Ni, J. H.; Zhong, H. J.; Si, G. Y.; Jin, X. L.; Tang, W.; Li, X. S.; Xong, S. M.; Shen, Z. X.; Sun, G. L.; Ma, J.; Zhang, P.; Zhang, T. D.; Gazin, C.; Naoe, T.; Chen, S. J.; Wang, Z. Y.; Chen, Z. *Blood* **1996**, *88*, 1052.
5. Shen, Z. X.; Chen, G. Q.; Ni, J. H.; Li, X. S.; Xiong, S. M.; Qiu, Q. Y.; Zhu, J.; Tang, W.; Sun, G. L.; Yang, K. Q.; Chen, Y.; Zhou, L.; Fang, Z. W.; Wang, Y. T.; Ma, J.; Zhang, P.; Zhang, T. D.; Chen, S. J.; Chen, Z.; Wang, Z. Y. *Blood* **1997**, *89*, 3354.
6. Soignet, S. L.; Maslak, P.; Wang, Z. G.; Jhanwar, S.; Calleja, E.; Dardashti, L. J.; Corso, D.; DeBlasio, A.; Gabrilove, J.; Scheinberg, D. A.; Pandolfi, P. P.; Warrell, R. P. *N. Engl. J. Med.* **1998**, *339*, 1341.
7. Jing, Y.; Dai, J.; Chalmers-Redman, R. M.; Tatton, W. G.; Waxman, S. *Blood* **1999**, *94*, 2102.
8. Zhu, X. H.; Shen, Y. L.; Jing, Y. K.; Cai, X.; Jia, P. M.; Huang, Y.; Tang, W.; Shi, G. Y.; Sun, Y. P.; Dai, J.; Wang, Z. Y.; Chen, S. J.; Zhang, T. D.; Waxman, S.; Chen, Z.; Chen, G. Q. *J. Natl. Cancer Inst.* **1999**, *91*, 772.
9. Dai, J.; Weinberg, R. S.; Waxman, S.; Jing, Y. *Blood* **1999**, *93*, 268.
10. The National Academy of Sciences–National Research Council: Washington, DC, 1980; p 40.
11. Nomoto, F.; Obase, H.; Takai, H.; Hirata, T.; Teranishi, M.; Nakamura, J.; Kubo, K. *Chem. Pharm. Bull.* **1990**, *38*, 1691.
12. Thomas, C. L. *Catalytic Processes and Proven Catalysts*; Academic: New York, 1970.
13. Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. *Tetrahedron Lett.* **1969**, *52*, 4555.
14. Yuki, H.; Kishikawa, T.; Tohira, Y.; Watanabe, K. *Chem. Pharm. Bull.* **1967**, *15*, 1052.
15. Andres, R. J.; Hamilton, C. S. *J. Am. Chem. Soc.* **1945**, *67*, 946.
16. Analytical data for lead compounds:
2-Methylthio-4-(2′-phenylarsonic acid)aminopyrimidine (15, PHI-P381). Yield 86%, mp 225–228 °C. ^{1}H NMR (DMSO-d_6) δ 10.94 (s, 1H, –NH), 8.46 (d, 1H, $J = 8.1$ Hz, 5-H), 8.21–7.23 (m, 4H, 3′, 4′, 5′, 6′-H), 6.47 (d, 1H, $J = 8.1$ Hz, 6-H), 2.49 (s, 3H,-SCH_3), ^{13}C NMR (DMSO-d_6) δ 170.7 (C-2), 159.0 (C-4), 156.1 (C-6), 141.7 (C-5), 133.9 (C-1′), 131.5 (C-6′), 123.3, 121.9, 121.0 (C-3′, 4′, 5′), 103.6 (C-2′), 13.8 (–SCH_3). UV (MeOH) λ_{max} 243 nm (ε = 1030). IR (KBr) ν_{max} 3550-3420, 2638, 1664, 1583 cm^{-1}. Found: C, 35.25, H, 3.62, N, 10.93. $C_{11}H_{12}AsN_3O_3S{\cdot}HCl$ requires: C, 35.01, H, 3.45, N, 11.14%.
2-Trichloromethyl-4-[4′-(4″-phenylazo)phenylarsonic acid]-aminoquinazoline (19, PHI-P518). Yield 77%, mp > 300 °C. ^{1}H NMR (DMSO-d_6) δ 10.94 (s, 1H,-NH), 8.45 (d, 2H, $J_{2'',3''} = 9.0$ Hz, 2″, 6″-H), 8.08–7.77 (m, 4H, 5, 6, 7, 8-H), 7.95 (s, 4H, 2′, 3′, 5′, 6′-H), 6.88 (d, 2H, $J_{3'',2''} = 9.0$ Hz, 3″, 5″-H). ^{13}C NMR (DMSO-d_6) δ 159.9 (C-2), 158.4 (C-4), 154.7 (C-1′), 154.5 (C-4″), 149.1 (C-4′), 147.7 (C-1″), 132.8 (C-9), 131.7 (C-10), 131.6 (C-2″, 6″), 128.6 (C-3′), 128.5 (C-5′), 126.2 (C-6,7), 123.8 (C-5), 123.1 (C-3″, 5″), 122.4 (C-2′, 6′), 122.2 (C-8), 114.4, 97.9 (C–CCl_3). UV (MeOH) λ_{max} 205 nm (ε = 5407). IR (KBr) ν_{max} 3441.6, 3013.3, 2292.2, 1630.7, 1598.2, 1538.6, 1408.4, 1262.0, 1169.9, 1099.4, 817.5 cm^{-1}. MS (EI) *m*/*z* 565 (M^+, 5), 564 ($M^+ - 1$, 7), 459 (54), 395 (12), 341 (53), 306 (100). Found: C, 35.59, H, 2.66, N, 9.88. $C_{21}H_{15}AsCl_3N_5O_3{\cdot}4HCl$ requires: C, 35.54, H, 2.68, N, 9.87%.
17. Narla, R. K.; Liu, X. P.; Myers, D. E.; Uckun, F. M. *Clin. Cancer Res.* **1998**, *4*, 1405.
18. Uckun, F. M.; Evans, W. E.; Forsyth, C. J.; Waddick, K. G.; Ahlgren, L. T.; Chelstrom, L. M.; Burkhardt, A.; Bolen, J.; Myers, D. E. *Science* **1995**, *267*, 886.
19. Uckun, F. M.; Chandan-Langlie, M.; Dockham, P. A.; Aeppli, D.; Sladek, N. E. *Leuk. Lymphoma* **1994**, *13*, 417.