Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 1749–1761
Bioorganic & Medicinal Chemistry

Synthesis, structure–activity relationship, and p210$^{bcr-abl}$ protein tyrosine kinase activity of novel AG 957 analogs☆

Gurmeet Kaur,[a] Ven L. Narayanan,[a] Prabhakar A. Risbood,[a,b] Melinda G. Hollingshead,[a] Sherman F. Stinson,[a] Ravi K. Varma[a,†] and Edward A. Sausville[a,*]

[a]*Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892, USA*
[b]*Starks associates Inc., 1280 Niagara St. Buffalo, NY 14213, USA*

Received 28 July 2004; revised 1 December 2004; accepted 1 December 2004
Available online 7 January 2005

Abstract—A series of novel, sterically hindered lipophilic analogs of AG 957 was designed and synthesized as potential protein tyrosine kinase (PTK) inhibitors. The in vitro activity, in vivo anti-leukemia activity, and pharmacology of these PTK inhibitors were studied. Some aspects of the structure–activity relationship associated with the carboxylic acid, phenol ring, and linker modifications are discussed. We have demonstrated that the 1,4-hydroquinone moiety is essential for activity and that sterically hindered esters contribute to enhanced in vivo efficacy. Adaphostin (NSC 680410) has emerged as the improved compound with the maximum in vivo anti-leukemia hollow fiber activity, concordant with the original lead compound AG 957. Currently, adaphostin is undergoing preclinical toxicology studies.

1. Introduction

The bcr-abl fusion gene that results from the t(9:22) chromosomal translocation (p210$^{bcr-abl}$) results in expression of a pathogenic protein tyrosine kinase (PTK) associated with the occurrence of chronic myelogenous leukemia (CML) and is therefore an attractive therapeutic target.[1,2] The p210$^{bcr-abl}$ chimeric protein (localized to the cytoplasm) possesses an activated Abl tyrosine kinase activity and has transforming properties as compared with the normal c-abl protein.[3] A myeloproliferative syndrome resulting from over expression of the fusion protein has been demonstrated in bone marrow cells of mice.[4,5] This is further evidence that p210$^{bcr-abl}$ over expression is sufficient to drive the disease phenotype, and reinforces the idea that a selective inhibitor of p210$^{bcr-abl}$ may suppress or reverse the CML disease phenotype.

Recently, Gleevec® has emerged as a small molecule antagonist of p210$^{bcr-abl}$ with evidence of clinical activity. [6] However, Gleevec® is marginally effective in certain blast transformation events in CML,[7] and resistance to Gleevec® through altered p210$^{bcr-abl}$ variants has been described.[8] Despite the success of Gleevec®, the duration of its useful effect remains uncertain, and the development of additional molecules with activity against cells expressing p210$^{bcr-abl}$ would be desirable as discussed in detail elsewhere.[9,10] In our initial efforts to derive a tyrosine kinase inhibitor for treatment of chronic myelogenous leukemia, we identified the tyrphostin AG 957 (**4**) as a lead molecule that inhibits the p210$^{bcr-abl}$ autokinase activity in the K562 CML-derived tumor cell line.[11] Initially AG 957 was characterized as an inhibitor of the epidermal growth factor receptor (EGF-R) with an IC_{50} of 0.25 μM.[12] Our findings confirmed that anti-PTK activity of AG 957 was a prominent feature of the drug's action,[13] and contributed to the hypothesis that compounds of this structural class might have therapeutic potential. However, AG

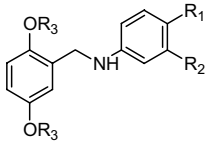
Keywords: PTK; NSC680410; Adaphostin; AG 957; p210$^{bcr-abl}$.
☆ US Patent Application for the this work is pending.[14]
* Corresponding author. Tel.: +410 328 7394; fax: +410 328 6896. Present address: Associate Director for Clinical Research, University of Maryland, Greenebaum Cancer Center, 22, S. Greene St., Baltimore, MD 21201-1595, USA; e-mail: esausville@umm.edu
† Present address: Revathy, Ettannil Temple Road, Puthiakavu, Tripunithura, Kerala 682301, India.

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.12.003

957 has serious solubility limitations, undesirable pharmacokinetic parameters, and was not active in preliminary in vivo models. We hypothesized that the low in vivo potency of AG 957 may be due to the facile hydrolysis of the methyl ester by endogenous esterase and the rapid elimination of the resulting acid. To mitigate this effect, we initiated a program for the synthesis of novel, sterically-hindered lipophilic analogs of AG 957.

These compounds fall into three structural categories; carboxylic acid modified (Table 1), phenol ring modified (Table 2), and linker modified analogs of AG 957 (Table 3). They include esters with varying degrees of steric hindrance, phenolic compounds substituted with electron donating and withdrawing groups, quinones, and compounds where the carbon–nitrogen link is replaced by carbon–carbon linkage. We also synthesized a few com-

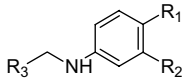
Table 1. Carboxylic acid modifications (compounds **1**–**18**)



No	NSC no	R_1	R_2	R_3	Assays	
					IC_{50} (μM) (a) 6d MTT	IC_{50} (μM) (b)
1	678027	Lavendustin A[18]			44.70 ± 0.93	41.07 ± 8.93
2	676538	COOH	H	H	41.93 ± 8.38	18.51 ± 1.72
3	654703*	$CONH_2$	H	H	20.90 ± 2.34	4.23 ± 0.73
4 (c)	654705	$COOCH_3$	H	H	16.63 ± 0.48	2.90 ± 0.60
5	677695*	$COOCH(CH_3)_2$	H	H	15.13 ± 1.04	4.45 ± 0.32
6	676622	COOH	H	CH_3	100.00 ± 0.3	>50 ± 0.0
7	676448	$COOCH_3$	H	CH_3	41.26 ± 3.23	>50 ± 0.0
8	678634	$COOCH_2Ph$	H	H	12.04 ± 0.83	36.56 ± 1.36
9	680779*	$COOCH[CH(CH_3)_2]_2$	H	H	9.98 ± 1.32	11.11 ± 1.11
10	677696*	$COOC(CH_3)_3$	H	H	16.38 ± 0.87	7.72 ± 1.15
11	680410*	COO-1-adamantyl	H	H	9.75 ± 0.81	13.6 ± 1.81
12	689857*	$COOCH_2$-1-adamantyl	H	H	12.84 ± 0.2	12.62 ± 1.7
13	687945*	$COOCH_3$	OH	H	17.71 ± 0.11	6.44 ± 1.78
14	686561	$COOCH(CH_3)_2$	Cl	H	21.60 ± 1.0	1.22 ± 0.15
15	689858*	$COCH_3$	H	H	13.00 ± 0.0	1.03 ± 0.08
16	689431*	$CON[CH(CH_3)_2]_2$	H	H	18.20 ± 0.5	2.36 ± 1.26
17	689001*	$CH(OH)CF_3$	H	H	14.32 ± 0.4	0.57 ± 0.29
18	688792*	$O{=}P(OCH_3)_2$	H	H	9.83 ± 0.3	0.49 ± 0.08

Results are expressed as mean IC_{50} ± SE of three or more experiments in 6 day growth inhibition assays of K562 cells (a) and in $p210^{bcr\text{-}abl}$ in vitro immune complex kinase assay (b). Compound **4** is the parent compound AG 957 (NSC 654705) (c).

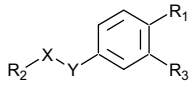
Table 2. Phenol ring modifications (compounds **19**–**33**)



No	NSC no	R_1	R_2	R_3	Assays	
					IC_{50} (μM) (a) 6d MTT	IC_{50} (μM) (b)
19	685985*	$COOCH_3$	Cl	2-Bromo-3,6-dihydroxy-phenyl	8.90 ± 0.50	3.56 ± 0.53
20	681541	$COOCH_3$	H	2-(1,4-Dihydroxy)-naphthyl	6.83 ± 1.68	>50 ± 0.00
21	681288	$COOCH_3$	H	1,4-Dihydro-1,4-dioxo-2-naphthelenyl	9.11 ± 1.00	>50 ± 0.00
22	676537	$COOCH_3$	H	3,6-Dioxo-1,4-cyclohexadienyl	15.11 ± 1.28	15.89 ± 2.91
23	688791*	$COOCH_3$	OH	3,6-Dioxo-1,4-cyclohexadienyl	17.00 ± 0.3	5.15 ± 1.01
24	679817	$CONH_2$	H	3,6-Dioxo-1,4-cyclohexadienyl	21.74 ± 2.27	5.10 ± 0.30
25	678636	$COOCH_2$–C_6H_6	H	3,6-Dioxo-1,4-cyclohexadienyl	22.75 ± .18	8.75 ± 1.25
26	678635	$COOCH(CH_3)_2$	H	3,6-Dioxo-1,4-cyclohexadienyl	17.41 ± 1.00	13.67 ± 5.78
27	678637	$COOC(CH_3)_3$	H	3,6-Dioxo-1,4-cyclohexadienyl	25.36 ± 1.88	25.88 ± 9.02
28	681719	$COOCH[CH(CH_3)_2]_2$	H	3,6-Dioxo-1,4-cyclohexadienyl	38.80 ± 12.80	34.03 ± 9.02
29	681151	COO-1-adamantyl	H	3,6-Dioxo-1,4-cyclohexadienyl	32 ± 0.50	>50 ± 0.00
30	683766	COO-1-adamantyl	H	2-Bromo-3,6-dioxo-1,4-cyclohexadienyl	24.80 ± 3.10	2.38 ± 0.45
31	685405	$COOC(CH_3)_3$	H	2-Bromo-3,6-dioxo-1,4-cyclohexadienyl	64.83 ± 1.22	2.68 ± 1.33
32	686334*	$COOCH_3$	Cl	2-Bromo-3,6-dioxo-1,4-cyclohexadienyl	17.50 ± 0.10	2.35 ± 0.41
33	687225	$COOCH(CH_3)_2$	Cl	3,6-Dioxo-1,4-cyclohexadienyl	27.70 ± 0.60	3.01 ± 1.75

Results are expressed as mean IC_{50} ± SE of three or more experiments in 6 day growth inhibition assays of K562 cells (a) and in $p210^{bcr\text{-}abl}$ in vitro immune complex kinase assay (b).

Table 3. Linker modifications (compounds **34–43**)



No	NSC no	R_1	R_2	X–Y	R_3	Assays	
						IC_{50} (μM) (a) 6d MTT	IC_{50} (μM) (b)
34	681152*	$COOCH_3$	2,5-Dihydroxyphenyl	CH_2–CH_2	H	18.47 ± 5.62	2.64 ± 0.99
35	684424*	$COOCH_3$	4-Bromo-2,5-dihydroxyphenyl	CH_2–CH_2	H	7.00 ± 0.50	4.42 ± 0.24
36	655255	COOH	2,5-Dihydroxyphenyl	CH_2–CH_2	OH	33.40 ± 4.80	12.95 ± 1.03
37	680649	$COOCH_3$	3,6-Dioxo-1,4-cyclohexadienyl	CH_2–CH_2	H	5.65 ± 0.80	50 ± 0.00
38	684496*	$COOCH(CH_3)_2$	3,6-Dioxo-1,4-cyclohexadienyl	CH_2–CH_2	H	19.90 ± 0.50	5.26 ± 2.09
39	685106	$COOCH(CH_3)_2$	4-Bromo-2,5-dihydroxyphenyl	CH_2–CH_2	H	20 ± 0.70	5.77 ± 0.53
40	685105	$COOCH_3$	4-Bromo-3,6-dioxo-1,4-cyclohexadienyl	CH_2–CH_2	H	36.50 ± 1.00	3.52 ± 0.16
41	685107	$COOCH(CH_3)_2$	4-Bromo-3,6-dioxo-1,4-cyclohexadienyl	CH_2–CH_2	H	59.80 ± 0.44	4.19 ± 0.07
42	680561	$COOCH_3$	2,5-Dihydoxyphenyl	CH=CH	H	9.72 ± 0.49	19.86 ± 9.94
43	680780*	$COOCH_3$	3,6-Dioxo-1,4-cyclohexadienyl	CH=CH	H	17.80 ± 0.22	5.47 ± 0.47

Results are expressed as mean IC_{50} ± SE of three or more experiments in 6 day growth inhibition assays of K562 cells (a) and in $p210^{bcr\text{-}abl}$ in vitro immune complex kinase assay (b).

pounds lacking the ester linkage. In this paper we describe the synthesis, in vitro activity, in vivo anti-leukemia activity in the hollow fiber assay, and pharmacology of these AG 957-related compounds. More recent detailed analyses of the in vitro cellular pharmacology of adaphostin (NSC-680410, **11**),[14] the most promising compound in the series, have been presented in prior reports.[15–17] Furthermore, adaphostin formulated in HPCD (β-hydroxypropylcyclodextran) is undergoing dose–range finding toxicology studies in mice and dogs using a three-day continuous infusion schedule.

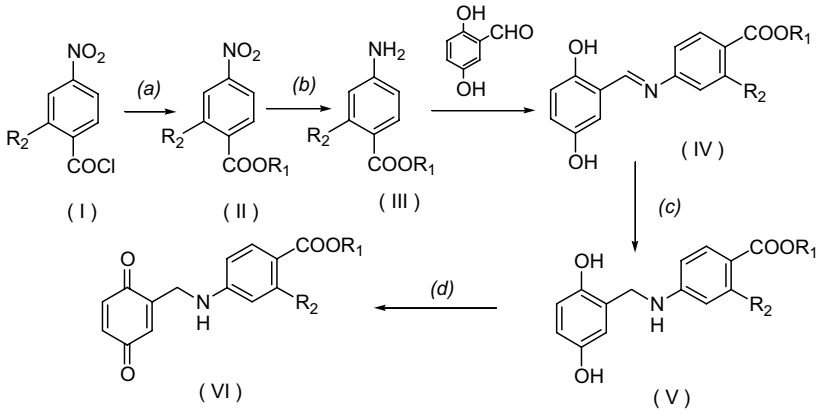
2. Synthesis

For each class of compounds, a representative example with experimental details is described. Lavendustin A[18] (**1**) was obtained from Sigma. Compounds (**2–33**) were prepared according to the reaction sequence shown in Scheme 1 using the appropriate starting materials. Compounds (**36–43**) were synthesized analogous to the procedures described in the literature.[19] The carboxylic acids groups on these compounds were esterified using standard procedures and further modified by substituting the appropriate alcohol. Bromine was introduced in compounds (**35**) and (**40**). A detailed procedure for the preparation of adaphostin (**11**) and its corresponding quinone (**29**) is described in the Experimental section.

3. Results

3.1. Growth inhibition and $p210^{bcr\text{-}abl}$ kinase inhibition studies

To identify analogs of AG 957 with greater potential for in vivo activity, we compared the K562 cell growth inhibition activity and the $p210^{bcr\text{-}abl}$ autokinase inhibition activity of 43 analog compounds. The mean IC_{50} for growth inhibition of K562 cells is shown for all 43 compounds, based upon their structural class, in Table 1 (carboxylic acid modifications), Table 2 (phenol ring variants), and Table 3 (linker modifications). All the compounds were tested at least three times in the 6-day MTT-based growth inhibition assay and the IC_{50} was calculated for each analog using regression analysis. The capacity of each compound to inhibit $p210^{bcr\text{-}abl}$



Scheme 1. Reagents and conditions: (a) ROH/pyridine; (b) and (c) H_2/Pd–C; (d) O_2/salcomine.

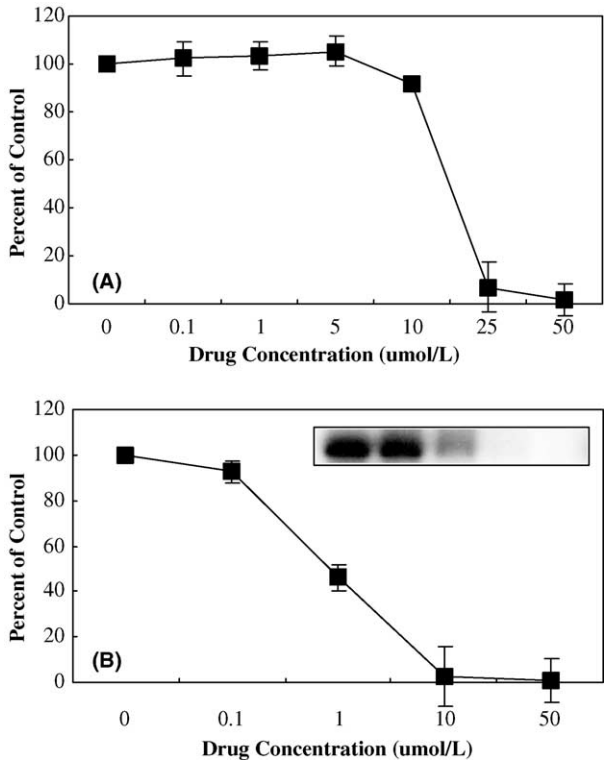


Figure 1. Effect of tyrphostin on K562 cells. (A) Growth inhibition curve showing the effect of NSC 654703 (**3**) on growth of K562 cells. Cells were incubated with various concentrations of drug for 6 days. Growth inhibition was quantitated using the MTT assay as described in Material and methods. (B) Inhibition of in vitro $p210^{bcr\text{-}abl}$ phosphorylation by NSC 654703 (**3**). Graph depicts the quantitation of the autoradiograph (inset) of phosphorylated $p210^{bcr\text{-}abl}$ protein resolved on 4–20% SDS-Page.

autokinase activity after addition to immunoprecipitates from untreated K562 cells is also shown in these Tables. The phosphorylated $p210^{bcr\text{-}abl}$ bands, quantitated from autoradiographs, were plotted as a percent of the untreated control. A typical growth inhibition curve after 6d exposure to compound **3** is shown in (Fig. 1A), and the phosphorylation study is shown in (Fig. 1B). Several compounds were active in both assays and are noted in the respective Tables by being starred.

3.2. Pharmacokinetic studies

A major criterion for selection of analogs for further study would be evidence of more favorable pharmacokinetic features compared to AG 957. The effect of chemical structural modifications of AG 957 on in vivo disposition was therefore investigated. The overall goal was to improve the mean residence time by reducing the rate of elimination, while at the same time maintaining high biological activity. Selected pharmacokinetic parameters derived from nonlinear regression analysis of plasma concentration–time data obtained following iv administration of analogs to mice are presented in Table 4. The lead molecule, AG 957 (NSC 654705, **4**), a methyl ester, exhibited a very rapid total plasma clearance rate, and a biological half-life of only 3 min. The mean residence time was 5 min. This rapid rate of elimination was believed to owe, at least in part, to facile cleavage of the methyl ester moiety by endogenous esterase activity. Attempts were made to sterically hinder esterase cleavage by substituting more structurally complex constituents for the methyl moiety. Particularly successful in this regard was the adamantyl ester adaphostin (NSC 680410, **11**). The plasma clearance rate was reduced by 50% compared to AG 957 (NSC 654705, **4**), resulting in an increase in the mean residence time to 36 min (Fig. 2). Other approaches involved replacing the ester moiety with nonester entities, and changing the nature of the linker between the two aromatic components of the molecule. Notable among these analogs was a trifluoroethanol derivative (NSC 689001, **17**) exhibiting a biological half-life of 111 min, and a mean residence time of 41 min.

3.3. Hollow fiber assay

The hollow fiber assay can aid in identifying compounds with a probability of demonstrating in vivo efficacy.[20,21] Several representative compounds (12 from Table 1, 2 from Table 2, and 8 from Table 3) were tested in the standard hollow fiber assay using a panel of 12 solid tumor lines[22] (data not shown). In this assay most of the compounds from Table 2 and Table 3 were inactive. Based on the in vitro anti-leukemia activity of these compounds, selected compounds were tested in a poten-

Table 4. Selected pharmacokinetic parameters observed following iv administration of analogs to mice

Compound no	NSC no.	R_1	X–Y	CL (mL/min/kg)	$t_{1/2}$ (min)	MRT (min)	Hollow fiber score
4	654705	$COOCH_3$	NH	275	3	5	8 + 2 = 10
5	677695	$COOCH(CH_3)_2$	NH	142	17	9	2 + 6 = 8
10	677696	$COOC(CH_3)_3$	NH	130	101	16	0 + 2 = 2
9	680779	$COOCH[CH(CH_3)]_2$	NH	212	30	30	8 + 4 = 12
11	680410	COO-1-adamantyl	NH	137	41	36	14 + 8 = 22
8	678634	$COOCH_2$-phenyl	NH	85	56	6	0 + 6 = 6
3	654703	$CONH_2$	NH	159	189	11	0 + 6 = 6
18	688792	$PO(OCH_3)_2$	NH	139	31	11	0 + 0 = 0
17	689001	$CHOHCF_3$	NH	227	111	41	4 + 4 = 8
16	689431	$CON[CH(CH_3)]_2$	NH	450	53	22	4 + 0 = 4
15	689858	$COCH_3$	NH	242	4	3	4 + 0 = 4
34	681152	$COOCH_3$	CH_2–CH_2	632	2	2	4 + 2 = 6

CL = total plasma clearance rate; $t_{1/2}$ = terminal disposition phase half-life (biological half-life); MRT = mean residence time. Hollow fiber score: ip score + sc score = total score.

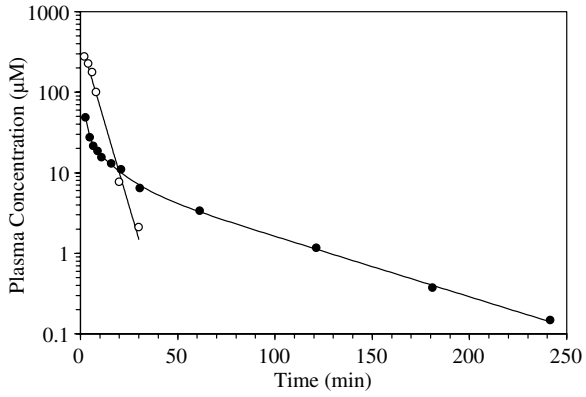


Figure 2. Geometric mean plasma concentrations and lines of best fit observed following iv administration of NSC 654705 (○) compound (**4**) and NSC 680410 (•) compound (**11**) to mice at equivalent molar doses (127 μmol/kg).

tially more pertinent leukemia specific hollow fiber assay. In this modification of the hollow fiber assay, 6 leukemia/lymphoma cell lines [CCRF-CEM, HL-60, SR, K562, RPMI-8226, and MOLT-4] were evaluated for sensitivity to the test agent. Eleven compounds with carboxylic acid modifications (Table 1) and one compound with linker modifications (Table 3) were selected for study. The data obtained form these studies are shown in Table 4. To allow simple presentation, activity is presented as a score. This value is determined by assigning a value of 2 each time a compound reduces the net cell growth by 50% or more when compared to the vehicle treated controls. Since each compound was assessed at 2 dose levels against 6 cell lines, the maximum possible score at each implant site is 24 (2 doses × 6 cell lines × score of 2). To calculate the total score for a compound, the intraperitoneal (ip) and subcutaneous (sc) scores are added. In these studies, the greatest activity against the leukemia cells was seen with adaphostin (NSC 680410, **11**, Table 4) as it scored a total of 22, which was over twice the score achieved by any other compound in this series including AG 957 (NSC 654705, **4**). Based upon this comparison, adaphostin clearly emerges as the most favorable compound to date from this series.

4. Discussion

This paper describes the design, synthesis, and structure–activity relationships of novel AG 957 analogs with the goal of finding potent and selective tyrosine kinase inhibitors. The initial activity of AG 957 was defined by inhibition of $p210^{bcr\text{-}abl}$ mediated gene products. Therefore, the current studies compared the effects of AG 957 analog treatment on K562 cell growth, $p210^{bcr\text{-}abl}$ autokinase activity, and in vivo anti-leukemia activity. To achieve this objective, carboxylic acid esters with varying degrees of steric hindrance and lipophilicity were synthesized to inhibit endogenous esterase activity. For comparative purposes, a few selected compounds lacking the ester function were also synthesized. We found that most of the hindered ester analogs with varied lipophilicity maintained the growth inhibitory property of the parent compound but they were less potent inhibitors of $p210^{bcr\text{-}abl}$ autokinase activity. Compounds lacking the ester function (NSC 689858, **15**; NSC 689431, **16**; NSC 689001, **17**; NSC 688792, **18**) demonstrated significantly increased anti-kinase activity (2–10-fold) while maintaining the growth inhibitory property (Table 1). However, these compounds were inactive in the hollow fiber assay. Adaphostin (NSC 680410, **11**) showed maximum cell growth inhibition both in vitro and in vivo while maintaining the autokinase activity. The plasma clearance rate of adaphostin (NSC 680410, **11**) was half that of AG 957 (NSC 654705, **4**), resulting in an increase in the mean residence time to 36 min (Fig. 2). The maximum hollow fiber activity for the leukemia panel was seen with adaphostin (NSC680410, **11**, score = 22), which was over twice the score achieved by any other compound in this series (Table 4).

Although adaphostin clearly inhibits $p210^{bcr\text{-}abl}$ autokinase activity in association with decreased cell growth, the actual range of its tyrosine kinase targets remains to be defined. Indeed, AG 957 can inhibit T-lymphoblast proliferation with prominent inhibition of c-*cbl* phosphorylation,[23] and inhibits the growth of non-$p210^{bcr\text{-}abl}$ expressing cells in vitro[24–26] and in hollow fibers (Table 4). The fact that adaphostin is not 'simply' a tyrosine kinase inhibitor is underscored by recent studies from Svingen et al.[15] who showed that along with the inhibition of kinase activity, adaphostin decreases the expression of $p210^{bcr\text{-}abl}$, in contrast to Gleevec®. Adaphostin also induced apoptosis very efficiently in $p210^{bcr\text{-}abl}$-expressing[15] and $p210^{bcr\text{-}abl}$-nonexpressing cell lines,[17,24–26] possibly by triggering redox-related cell killing mechanisms, in addition to tyrosine kinase inhibition. In addition, Avramis et al.[25,26] have demonstrated that adaphostin can also down regulate elaboration of the vascular endothelial growth factor (VEGF). These findings further emphasize that the full range of adaphostin-related killing mechanisms needs further exploration. Nonetheless, adaphostin has emerged from this study as the best candidate for further pre-clinical evaluation with the aim of evaluation in human clinical trials.

Other features of the structure–activity relationship described here are of interest. Phenol ethers (NSC 676622, **6**; NSC 676448, **7**) exhibited complete abrogation of growth and kinase inhibitory activity. The quinones (NSC 681151, **29**; NSC 676537, **22**), corresponding to adaphostin and AG 957, respectively, showed lesser degrees of anti-kinase activity, and showed decreased growth inhibitory activity. In addition, making the phenol ring bulkier (NSC 681541, **20**) had the same negative effect as that of the quinones. Some of the analogs with linker modifications maintained the properties of the parent compound (AG 957) whereas in other cases the growth inhibitory and/or kinase activity was lost (Table 3). However these compounds did not demonstrate in vivo anti-leukemic activity in the hollow fiber assay (Table 4).

We thus demonstrate that the 1,4-dihydroquinone moiety is essential for $p210^{bcr\text{-}abl}$ inhibition, inhibition of K562 cell growth, and in vivo activity. A potential mechanism for these analogs is to act as a covalent modifier of target proteins by intracellular oxidation to their respective quinones, which then could undergo 1,4 addition reactions with biological nucleophiles. Indeed, recent studies have documented the ability of adaphostin to generate reactive oxygen species while altering the physical state of $p210^{bcr\text{-}abl}$ likely inducing its degradation.[17] This occurs in CML cells which are resistant to Gleevec®,[17,27] or which do not express $p210^{bcr\text{-}abl}$.

Quinone derivatives themselves are inactive, probably because of their inability to enter the cell. Most of the hindered (ester) analogs maintained the growth inhibitory property of the parent compound but they were less potent inhibitors of $p210^{bcr\text{-}abl}$ protein kinase. The compound with the adamantyl ester function (Adaphostin, NSC 680410, **11**) has emerged as the optimal compound from these structure activity studies. Adaphostin (NSC 680410, **11**) inhibits K562 cell growth and $p210^{bcr\text{-}abl}$ phosphorylation in vitro, and it has favorable in vivo behavior in the leukemia-directed hollow fiber assay. This selectivity of adaphostin in comparison to its effects in solid tumor cell lines presents added stimulus to develop this compound further and to test it in clinical settings for hematologic malignancies. It is likely to possess anti-tyrosine kinase activity with selectivity for $p210^{bcr\text{-}abl}$, although the complete spectrum of its anti-phospho tyrosine activity remains to be established. In addition, it might cause the elaboration of reactive oxygen intermediates through pathways yet to be determined, which may augment its cytotoxic potential in the appropriate cellular context.

5. Material and methods

5.1. Cell culture and cell growth assay

The human leukemia Philadelphia chromosome positive CML cell line K562 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in RPMI-1640 medium supplemented with 10% Fetal Bovine serum, 2 mM glutamine, 100 units/mL of penicillin, and 100 units/mL of streptomycin. All cultures were maintained at 37 °C in a 5% CO_2 incubator. Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO), aliquoted, and stored at −20 °C.

Cells (1×10^3 cells/well) were incubated with increasing concentrations of compounds in a final volume of 200 μL in 96-well plates. Control cells were incubated with medium containing identical concentrations of the solvent, DMSO. Growth of K562 cells was assessed after 6 days by the ability of living cells to reduce the yellow dye 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan product,[28] and absorbance was measured at 570 nm. Data was plotted using Sigmaplot software and the IC_{50} was calculated using regression analysis.

5.2. $p210^{bcr\text{-}abl}$ Immunoprecipitation

Cells were washed twice in phosphate buffered saline (PBS) and were lysed in lysis buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, and 1 mM phenylmethanesulfonyl fluoride] as described previously.[13] Cell lysates were centrifuged at 100,000*g* for 45 min and supernatants were collected. Protein concentrations of the clarified supernatants were determined by the Bradford protein assay (Pierce Biotechnology Inc.). Cell lysates were incubated and gently rocked at 4 °C overnight with anti-bcr antibody (Ab-2, Calbiochem). Immune complexes were collected by incubation with protein A-Sepharose beads (Amersham Biosciences), and centrifugation.

5.3. In vitro immune complex kinase assay

The $p210^{bcr\text{-}abl}$ protein immunoprecipitates were washed twice with HNTG buffer [20 mM HEPES (pH 7.5), 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, and 1 mM sodium orthovanadate] to remove unbound material. Immuno-precipitates were washed once with 50 mM Tris (pH 7.0) and resuspended in 20 μL of 20 mM PIPES [piperzine-N,N′-bis(2-ethanesulfonic acid)] (pH 7.0) plus 20 mM $MnCl_2$. Five micrograms (1 μg/μL) of denatured rabbit muscle enolase were added to all the samples as an exogenous substrate for $p210^{bcr\text{-}abl}$ kinase. Ten microliter of each analog were added at four times the final concentration to each reaction mixture. Reactions were initiated with addition of 10 μL of [γ-^{32}P]ATP (10 μCi per sample, 3000 Ci/mmol; Amersham Corp.), incubated for 20 min at 30 °C, stopped by addition of 10 μL of 5X SDS gel loading buffer, and heated at 95 °C for 5 min. Proteins were resolved on 4–20% Novex mini gels (Invitrogen) and proteins were analyzed by autoradiography. Quantitation of phosphorylated $p210^{bcr\text{-}abl}$ protein was performed using an IS1000 Digital Imaging System (Alpha Innotech Corp.) and statistical analysis was performed using linear regression analysis.

5.4. Pharmacokinetics studies

Male, young adult (average body weight approximately 25 g), CD2F1 mice (Harlan Sprague–Dawley, Frederick, MD) were used for all studies. Animals were maintained on hardwood chip bedding in temperature controlled rooms (20 °C) with a 12 h light–dark cycle. Standard diet (rat and mouse 18% protein diet, PMI Nutrition International, Inc., Brentwood, MO) and water were provided ad libitum. Housing, animal care and all experimental procedures and manipulations were carried out in strict compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. AG 957 analogs were dissolved in 100% DMSO and administered to mice via the tail vein as a 0.5 min infusion at a dose of 127 μmol/kg, using a dose volume of 1 mL/kg body weight. At selected intervals from 2 through 360 min, groups of 3 mice were

anesthetized with methoxyfluorane and exsanguinated via the retro-orbital sinus. Blood was collected into heparinized microfuge tubes, plasma was separated by centrifugation, and samples were stored at −70 °C until assayed. Plasma concentrations of AG 957 analogs were determined by reverse-phase HPLC with ultraviolet detection. Plasma concentration versus time profiles were constructed using the geometric mean of the plasma concentrations for the individual animals at each time point, and the mean of the times of collection. The data were analyzed by noncompartmental methods. The line of best fit was determined by nonlinear regression analysis, and pharmacokinetic parameters were calculated by standard equations as previously described.[29]

5.5. Hollow fiber assays

The hollow fiber assay[22] has been adopted as a rapid and inexpensive means of obtaining initial evidence of in vivo activity. Cells, loaded into differentially permeable ($M_r < 500{,}000$) polyvinylidene fluoride hollow fibers (Spectrum Medical Corp, Los Angeles, CA) are placed into the peritoneal and subcutaneous compartments of mice. Drug is administered to the animal for 4 days, and then the cell mass in the fibers is determined by a colorimetric assay.[30] The compounds synthesized here were evaluated in the hollow fiber assay as described previously with the exception that the target cell lines were 6 human leukemia/lymphoma cell lines rather than the standard panel of 12 solid tumor lines. The cell lines used for this assay included CCRF-CEM, HL-60, SR, K562, RPMI-8226, and MOLT-4. The doses evaluated were 50 and 33.5 mg/kg/dose prepared in 10% DMSO in saline with 0.05% Tween 80® and given once daily for 4 days starting on the 3rd day after hollow fiber implantation. On day 7, the fibers were collected, and the viable cell mass in the treated fibers was compared to that of the vehicle treated fibers. Each time the treated group had a 50% or greater reduction in viable cell mass compared to control it was assigned a score of 2.[22] Each compound score was determined by summing the intraperitoneal and subcutaneous values obtained against each of the six cell lines at both dose levels and then calculating a total score from those. The maximum a compound could score is 48 since there are a total of 24 combinations of cell lines, doses and implant sites. Based on prior hollow fiber experience, a score of 10 or greater was considered preliminary evidence of in vivo activity therefore justifying further evaluation in vivo.[30]

6. Experimental section

6.1. General

Melting points were determined on a Fisher Johns hot stage melting point apparatus or MEL-TEMP apparatus using Pyrex capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DRX-500 instrument or on a Varian Inova-400 instrument and chemical shifts (δ) are reported relative to TMS and/or referenced to the solvent in which they were run. Infrared spectra were obtained on Beckman IR-33 or Bio-Rad FRS-40 Fourier transform IR. Mass spectra were recorded on VG Analytical ZAB-E or VG Analytical ZAB-R instruments. Microanalyses were performed by Atlantic Microlab Inc., Norcross, GA or by Galbraith Laboratories, Knoxville, TN, and are within ±0.4% of theoretical values unless otherwise indicated. Extremely minor traces (less than 0.01%) of the residual solvents observed in the ^{1}H NMR were not factored in the combustion analyses calculations, but are mentioned in the text. Starting materials for the synthesis and other chemicals were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI and other commercial sources. Elemental analysis and ^{1}H NMR spectra showed that fractional molar amounts of organic solvents or water were retained by some of the analytical samples even after careful drying. Melting point and elemental analysis are given in Table 5.

6.2. 1-Adamantyl 4-nitrobenzoate

A solution of 1-adamantanol (15.2 g, 0.1 mol) in THF (100 mL) containing pyridine (25 mL) was cooled to 0–5 °C and 4-nitrobenzoyl chloride (18.55 g, 0.1 mol) was added portion wise (over 15 min). The resulting slurry was stirred at 5 °C for 1 h then at room temperature for 16 h. The reaction mixture was filtered and the collected solid was washed with EtOAc (3×20 mL). The combined filtrate and washings were concentrated in vacuo to give a residual solid. This solid was dissolved in CH_2Cl_2 (200 mL) and the solution was applied to a pad of silica gel ($4'' \times 3''$) and eluted with CH_2Cl_2 (10×200 mL). Fractions containing pure product as indicated by TLC were concentrated in vacuo to obtain a solid (19 g). This solid was recrystallized from EtOAc (200 mL), to give 13 g (43%) of pure product.

6.3. 1-Adamantyl 4-aminobenzoate

A suspension (semi-solution) of 1-adamantyl 4-nitrobenzoate (6.1 g, 20.2 mmol) in EtOH (100 mL) containing 5% Pd/C (300 mg) was hydrogenated at 30 psi for 3 h. The reaction mixture was clarified by filtration through a Celite pad, and the pad was washed with EtOH (3×25 mL). The clear colorless filtrate was concentrated in vacuo to give a residual white solid 5.3 g (96%), as a pure product.

6.4. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)methylene]-amino]-, 1-adamantyl ester

To a solution of 1-adamantyl 4-aminobenzoate (5 g, 18.4 mmol) in EtOH (150 mL) containing $NaHCO_3$ (2–3 mg) was added 2,5-dihydroxybenzaldehyde (2.54 g, 18.4 mmol) and the resulting solution was stirred at room temperature for 20 h. The reaction mixture was partially concentrated in vacuo to remove ~50 mL of EtOH. The residual slurry was diluted with hexanes, and the yellow solid that separated was collected by filtration, washed with hexane and dried to give 6.25 g (86%) of pure product as a yellow solid.

Table 5. Elemental analyses-found values and melting point

No	NSC no	Emperial formula	Found values			Other elements	Mp (°C)
			C	H	N		
1	678027	Commercial sample					
2	676538	$C_{14}H_{13}NO_4 \cdot 0.2\ H_2O$	63.94	5.17	5.34		231–232
3	654703	$C_{14}H_{14}N_2O_3 \cdot 0.1H_2O$, 0.13EtOH	64.57	5.67	10.58		205–210 (d)
4	654705	$C_{14}H_{15}NO_4$	65.72	5.58	5.02		55–157
5	677695	$C_{17}H_{19}NO_4$	67.76	6.37	4.58		Gummy solid
6	676622	$C_{16}H_{17}NO_4$	66.79	6.01	4.88		150–153
7	676448	$C_{17}H_{19}NO_4$	67.62	6.34	4.79		111–113
8	678634	$C_{21}H_{19}NO_4 \cdot 0.3H_2O$, 0.15EtOH	70.3	5.87	3.66		Gummy solid
9	680779	$C_{21}H_{27}NO_4$	71	7.92	3.69		78–80
10	677696	$C_{18}H_{21}NO_4$	68.56	6.75	4.37		158–160
11	680410	$C_{24}H_{27}NO_4$	73.3	7.05	3.41		113–115
12	689857	$C_{25}H_{29}NO_4$	73.41	7.11	3.45		183–185 (d)
13	687945	$C_{15}H_{15}NO_5$	62.19	5.29	4.74		179–180
14	686561	$C_{17}H_{18}ClNO_4$	60.77	5.36	4.17	Cl, 10.58	135–137 (d)
15	689858	$C_{15}H_{15}NO_3$	69.74	5.89	5.35		197–199 (d)
16	689431	$C_{20}H_{26}N_2O_3$	70.27	7.81	8.21		168–170
17	689001	$C_{15}H_{14}F_3N_3$	57.18	4.93	4.25	F, 15.89	118–120
18	688792	$C_{15}H_{18}NO_5P$	55.26	5.74	4.26	P, 9.23	140–142
19	685985	$C_{15}H_{13}NBr_2ClO_4$	46.84	3.55	3.57		167–169
20	681541	$C_{19}H_{17}NO_4$	70.62	5.28	4.35		135–137 (d)
21	681288	$C_{19}H_{15}NO_4$	70.89	4.88	4.23		158–159 (d)
22	676537	$C_{15}H_{13}NO_4$	66.31	4.88	5.14		158–160(d)
23	688791	$C_{15}H_{13}NO_5$	62.69	4.61	4.83		148–160
24	679817	$C_{14}H_{12}N_2O_3$	65.67	4.74	10.93		155–158
25	678636	$C_{21}H_{17}NO_4$	72.47	4.92	4.02		145–147
26	678635	$C_{17}H_{17}NO_4$	68.04	5.78	4.64		111–113
27	678637	$C_{18}H_{19}NO_4$	69.05	6.12	4.51		125–127
28	681719	$C_{21}H_{25}NO_4$	70.83	7.09	3.93		Gummy solid
29	681151	$C_{24}H_{25}NO_4 \cdot 0.3H_2O$	72.64	6.48	3.52		166–168 (d)
30	683766	$C_{24}H_{24}BrNO_4$	61.32	5.15	2.94	Br, 17.10	156–158 (d)
31	685405	$C_{18}H_{18}BrNO_4 \cdot 0.09$hexane	4.99	3.69	3.69		110–112
32	686334	$C_{15}H_{11}BrClNO_4$	3.03	3.5	3.5		102–105
33	687225	$C_{17}H_{16}ClNO_4$	61.1	4.93	4.11	Cl, 10.75	112–113
34	681152	$C_{16}H_{16}O_4 \cdot 0.04$EtOAc	70	6.04			143–146
35	684424	$C_{16}H_{15}BrO_4$	54.74	4.3		Br, 22.63	178–180
36	655255	$C_{15}H_{14}O_4 \cdot 0.25\ H_2O$	64.69	5.31			180–184
37	680649	$C_{16}H_{14}O_4$	70.83	5.37			123–124
38	684496	$C_{18}H_{18}O_4$	71.97	6.19			71–73
39	685106	$C_{18}H_{19}BrO_4$	56.98	5.06		Br, 21.11	122–124
40	685105	$C_{16}H_{13}BrO_4$	55.1	3.75		Br, 22.81	131–133
41	685107	$C_{18}H_{17}BrO_4$	57.26	4.58		Br, 21.05	106–107
42	680561	$C_{16}H_{14}O_4$	70.81	5.37			205–215 (d)
43	680780	$C_{16}H_{12}O_4$	71.5	4.56			153–156 (d)

6.5. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)methyl]-amino]-, 1-adamantyl ester (11)

A semi-solution of benzoic acid, 4-[[(2,5-dihydroxyphenyl)methylene]amino]-, 1-adamantyl ester (3.27 g, 8.35 mmol) in EtOH (200 mL) containing 5% Pd/C (400 mg) was hydrogenated using a Parr hydrogenator, at 19 psi, for 1.75 h. The reaction mixture was clarified by filtration through a Celite pad and the pad was washed with EtOH (3×20 mL). The combined filtrate and washings were concentrated in vacuo to give 3 g of a foamy solid. This was dissolved in CH_2Cl_2 (30 mL) when the product crystallized upon stirring. The crystalline product was filtered, washed with hexane (5×10 mL), and dried in vacuo to give 2.95 g (90%) of pure product (**11**) as a light gray (beige) solid.

6.6. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclohexadienyl)-methyl]-amino-, 1-adamantyl ester (29)

Air was bubbled through a stirred solution of benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]amino]-, 1-adamantyl ester (**11**) (1.0 g, 2.54 mmol) in MeOH (100 mL) in the presence of N,N′-bis(salicylidene)-ethylenediaminocobalt (II) hydrate (200 mg) at room temperature for 1 h. The reaction mixture was concentrated in vacuo to give a dark brown solid. This solid was dissolved in MeOH (5 mL) and CH_2Cl_2 (150 mL) and the solution was applied to a silica gel column (3×15 cm), which was eluted with CH_2Cl_2 (200 mL) followed by EtOAc/hexanes (1:1). Fractions containing pure product as indicated by TLC were combined and concentrated to give a red solid, which was triturated with CH_2Cl_2 (5 mL) and diluted with hexane

(20 mL). The red solid that separated was filtered, washed with hexane and dried in vacuo at ~35 °C to give 710 mg (70%) of pure product (**29**) as a red crystalline powder.

An alternate method was also used for converting hydroquinone to its corresponding quinone. In a typical experiment compound (**19**) was converted to (**32**) using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Compounds (**24**), (**28**), and (**31**) were also prepared using similar protocol.

6.7. Benzoic acid, 2-chloro-4-[[(2-bromo-3,6-dioxo-1,4-cyclohexadienyl)methyl]amino-, methyl ester (NSC 686334) (32)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.44 g, 2.1 mmol) was added to a stirred solution of (**19**) (0.80 g, 2.1 mmol) in Et_2O (120 mL) and EtOAc (10 mL) at room temperature. The resulting reaction mixture was stirred for 30 min then diluted with hexanes (100 mL). The insolubles were removed by filtration and discarded. The filtrate was passed through a silica gel column and eluted with Et_2O/hexane (1:1). The appropriate fractions were combined and the solvent was removed in vacuo to leave 609 mg (76%) of purified product as a dark purple solid; mp 102–105 °C.

6.8. Benzoic acid, 4-[2-(2,5-dihydroxyphenyl)-ethyl]-, methyl ester (NSC 681152) (34)

A solution of benzoic acid, 4-[2-[2,5-dihydroxyphenyl]ethyl]- (2.1 g, 8.1 mmol) in MeOH (100 mL) was cooled to ~5 °C while stirring and concentrated H_2SO_4 (4.0 mL) was added drop wise over 15 min. Upon completion of the H_2SO_4 addition, the reaction mixture was allowed to warm to room temperature, and then it was heated to a gentle reflux for 2 h. The reaction mixture was again cooled to ~5 °C and neutralized to pH 8.5 by adding saturated aqueous $NaHCO_3$ (150 mL). The precipitate formed was removed by filtration, washed successively with water (200 mL), hexanes (200 mL), and dried in vacuo at room temperature to give 1.5 g (68%) of the crude product. Chromatography on silica gel using ethyl acetate/hexanes (1:1) gave 284 mg of the analytically pure sample.

6.9. Benzoic acid, 4-[2-(3, 6-dioxo-1,4-cyclo-hexadienyl)ethyl]-, methyl ester (NSC 680649) (37)

Air was bubbled through a stirred solution of (**34**) (1.0 g, 3.7 mmol) in MeOH (100 mL) in the presence of salcomine (300 mg) at room temperature for 1 h. The reaction mixture was concentrated in vacuo to give a dark brown solid. This solid was dissolved in a small amount of CH_2Cl_2, and the solution was applied to a silica gel column (200 g), which was eluted with CH_2Cl_2. The appropriate fractions were combined, and the solvent was removed in vacuo to leave 430 mg of partially purified material. This material was reprecipitated from a mixture of CH_2Cl_2 (5 mL) and hexane (300 mL). The solid that precipitated was collected by filtration, washed with hexanes (100 mL), and dried in vacuo to constant weight to give 354 mg (35%) of pure product (**37**). Compound (**43**) was prepared from (**42**) using above procedure.

6.10. Methyl 4-[2-[4-bromo-2,5-dihydroxyphenyl]-ethyl]benzoate (NSC 684424) (35) and methyl 4-[2-[4-bromo-3,6-dioxo-1,4-cyclohexadienyl]ethyl]benzoate (NSC-685105) (40)

To a stirred, argon blanketed solution of (**38**) (1.8 g, 6.6 mmol) in CH_2Cl_2 (1.8 L) was added a solution of Br_2 (18 g/L in CH_2Cl_2) (60 mL, 6.8 mmol) drop wise over 1.5 h. The mixture was stirred at 25 °C for 1.5 h. Additional Br_2 solution (18 g/L in CH_2Cl_2) (60 mL, 6.8 mmol) was added drop wise and stirring continued at 25 °C for 1 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (900 mL), dried over $MgSO_4$ (10 g), and concentrated in vacuo to a volume to ~50 mL. The solids were collected by filtration, washed with hexanes (2 × 25 mL) and dried to constant weight in vacuo to give 760 mg (33.0%) of product (**35**). The filtrate, mostly containing the oxidation product (**40**) was spin-evaporated in vacuo and the residual material was chromatographed on a silica gel column packed and eluted with EtOAc/hexane (1:6). Appropriate fractions as determined by TLC were combined and concentrated to a volume of ~5 mL. The residue was diluted with hexanes (30 mL). The resulting crystalline solids were collected by filtration and dried to constant weight in vacuo to give 943 mg (40.9%) of pure (**40**). Compounds (**39**) and (**41**) were prepared in a similar fashion.

6.11. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-, (NSC 676538) (2)

Mp 231–232 °C. ^{1}H NMR; DMSO–d_6: δ 11.96 (s, 1H, –COOH); 8.81 and 8.57 (two s, 2H, –OH phenolic); 7.62 (d, 2H); 6.84 (t, 1H, –NH); 6.60–6.40 (m, 5H); 4.15 (d, 2H). Anal. Calcd ($C_{14}H_{13}NO_4$) C, H, N.

6.12. Benzamide, 4-[[(2,5-dihydroxyphenyl)methyl]-amino]- (NSC 654703) (3)

Mp 205–210 °C (decomposes). ^{1}H NMR; DMSO–d_6: δ 8.82 and 8.59 (two s, 2H, D_2O exchangeable, –OH); 7.66 (d, 2H, aromatic-H); 7.53 and 6.85 (two br s, D_2O exchangeable, –$CONH_2$); 6.67 (d, 1H, aromatic-H); 6.63 (d, 1H, aromatic-H); 6.61 (m, 1H, D_2O exchangeable, –NH); 6.57 (d, 2H, aromatic-H); 6.49 (dd, 1H, aromatic-H), 4.21 (d, 2H, –CH_2). A trace of EtOH and EtOAc are also observed. Anal. Calcd ($C_{14}H_{14}N_2O_3 \cdot 0.1H_2O \cdot 0.13EtOH$) C, H, N.

6.13. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-, methyl ester (NSC 654705) (4)

Mp 155–157 °C. ^{1}H NMR; DMSO–d_6: δ 8.85 and 8.62 (two br s, 2H, –OH, D_2O exchangeable); 7.72 (d, 2H); 6.96 (t, 1H, –NH, D_2O exchangeable); 6.68–6.62 (m, 4H); 6.49 (m, 1H); 4.23 (d, 2H, –CH_2); 3.77 (s, 3H, –CH_3). Anal. Calcd ($C_{14}H_{15}NO_4$) C, H, N.

6.14. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-, isopropyl ester (NSC 677695) (5)

Gummy solid. ^{1}H NMR; DMSO–d_6: δ 8.84 and 8.60 (two s, 2H, D_2O exchangeable –OH); 7.70 (d, 2H, aromatic-H); 6.92 (t, 1H, D_2O exchangeable –NH); 6.68 (d, 1H, aromatic-H); 6.63–6.16 (m, 3H, aromatic-H); 6.50–6.48 (dd, 1H, aromatic-H); 5.07 (m, 1H, –CH); 4.23 (d, 2H, $–CH_2$). Anal. Calcd ($C_{17}H_{19}NO_4$) C, H, N.

6.15. Benzoic acid, 4-[[(2,5-dimethoxyphenyl)-methyl]-amino]-, (NSC 676622) (6)

Mp 150–153 °C. ^{1}H NMR; DMSO–d_6: δ 11.98 (s, 1H, D_2O exchangeable –COOH); 7.63 (d, 2H, aromatic-H); 6.91–6.89 (m, 1H, aromatic-H); 6.85 (t, 1H, D_2O exchangeable, –NH); 6.75 (m, 2H, aromatic-H); 6.54 (d, 2H, aromatic-H); 4.23 (d, 2H, $–CH_2$); 3.76 and 3.60 (two s, 6H, $–OCH_3$). Anal. Calcd ($C_{16}H_{17}NO_4$) C, H, N.

6.16. Benzoic acid, 4-[[(2,5-dimethoxyphenyl)-methyl]-amino]-, methyl ester (NSC 676448 (7)

Mp 111–113 °C. ^{1}H NMR; $CDCl_3$: δ 7.85 (d, 2H); 6.85 (d, 1H); 6.82 (d, 1H); 6.76 (dd, 1H); 6.59 (d, 2H); 4.55 (br s, 1H, D_2O exchangeable, –NH); 4.36 (d, 2H); 3.83, 3.82, and 3.72 (3s, 9H). Anal. Calcd ($C_{17}H_{19}NO_4$) C, H, N.

6.17. Benzoic acid, 4-[[(2,5-dihidroxyphenyl)-methyl]-amino]-, benzyl ester (NSC 678634) (8)

Gummy solid. ^{1}H NMR; DMSO–d_6: δ 8.74 (br s, 2H, D_2O exchangeable, –OH); 7.77 (d, 2H, aromatic-H); 7.48–7.36 (m, 5H, aromatic-H); 6.99 (br s, 1H, D_2O exchangeable, –NH); 6.69–6.62 (m, 4H, aromatic-H); 6.52–6.48 (dd, 1H, aromatic-H); 5.29 (s, 2H, $–CH_2$); 4.25 (d, 2H, CH_2–N–). Anal. Calcd ($C_{21}H_{19}NO_4 \cdot 0.15$ EtOH, 0.3 H_2O) C, H, N.

6.18. Benzoic acid, 4-[[(2,5-dihidroxyphenyl)-methyl]-amino]-, 2,4-dimethyl-3-pentanyl ester (NSC 680779) (9)

Mp 78–80 °C. ^{1}H NMR; DMSO–d_6: δ 8.84 (s, 1H, –OH); 8.59 (s, 1H, –OH); 7.73 (d, 2H); 6.94 (t, 1H, –NH); 6.64 (m, 4H); 6.49 (dd, 1H); 4.68 (t, 1H); 4.24 (d, 2H); 1.99 (m 2H); 0.911, 0.909, 0.898, and 0.895 (4s, 12H). Anal. Calcd ($C_{21}H_{27}NO_4$) C, H, N.

6.19. Benzoic acid, 4-[[(2,5-dihidroxyphenyl)-methyl]-amino]-, *tert*-butyl ester (NSC 677696) (10)

Mp 158–160 °C. ^{1}H NMR; DMSO–d_6: δ 8.83 and 8.59 (two s, 2H, D_2O exchangeable, –OH); 7.65 (d, 2H, aromatic H); 6.86 (t, 1H, –NH); 6.67 (d, 1H, aromatic-H); 6.60–6.59 (m, 3H, aromatic-H); 6.50–6.48 (dd, 1H, aromatic-H); 4.23 (d, 2H, $–CH_2$); 1.53 (s, 9H, $–(CH_3)_3$. Anal. Calcd ($C_{18}H_{21}NO_4$) C, H, N.

6.20. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-, 1-adamantyl ester (NSC 680410) (11)

Mp 113–115 °C. ^{1}H NMR; DMSO–d_6: δ 8.85 and 8.60 (two br s, 2H, –OH); 7.64 (d, 2H); 6.87 (t, 1H, –NH); 6.67 (d, 1H); 6.60–6.58 (m, 3H); 6.48 (dd, 1H); 4.22 (d, 2H); 2.19 (s, 10H); 1.69 (s, 5H). Anal. Calcd ($C_{24}H_{27}NO_4$) C, H, N.

6.21. Benzoic acid, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-, 1-adamantanemethyl ester (NSC 689857) (12)

Mp 183–185 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 8.85 (br s, 1H, –OH); 8.61 (br s, 1H, –OH); 7.73 (d, 2H); 6.96 (t, 1H, –NH); 6.68–6.61 (m, 4H); 6.50 (dd, 1H); 4.24 (d, 2H, $–CH_2N–$); 3.81 (s, 2H, $–OCH_2–$); 2.00 (s, 3H); 1.75–1.61 (m, 12H). Anal. Calcd ($C_{25}H_{29}NO_4$) C, H, N.

6.22. Benzoic acid, 2-hydroxy-4-[[(2,5-dihydroxy-phenyl)methyl]amino]-, methyl ester (NSC 687945) (13)

Mp 179–180 °C. ^{1}H NMR, DMSO–d_6: δ 10.84 (s, 1H, –OH); 8.86 (s, 1H, –OH); 8.62 (s, 1H, –OH); 7.51 (d, 1H); 7.12 (t, 1H, –NH); 6.68 (d, 1H); 6.60 (d, 1H); 6.50 (dd, 1H); 6.26 (dd, 1H); 5.99 (d, 1H); 4.20 (d, 2H); 3.83 (s, 3H). Anal. Calcd ($C_{15}H_{15}NO_5$) C, H, N.

6.23. Benzoic acid, 2-chloro-4-[[(2,5-dihydroxyphe-nyl)methyl]amino]-, isopropyl ester (NSC 686561) (14)

Mp 135–137 °C. ^{1}H NMR, DMSO–d_6: δ 8.89 (s, 1H, OH); 8.62 (s, 1H, OH); 7.67 (d, 1H, ArH); 7.11 (s, 1H, NH); 6.67 (m, 2H, ArH); 6.59 (m, 2H, ArH); 6.52 (dd, 1H, ArH); 5.07 (m, 1H, –CH); 4.22 (d, 2H, CH_2); 1.31 (d, 6H, $–CH_3$) ppm. Anal. Calcd ($C_{17}H_{18}ClNO_4$) C, H, N.

6.24. Acetophenone, 4′-[[(2,5-dihydroxyphenyl)-methyl]-amino]-(NSC 689858) (15)

Mp 197–199 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 8.87 (s, 1H, –OH); 8.62 (s, 1H, –OH); 7.74 (d, 2H); 7.04 (t,1H, –H); 6.68 (d, 1H); 6.62–6.60 (m, 3H); 6.49 (dd, 1H); 4.25 (d, 2H); 2.42 (s, 3H). Anal. Calcd ($C_{15}H_{15}NO_3$) C, H, N.

6.25. N,N-Diisopropylbenzamide, 4-[[(2,5-dihydroxyphe-nyl)methyl]amino]-(NSC-689431) (16)

Mp 168–170 °C. ^{1}H NMR, DMSO–d_6: δ 8.79 (s, 1H, –OH); 8.59 (s, 1H, –OH); 7.06 (d, 2H); 6.65 (m, 2H); 6.58 (d, 2H); 6.49 (dd, 1H); 6.34 (t, 1H, –NH); 4.18 (d, 2H, $–CH_2$); 3.70 (br s, 2H, –CH); 1.29 and 1.28 (two s, 12H). Anal. Calcd ($C_{20}H_{26}N_2O_3$) C, H, N.

6.26. Benzenemethanol, 4-[[(2,5-dihydroxyphenyl)-methyl]-amino]-.alpha.-(trifluoromethyl)- (NSC 689001) (17)

Mp 118–120 °C. ^{1}H NMR, DMSO–d_6: δ 8.77 (s, 1H, –OH); 8.54 (s, 1H, –OH); 7.18 (d, 2H); 6.65 (m, 2H); 6.59 (d, 2H); 6.48 (m, 2H); 6.19 (t, 1H, –NH); 4.89 (d,

1H); 4.17 (d, 2H). Anal. Calcd ($C_{15}H_{14}F_3NO_3$) C, H, F, N.

6.27. Phenylphosphonic acid, 4-[[(2,5-dihydroxy-phenyl)-methyl]amino]-, dimethyl ester (NSC 688792) (18)

Mp 140–142 °C. ^{1}H NMR, DMSO–d_6: δ 8.85 (s, 1H, –OH); 8.62 (s, 1H, –OH); 7.40 (m, 2H); 6.86 (t, 1H, –NH); 6.86–6.66 (m, 3H); 6.63 (d, 1H); 6.50 (dd, 1H); 4.22 (d, 2H, –CH_2–); 3.61 and 3.59 (two s, 6H, –OCH_3). Anal. Calcd ($C_{15}H_{18}NO_5P$) C, H, N, P.

6.28. Benzoic acid, 2-chloro-4-[[(2-bromo-3,6-dihydroxy-phenyl)methyl]amino]-, methyl ester (NSC 685985) (19)

Mp 167–169 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 9.45 (2s, 2H, –OH), 7.72 (d, 1H, $J = 8.79$); 6.87 (d. 1H, $J = 2.30$); 6.83 (d, 1H, $J = 7.74$); 6.78 (d, 1H, $J = 8.75$); 6.75–6.72 (m, 2H); 4.34 (d, 2H); 3.78 (s, 3H). Anal. Calcd ($C_{15}H_{13}BrClNO_4$) C, H, N.

6.29. Benzoic acid, 4-[[2-(1,4-dihydroxynaphthalenyl)-methyl]amino]-, methyl ester (NSC 681541) (20)

Mp 135–137 °C (decomposes). ^{1}H NMR, $CDCl_3$: δ 8.17 (d, 1H); 8.10 (d, 1H); 7.91 (d, 2H); 7.52 (m, 3H, 1D_2O exchangeable); 6.81 (d, 2H); 6.67 (s, 1H); 4.93 (s, 1H, D_2O exchangeable); 4.53 (d, 2H –CH_2); 4.39 (m, 1H, D_2O exchangeable); 3.87 (s, 3H, –CH_3). Anal. Calcd ($C_{19}H_{17}NO_4$) C, H, N.

6.30. Benzoic acid, 4-[[(1,4-dihydro-1,4-dioxo-2-naph-thalenyl)methyl]amino]-, methyl ester (NSC 681288) (21)

Mp 158–159 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 8.09 (m, 1H); 8.02 (m, 1H); 7.92 (m, 2H); 7.74 (m, 2H); 7.03 (m, 1H, –NH); 6.73 (m, 3H); 4.36 (d, 2H, –CH_2); 3.79 (s, 3H, –OCH_3). Anal. Calcd ($C_{19}H_{15}NO_4$) C, H, N.

6.31. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclo-hexadien-yl)methyl]amino]-, methyl ester (NSC 676537) (22)

Mp 158–160 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 7.75 (d, 2H); 6.98–6.87 (m, 3H); 6.69 (d, 2H); 6.51 (s, 1H); 4.20 (d, 2H); 3.78 (s, 3H). Anal. Calcd ($C_{15}H_{13}NO_4$) C, H, N.

6.32. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclohexa-dienyl)-methyl]amino]-2-hydroxy-, methyl ester (NSC 688791) (23)

Mp 148–160 °C. ^{1}H NMR, DMSO–d_6: δ 10.85 (s, 1H, –OH); 7.55 (d, 1H); 7.07 (t, 1H, –NH); 6.98 (d, 1H); 6.89 (d, 1H); 6.49 (s, 1H); 6.29 (dd, 1H); 6.07 (d, 1H); 4.18 (d, 2H); 3.84 (s, 3H). Anal. Calcd ($C_{15}H_{13}NO_5$) C, H, N.

6.33. Benzamide, 4-[[(3,6-dioxo-1,4-cyclohexa-dienyl)-methyl]amino]- (NSC 679817) (24)

Mp 155–158 °C. ^{1}H NMR, DMSO–d_6: δ 7.69–7.67 (d, 2H), 7.60 (br s, 1H, amide- H); 6.97–6.95 (d, 1H); 6.91–6.86 (m, 2H, amide-H and aromatic-H); 6.63–6.59 (m, 3H, –CH_2N*H*–, and aromatic-H); 6.51 (d, 1H); 4.18–4.17 (2d, 2H, –C*H*$_2$–N). Anal. Calcd ($C_{14}H_{12}N_2O_3$) C, H, N.

6.34. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclo-hexadienyl)-methyl]amino]-, benzyl ester (NSC 678636) (25)

Mp 145–147 °C. ^{1}H NMR, DMSO–d_6: δ 7.79–7.76 (d, 2H, aromatic-H); 7.48–7.37 (m, 5H, aromatic-H); 7.01–6.86 (m, 3H, aromatic-H and –NH); 6.71–6.68 (d, 2H, aromatic-H); 6.50 (d, 1H, aromatic-H); 5.30 (s, 2H, –CH_2); 4.21 (d, 2H, –*CH*$_2$–N–). Anal. Calcd ($C_{21}H_{17}NO_4$) C, H, N.

6.35. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclo-hexadienyl)-methyl]amino]-, isopropyl ester (NSC 678635) (26)

Mp 111–113 °C, ^{1}H NMR, DMSO–d_6: δ 7.73 (d, 2H); 6.99–6.89 (m, 3H); 6.69 (d, 2H); 6.49 (d, 1H); 5.08 (m, 2H); 4.20 (d, 2H); 1.31 and 1.29 (two s, 6H). Anal. Calcd ($C_{17}H_{17}NO_4$) C, H, N.

6.36. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclo-hexadienyl)-methyl]amino]-, *tert*-butyl ester (NSC 678637) (27)

Mp 125–127 °C. ^{1}H NMR, DMSO–d_6: δ 7.69 (d, 2H, aromatic-H); 6.94–6.95 (d, 1H, aromatic-H); 6.90–6.86 (m, 2H, aromatic-H and –NH); 6.67 (d, 2H, aromatic-H); 6.48 (d, 1H, aromatic-H); 4.20 (d, 2H, –CH_2); 1.53 (s, 9H, *t*-butyl). Anal. Calcd ($C_{18}H_{19}NO_4$) C, H, N.

6.37. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclohexa-dienyl)-methyl]amino]-, 2,4-dimethyl-3-pentanyl ester (NSC 681719) (28)

Gummy red solid. ^{1}H NMR, DMSO–d_6: δ 7.77 (d, 2H); 6.98 (d, 1H); 6.92 (t, 1H, –NH); 6.89 (dd, 1H); 6.70 (d, 2H); 6.52 (d, 1H); 4.69 (t, 1H); 4.20 (d, 2H); 2.00 (m, 1H); 0.91 and 0.89 (two s, 12H). A few extraneous minor signals of unknown origin are also observed. Anal. Calcd ($C_{21}H_{25}NO_4$) C, H, N.

6.38. Benzoic acid, 4-[[(3,6-dioxo-1,4-cyclo-hexadienyl)-methyl]amino]-, 1-adamantyl ester (NSC 681151) (29)

Mp 166–168 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 7.67 (d, 2H); 6.98 (d, 1H); 6.89–6.85 (m, 2H); 6.66 (d, 2H); 6.49 (d, 1H); 4.20 (d, 2H); 2.19 (s, 9H); 1.69 (s, 6H). Anal. Calcd ($C_{24}H_{25}NO_4$) C, H, N.

6.39. Benzoic acid, 4-[[(2-bromo-3,6-dioxo-1,4 cyclo-hexadienyl)methyl]amino]-, 1-adamantyl ester (NSC 683766) (30)

Mp 156–158 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 7.69 (d, 2H); 7.18 (d, 1H); 7.08 (d, 1H); 6.69 (d, 2H); 6.57 (t, 1H, –NH); 4.27 (d, 2H); 2.20 (s, 9H); 1.70 (s, 6H). Anal. Calcd ($C_{24}H_{24}BrNO_4$) C, H, N.

6.40. Benzoic acid, 4-[[(2-bromo-3,6-dioxo-1,4-cyclohexadienyl)methyl]amino]-, *tert*-butyl ester (NSC 685405) (31)

Mp 110–112 °C. ^{1}H NMR, DMSO–d_6: δ 7.70 (d, 2H, J = 8.73); 7.18 (d, 1H, J = 10.03); 7.08 (d, 1H, J = 10.2); 6.70 (d, 2H, J = 8.77); 6.57 (t, 1H, –NH); 4.27 ($C_{18}H_{18}BrNO_4 \cdot 0.09$ d, 2H, J = 4.93); 1.54 (s, 9H, *t*-butyl). Anal. Calcd ($C_{18}H_{18}BrNO_4 \cdot 0.09$hexane) C, H, N.

6.41. Benzoic acid, 2-chloro-4-[[(2-bromo-3,6-dioxo-1,4-cyclohexadienyl)methyl]amino]-, methyl ester (NSC 686334) (32)

Mp 102–105 °C. ^{1}H NMR, DMSO–d_6: δ 7.75 (d, 1H, J = 8.76); 7.19 (d, 1H, J = 10.05); 7.08 (d, 1H, J = 10.05); 6.89 (t, 1H, –NH); 6.79 (d, 1H, J = 2.33); 6.70–6.67 (dd, 1H, J = 8.8 and 2.37); 4.29 (d, 2H, $–CH_2$); 3.79 (s, 3H, $–OCH_3$). Anal. Calcd ($C_{15}H_{11}BrClNO_4$) C, H, N.

6.42. Benzoic acid, 2-chloro-4-[[(3,6-dioxo-1,4-cyclohexadienyl)methyl]amino]-, isopropyl ester (NSC 687225) (33)

Mp 112–113 °C. ^{1}H NMR, DMSO–d_6: δ 7.61 (d, 1H, Ar); 7.01 (t, 1H, NH); 6.91 (d, 1H, Ar); 6.82 (dd, 1H, Ar); 6.67 (d, 1H, Ar); 6.57 (dd, 1H, Ar); 6.42 (d, 1H, Ar); 5.00 (m, 1H, CH); 4.12 (d, 2H, CH_2); 1.23 (d, 6H, CH_3) ppm. Anal. Calcd ($C_{17}H_{16}ClNO_4$) C, H, Cl, N.

6.43. Benzoic acid, 4-[2-(2,5-dihydroxyphenyl]-ethyl]-, methyl ester (NSC 681152) (34)

Mp 143–146 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 8.60 (s, 1H, –OH); 8.53 (s, 1H, –OH); 7.92 (d, 2H); 7.40 (d, 2H); 6.64 (d, 1H); 6.49 (d, 1H); 6.46–6.44 (dd, 1H); 4.07 (s, 3H, $–OCH_3$); 2.94–2.91 (m, 2H); 2.80–2.77 (m, 2H); a trace of EtOAc is also observed. Anal. Calcd ($C_{16}H_{16}O_4 \cdot 0.4$EtOAc) C, H.

6.44. Methyl 4-[2-[4-bromo-2,5-dihydroxyphenyl]-ethyl]-benzoate (NSC 684424) (35)

Mp 178–180 °C. ^{1}H NMR, DMSO–d_6: δ 9.22 (s, 1H, OH); 9.08 (s, 1H, OH); 7.92 (m, 2H, ArH); 7.39 (d, 2H, ArH); 6.92 (s, 1H, ArH); 6.65 (s, 1H, ArH); 3.88 (s, 3H, $–OCH_3$); 2.91 (m, 2H, $–CH_2$); 2.78 (m, 2H, $–CH_2$). Anal. Calcd ($C_{16}H_{15}BrO_4$) C, H, Br.

6.45. 1-(3′-Carboxy-4′-hydroxyphenyl)-2-(2″,5″-dihydroxyphenyl)ethane (NSC 655255) (36)

Mp 180–184 °C. ^{1}H NMR, DMSO–d_6: δ 12.2, 8.56, 8.52 (3 br s, 4H, –OH, D_2O exchangeable); 7.65 (d, 1H); 7.38 (dd, 1H); 6.90 (d, 1H); 6.63 (d, 1H); 6.50 (d, 1H); 6.44 (dd, 1H). Anal. Calcd ($C_{15}H_{14}O_5$) C, H.

6.46. Benzoic acid, 4-[2-(3,6-dioxo-1,4-cyclohexadienyl)-ethyl]-, methyl ester (NSC 680649) (37)

Mp 123–124 °C. ^{1}H NMR, DMSO–d_6: δ 7.93 (d, 2H); 7.40 (d, 2H); 6.91 (d, 1H); 6.85 (dd, 1H); 6.69 (d, 1H); 3.88 (s, 3H); 2.92 (t, 2H); 2.73 (t, 2H). Anal. Calcd ($C_{16}H_{14}O_4$) C, H.

6.47. Benzoic acid, 4-[2-(3,6-dioxo-1,4-cyclohexa-dienyl)ethyl]-, isopropyl ester (NSC 684496) (38)

Mp 71–73 °C. ^{1}H NMR, DMSO–d_6: δ 7.92 (d, 2H); 7.42 (d, 2H); 6.93 (d, 1H); 6.86 (dd, 1H); 6.69 (d, 1H); 5.16 (m, 1H); 2.92 (t, 2H); 2.74 (t, 2H); 1.36 (s, 3H); 1.35 (s, 3H). Anal. Calcd ($C_{18}H_{18}O_4$) C, H.

6.48. Isopropyl 4-[2-[4-bromo-2,5-dihydroxy-phenyl]ethyl] benzoate (NSC 685106) (39)

Mp 122–124 °C. ^{1}H NMR, DMSO–d_6: δ 9.22 (s, 1H, OH); 9.08 (s, 1H, OH); 7.91 (d, 2H, Ar); 7.36 (d, 2H, Ar); 6.92 (s, 1H, Ar); 6.65 (s, 1H, Ar); 5.17 (m, 1H, CH); 2.92 (m, 2H, CH_2); 2.77 (m, 2H, CH_2); 1.36 (d, 6H, CH_3) ppm. Anal. Calcd ($C_{18}H_{19}BrO_4$) C, H, Br.

6.49. Methyl 4-[2-[4-bromo-3,6-dioxo-1,4-cyclo-hexadienyl]-ethyl]benzoate (NSC 685105) (40)

Mp 131–133 °C. ^{1}H NMR, DMSO–d_6: δ 7.93 (d, 2H, ArH); 7.58 (s, 1H, ArH); 7.42 (d, 2H, ArH); 6.92 (s, 1H, ArH); 3.88 (s, 3H, $–OCH_3$); 2.91 (m, 2H, $–CH_2–$); 2.74 (m, 2H, $–CH_2–$) ppm. Anal. Calcd ($C_{18}H_{13}BrO_4$) C, H, Br.

6.50. Isopropyl 4-[2-[4-bromo-3,6-dioxo-1,4-cyclohexa-dienyl]ethyl] benzoate (NSC 685107) (41)

Mp 106–107 °C. ^{1}H NMR, DMSO–d_6: δ 7.91 (d, 2H, Ar); 7.58 (s, 1H, Ar); 7.41 (d, 2H, Ar); 6.92 (s, 1H, Ar); 5.17 (m, 1H, CH); 2.92 (m, 2H, CH_2); 2.74 (m, 2H, CH_2); 1.36 (d, 6H, CH_3) ppm. Anal. Calcd ($C_{18}H_{17}BrO_4$) C, H, Br.

6.51. Benzoic acid, 4-[2-(2,5-dihydroxyphenyl)-ethenyl]-, methyl ester, (E)- (NSC 680561) (42)

Mp 205–215 °C (decomposes). ^{1}H NMR, DMSO–d_6: δ 9.19 (s, 1H, –OH); 8.81 (s, 1H, –OH); 7.99 (d, 2H); 7.73 (d, 2H); 7.57 (d, 1H, J = 16.51 Hz); 7.23 (d, 1H, J = 16.51 Hz); 7.03 (d, 1H); 6.76 (d, 1H); 6.64 (dd, 1H); 3.90 (s, 3H). Anal. Calcd ($C_{16}H_{14}O_4$) C, H.

6.52. Benzoic acid, 4-[2-(3,6-dioxo-1,4-cyclohexadienyl)-ethenyl]-, methyl ester (E)- (NSC 680780) (43)

Mp 153–156 °C (decomposes). ^{1}H NMR, $CDCl_3$: δ 8.05 (d, 2H); 7.61 (d, 2H); 7.50 (d, 1H, J = 16.5 Hz, vinyl-H); 7.26 ($CHCl_3$); 7.20 (d, 1H, J = 16 Hz, vinyl-H); 6.90 (d, 1H); 6.80 (m, 2H); 3.93 (s, 3H, CH_3). Anal. Calcd ($C_{16}H_{12}O_4$) C, H.

Acknowledgements

This study was supported by National Cancer Institute, NIH, under contract N01-CO-12400 and N0-CM-47015.

References and notes

1. de Klein, A.; van Kessel, A. G.; Grosveld, G.; Bartram, C. R.; Hagemeijer, A.; Bootsma, D.; Spurr, N. K.; Heisterkamp, N.; Groffen, J.; Stephenson, J. R. *Nature* **1982**, *300*, 765.
2. Bartram, C. R.; de Klein, A.; Hagemeijer, A.; van Agthoven, T.; Geurts van Kessel, A.; Bootsma, D.; Grosveld, G.; Ferguson-Smith, M. A.; Davies, T.; Stone, M., et al. *Nature* **1983**, *306*, 277.
3. Konopa, J. B.; Watanabe, S. M.; Witte, O. N. *Cell* **1984**, *37*, 1035.
4. Daley, G. Q.; Van Etten, R. A.; Baltimore, D. *Science* **1990**, *247*, 824.
5. Daley, G. Q.; Van Etten, R. A.; Baltimore, D. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11335.
6. Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. *New Engl. J. Med.* **2001**, *344*, 1031.
7. Druker, B. J.; Sawyers, C. L.; Kantarjian, H.; Resta, D. J.; Reese, S. F.; Ford, J. M.; Capdeville, R.; Talpaz, M. *New Engl. J. Med.* **2001**, *344*, 1038.
8. Gorre, M. E.; Mohammed, M.; Ellwood, K.; Hsu, N.; Paquette, R.; Rao, P. N.; Sawyers, C. L. *Science* **2001**, *293*, 876.
9. Sausville, E. A. *Lancet* **2003**, *361*, 1400.
10. Sausville, E. *Clin. Cancer Res.* **2003**, *9*, 1233.
11. Kaur, G.; Gazit, A.; Levitzki, A.; Stowe, E.; Cooney, D. A.; Sausville, E. A. *Anti-Cancer Drug.* **1994**, *5*, 213.
12. Anafi, M.; Gazit, A.; Gilon, C.; Ben-Neriah, Y.; Levitzki, A. *J. Biol. Chem.* **1992**, *267*, 4518.
13. Kaur, G.; Sausville, E. A. *Anti-Cancer Drug.* **1996**, *7*, 815.
14. Narayanan, V. L.; Sausville, E. A.; Kaur, G.; Varma, R. K. Preparation of disubstituted Lavendustin A analogs and their pharmaceutical composition. United States Department of Health and Human Services, USA. PCT Int. Appl. WO 9943636 A2 19990902, 1999.
15. Svingen, P. A.; Tefferi, A.; Kottke, T. J.; Kaur, G.; Narayanan, V. L.; Sausville, E. A.; Kaufmann, S. H. *Clin. Cancer Res.* **2000**, *6*, 237.
16. Mow, B. M.; Chandra, J.; Svingen, P. A.; Hallgren, C. G.; Weisberg, E.; Kottke, T. J.; Narayanan, V. L.; Litzow, M. R.; Griffin, J. D.; Sausville, E. A.; Tefferi, A.; Kaufmann, S. H. *Blood* **2002**, *99*, 664.
17. Chandra, J.; Hackbarth, J.; Le, S.; Loegering, D.; Bone, N.; Bruzek, L. M.; Narayanan, V. L.; Adjei, A. A.; Kay, N. E.; Tefferi, A.; Karp, J. E.; Sausville, E. A.; Kaufmann, S. H. *Blood* **2003**, *102*, 4512.
18. Onoda, T.; Iinuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Naganawa, H.; Takeuchi, T.; Tatsuta, K.; Umezawa, K. *J. Nat. Prod.* **1989**, *52*, 1252.
19. Smyth, M. S.; Stefanova, I.; Hartmann, F.; Horak, I. D.; Osherov, N.; Levitzki, A.; Burke, T. R. *J. Med. Chem.* **1993**, *36*, 3010.
20. Johnson, J. I.; Decker, S.; Zaharevitz, D.; Rubinstein, L. V.; Venditti, J. M.; Schepartz, S.; Kalyandrug, S.; Christian, M.; Arbuck, S.; Hollingshead, M.; Sausville, E. A. *Brit. J. Cancer* **2001**, *84*, 1424.
21. Decker, S.; Hollingshead, M.; Bonomi, C. A.; Carter, J. P.; Sausville, E. A. *Eur. J. Cancer* **2004**, *40*, 821.
22. Hollingshead, M. G.; Alley, M. C.; Camalier, R. F.; Abbott, B. J.; Mayo, J. G.; Malspeis, L.; Grever, M. R. *Life Sci.* **1995**, *57*, 131.
23. Losiewicz, M. D.; Kaur, G.; Sausville, E. A. *Biochem. Pharmacol.* **1999**, *57*, 281.
24. Urbano, A. G. G.; Foss, F. *Biochem. Pharmacol.* **2002**, *63*, 689.
25. Avramis, I. A.; Christodoulopoulos, G.; Suzuki, A.; Laug, W. E.; Gonzalez-Gomez, I.; McNamara, G.; Sausville, E. A.; Avramis, V. I. *Cancer Chem. Pharmacol.* **2002**, *50*, 479.
26. Avramis, I. A.; Laug, W. E.; Sausville, E. A.; Avramis, V. I. *Cancer Chemother. Pharmacol.* **2003**, *52*, 307.
27. Yu, C. R. M.; Almenara, J.; Sausville, E. A.; Dent, P.; Grant, S. *Oncogene* **2004**, *23*, 1364.
28. Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.
29. Stinson, S. F.; House, T.; Bramhall, C.; Saavedra, J. E.; Keefer, L. K.; Nims, R. W. *Xenobiotica* **2002**, *32*, 339.
30. Hollingshead, M.; Plowman, J. *The Hollow Fiber Assay* **1999**, *54*.