Design, Synthesis, and Anticancer Properties of 4,4′-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone and Analogues

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4,4′-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) has recently completed a phase I clinical trial in advanced cancer with minimal toxicity, and impressive objective responses were noted. A-007 possesses three moieties that appear to have an influence on its anticancer activities: diphenylmethane, hydrazone, and dinitrophenyl. The goals of this study were to modify A-007's chemical moieties with the ultimate goal of maximizing its anticancer activity through increased planarity and introduction of functional groups. Thirty-five phenylhydrazone analogues of A-007 were synthesized and evaluated in vitro in a human primary cancer explant assay. Anticancer activities for selected analogues were also assayed for activity vs established human/murine cell lines. One-hundred-eighty-six fresh human solid tumors were used to screen for anticancer activity. Selected analogues were assayed for therapeutic indices (vs GM-CFC from bone marrow) in preparation for preclinical studies. Several polyaryl phenylhydrazones demonstrated improved cytotoxic activities by factors of 10^2-10^3 when compared with A-007**.** However, the polyaryl quinone moieties of the latter analogues introduced potential toxic properties (cardiac, hematological) that do not exist with A-007.

Introduction

4,4′-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007, **1**, Chart 1) has recently completed a phase I clinical trial, and objective responses were seen in advanced breast cancer, melanoma, and non-Hodgkin's lymphoma (NHL). **1** was applied topically to the sites of cancer involvement as a 0.25% gel and was well tolerated, without toxicity.[1,2]

X-ray crystallography data revealed that **1** exists as monoclinic crystals (Chart 1).[3] Furthermore, **1** exits as two unique molecules, which differ only in the orientation within the diphenylmethane moiety, where the rings are approximately perpendicular to each other (and rotated approximately 90° from the orientation of the rings in each rotamer). Both rotamers show strong intramolecular hydrogen bonding between the −NH of the −HN−N═C− moiety and an oxygen of the *o*-nitro group.[3] Thus, there are at least three unique moieties present in **1** that may contribute to its overall biological activity: a *dihydroxydiphenylmethane*, a *hydrazone*, and a *dinitrophenyl*. An impressive characteristic of **1** is that despite the significant electronegativity that exits within the structure, it is a relatively stable molecule; e.g., **1** is excreted unchanged in the urine.[4,5]

Rationale

1 was initially designed as an antiestrogen that was similar isosterically to tamoxifen but unable to isomerize in vivo to an estrogen, as does tamoxifen.[6] The skeletal backbones for **1** and tamoxifen are similar (Chart 2); thus, it was anticipated that the former might have similar binding properties to the latter with the antiestrogen receptor.[7] Both the N^+-O^- group of **1** and the N^+-H group of tamoxifen form strong hydrogen bonds with complement groups. The latter can form a pseudoring, similar to the third ring of **1** (Chart 2). The overall similarities are highlighted for comparison. The distances between the two electrostatically rich comple-

Chart 1. X-ray Crystallographic Characteristics of **1**[3]

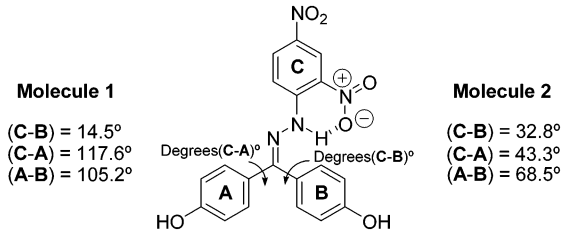
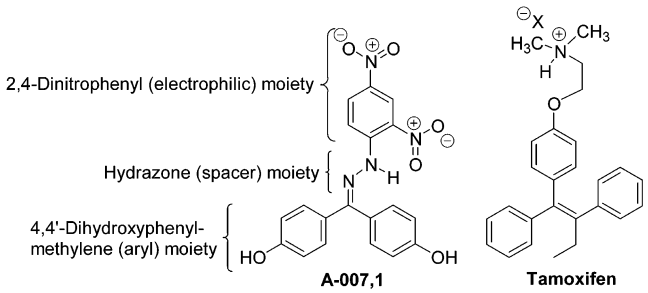


Chart 2. Comparative Structures for **1** and Tamoxifen (under Physiological Conditions)



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mentary centers (aryl vs electrophilic) are the same in both structures. Unfortunately, **1**'s antiestrogen activity was minimal and it did not bind effectively to the antiestrogen receptor.[8] **1** inhibits thioredoxin reductase and blocks [^{14}C]cytidine uptake/incorporation.[9] The exact mechanism(s) for **1**'s anticancer activity is still unknown.[10] However, the recent finding that **1** is able to induce cell membrane modulations through interactions with surface tumor markers and clusters of differentiations (CD) expands its therapeutic potential horizons.[11]

Easmon et al. have reported on **1**'s wide spectrum of activities, including activity vs Burkitt's lymphoma, MCF-7 breast cancer, and HeLa cells.[9] Easmon's group noted improved activity for **1** analogues when both electron-withdrawing and donating groups (amino vs methoxy) were substituted into the meta-5-position of **1**'s dinitrophenyl ring. A chlorine inserted in the 5-position (ortho and para to the 2- and 4-nitro groups, respectively) of the phenylhydrazone moiety improved activity by ~800-fold vs **1**.[9] The Easmon group has further demonstrated that replacement of one or both of the diphenyl rings with a *N*-heteroaryl moiety (i.e., pyridyl) did not improve activity. Acute lymphoblastic leukemia, Burkitt's lymphoma, colon, cervical, and breast adenocarcinomas and melanoma cell lines were used by the Easmon group to document activities.[9]

In the present paper, three of the potential pharmacophores that are present in **1** have been modified in an attempt to optimize and understand the basis for **1**'s activity.

Chemistry

Chemical modifications of **1**'s moieties are shown in structures **2**−**36**, which include (a) changes to the benzophenone moiety (analogues **2**−**17**), (b) coplanar transformations for the diphenyl rings (analogues **18**−**36**), and (c) changes to the hydrazine moiety (analogues **23**, **35**, and **36**) (Chart 3). The hydrazones were prepared via standard procedures, as well as with a new cationic resin column method. The latter procedure allows purer hydrazones to be generated with minimal steps.

The influence that functional group substitutions have on **1**'s intra-/intermolecular hydrogen bonding and electrostatic interactions was studied through the introduction of hydroxyl, amine, methyl, and chlorine groups (analogues **2**−**17**). The two phenyl rings in **1**'s diphenylmethane moiety are almost perpendicular to each other, providing a reason to fuse the two rings, introduce coplanarity, and document the respective influences. Since polyaryl structures can influence binding to DNA, anthraquinone, xanthone, and thioxanthone analogues were introduced in place of the diphenylmethane moiety (analogues **18**−**34)**. Coplanarity was introduced into **1** by fusing the ═N−NH− group to the benzophenone ring system, resulting in anthrapyridazinones **35** and **36.**[12] Analogue **33** is an anthrapyrazole dinitrophenylhydrazone that further challenges the influences of planarity in the system.

Analogue **35** was synthesized by first converting 1,8-dichloroanthraquinone into 8-hydroxyanthraquinone-1-carboxylic acid.[11] The latter was then treated with 2-nitrophenylhydrazine followed by thionyl chloride to obtain the respective anthrapyridazinone **35** (via modifications of ref 13). Analogue **36** was synthesized by using a similar scheme as described for **35.** The last two heterocyclic analogues are polyaryl hydrazones possessing **1**'s diphenyl skeletal moiety.

Biological Evaluations. All of the described new phenylhydrazone analogues were tested in vitro for cytotoxicity vs a battery of human cancers removed at surgery and grown as primary explants in culture. Selected analogues were also tested vs various established human tumor lines, and therapeutic indices were calculated vs normal FDCP-1 murine GM-CFC cells. The human primary cancers used were obtained at the time of surgery and placed fresh into culture. The CYTOTEC assay, developed by DEKK-TEC, was used to measure the anticancer activities.[14] With use of DEKK-TEC'S CYTOTEC monolayer tissue culture assay, 186 fresh human cancer tissues were grown as explants in tissue culture and analogues were evaluated for in vitro activities using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue)) assay to determine IC_{50} values.[15−18] All human specimens were collected in accordance with an approved Human Investigational Review Committee protocol. A total of 186 human cancers were used to obtain the data reviewed in this paper. Doxorubicin (DOX) and cisplatinum (*cis*-DDP) were selected as positive controls, with concentrations in the 0.1−10 μg/mL range. For IC_{50} values greater than 5 μg/mL, SD values are not included.

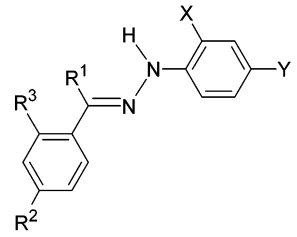
Fresh human tumor explants were selected as a screening system because of the cellular heterogeneity, i.e., lymphocytes, stromal components, cancer cells, etc. present in primary cancer tissue, compared to established xenograft cell lines.[8,14] In addition, visualization of each CYTOTEC plate allows for individual appreciation of the effects/impact that the analogues may have had on the entire ecology of the malignant explants, i.e., cancer cells, lymphocytes, fibroblasts, endothelial cells, etc.[8,14]

Results and Discussion

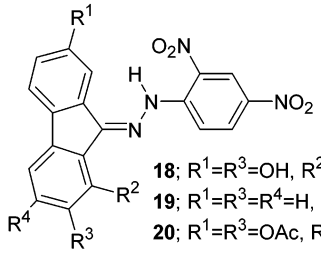
In this paper, the synthesis, characterization, and evaluation of 36 substituted hydrazones for anticancer activity in a human cancer explant assay are described. A total of 186 fresh primary human cancer tissues were grown as explants, and the analogues were evaluated for in vitro activities. Selected analogues were assayed for comparative therapeutic indices vs normal bone marrow and a battery of human cancer cell lines.

The major objective of this study was to seek correlations between the electronic, planar, hydrophobic, and steric properties vs anticancer activities for substituted aryl phenylhydrazones. Early studies with the simple nonaryl hydrazones demonstrated minimal antiestrogenic activity.[8] However, the introduction of the phenylhydrazone group significantly improved anticancer activity, with **1** emerging as a novel agent possessing novel potential for development.[10,11] To appreciate structural reasons for **1**'s cytotoxic activity, a wide range of substitutions/modifications has been made to both the diphenylmethane (benzophenone) and the phenylhydrazone moieties. The data that have been generated may serve to develop future structure−activity relationships and may suggest directions for the development of novel anticancer agents with minimal toxicity.

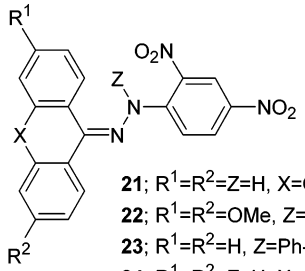
Chart 3. Structural Analogues of **1**



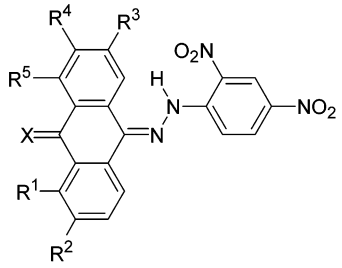
2; R^1=Ph, R^2=R^3=H, X=Y=NO_2
3; R^1=Ph, R^2=OH, R^3=H, X=Y=NO_2
4; R^1=2,4-di-HO-Ph, R^2=R^3=OH, X=Y=NO_2
5; R^1=2-HO-Ph, R^2=H, R^3=OH, X=Y=NO_2
6; R^1=4-HO-Ph, R^2=Cl, R^3=H, X=Y=NO_2
7; R^1=2-HO-4-MeOPh, R^2=OMe, R^3=OH, X=Y=NO_2
8; R^1=Ph, R^2=R^3=OH, X=Y=NO_2
9; R^1=4-AcOPh, R^2=AcO, R^3=H, X=Y=NO_2
10; R^1=4-HO-Ph, R^2=OH, R^3=H, X=Y=Me
11; R^1=4-HO-Ph, R^2=OH, R^3=Y=H, X=NO_2
12; R^1=4-HO-Ph, R^2=OH, R^3=X=Y=H
13; R^1=Me, R^2=OH, R^3=X=H, Y=NO_2
14; R^1=4-NH_2-Ph, R^2=NH_2, R^3=H, X=Y=NO_2
15; R^1=4-Me_2N-Ph, R^2=NMe_2, R^3=H, X=Y=NO_2
16; R^1=4-Et_2N-Ph, R^2=Et_2N, R^3=H, X=Y=NO_2
17; R^1=4-AcHN-Ph, R^2=AcHN, R^3=H, X=Y=NO_2



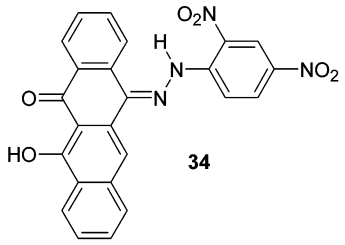
18; R^1=R^3=OH, R^2=R^4=H
19; R^1=R^3=R^4=H, R^2=OH
20; R^1=R^3=OAc, R^2=R^3=H



21; R^1=R^2=Z=H, X=O
22; R^1=R^2=OMe, Z=H, X=O
23; R^1=R^2=H, Z=Ph-CH_2, X=O
24; R^1=R^2=Z=H, X=SO



25; R^1=R^2=OH, R^3=R^4=R^5=H, X=O
26; R^1=R^5=OH, R^2=R^3=R^4=H, X=O
27; R^1=R^5=OAc, R^2=R^3=R^4=H, X=O
28; R^1=OAc, R^2=R^3=R^4=H, R^5=OH, X=O
29; R^1=OAc, R^2=R^3=R^4=H, R^5=$OCH_2CH{=}CH_2$, X=O
30; R^1=R^4=R^5=H, R^2=R^3=OH, X=2,4-$(NO_2)_2$-Ph-NHN
31; R^1=R^2=NH_2, R^3=R^4=R^5=H, X=O
32; R^1=R^4=R^5=H, R^2=R^3=NH_2, X=2,4-$(NO_2)_2$-Ph-NHN
33; R^1-X=NH-N, R^2=R^3=R^4=R^5=H



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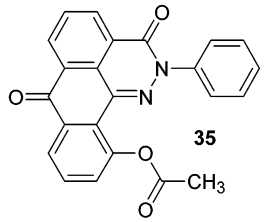

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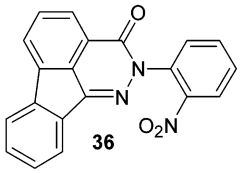

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Table 1 represents results from the testing of **1** vs 16 simple biosteric substituted analogues. Functional substitutions that could influence intra-/intermolecular hydrogen bonding and electrostatic interactions were introduced into the basic structure of **1** (Table 1). Analogue **13** is an acetophenone phenylhydrazone base structure (in which the diphenylmethane moiety of **1** has been converted to a phenylethane structure in which a phenyl has been replaced with a methyl group). However, the other 15 analogues in Table 1 represent substitutions into the benzophenone phenylhydrazone ring system of **1**. These analogues were designed to influence electronic and intramolecular hydrogen bonding, i.e., analogues **8** and **11.** Analogue **8** is of interest because it possesses a hydroxyl group *juxta*positioned such that hydrogen bonding occurs with the −NH of the hydrazone moiety (Chart 4). In contrast, analogue **11** displays intramolecular hydrogen bonding between a 2-nitro group and the −N═NH moiety.

X-ray crystallography supports the structure shown in Chart 4 with two sets of strong hydrogen bonds in analogue **8.** Analogue **8** has a composite of intramolecular hydrogen bondings that exist separately in **5** and **7**, i.e., between an *o*-hydroxyl and the ═*N*−*NH*−, as well as between the −NH and 2-NO_2 groups.[3] Analogue **5** is a 2,2′-isostere of **1** and possessed anticancer activities similar to, but with no improvement over, those of **1** (Chart 3). The 5-Cl-substituted analogue (ANG-36) of Easman et al. was not available for testing at the time that these studies were conducted.[9] Analogues **14**−**17** contain isosteric substitutions of amines for hydroxyls in **1**. The 4,4′-diamino analogue **14** had improved activity (∼1 log) vs **1**; N-substitutions reduced activity (analogues **15**−**17**). The activities for the other ana-

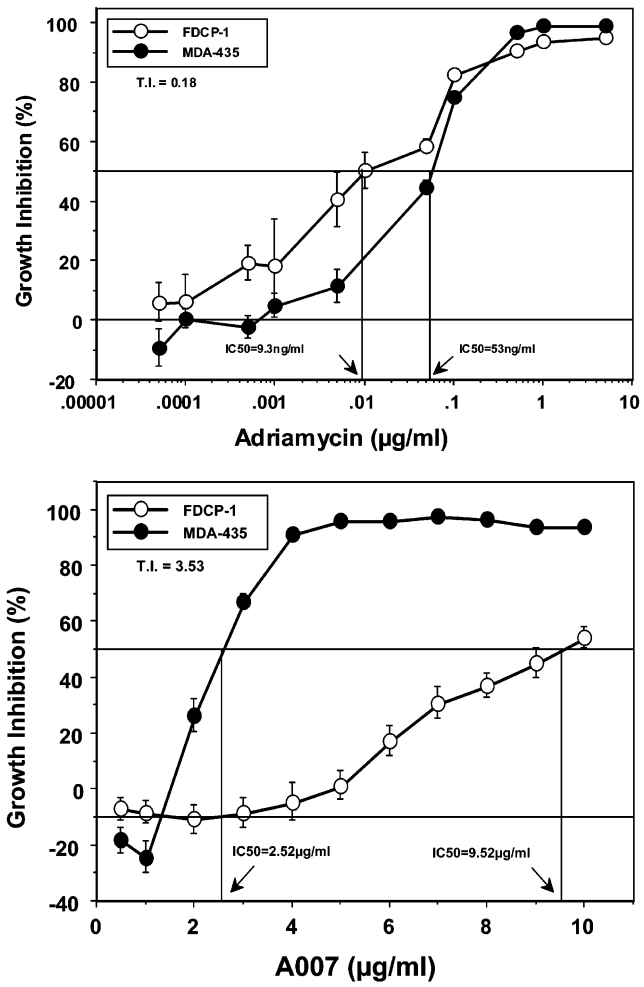


Figure 1. Inhibition of FDCP-1 and MDA-435 by doxorubin (adriamycin) and **1**.

logues in Table 1 were not significantly different from the activities of **1**, with the possible exception of analogue **8**, which is in preclinical studies to firmly document anticancer activity. Substitutions on the phenylhydrazone ring had significant influences on activity; i.e., absence of or replacement of nitro groups with methyl groups further reduced activity. Isosteric/functional substitution in any of the diphenylmethane rings with 2- and/or 4-chloro, methoxy, methyl, or acetoxy groups reduced the anticancer activity of **1**. Analogue **14** is a diamine isostere of **1**. Analogue **3** is a simple derivative of **1** that possesses a single 4-hydroxyl and has improved anticancer activities toward ovarian cancer cells. Both analogues **3** and **14** deserve more attention.[19]

Tables 2 and 3 describe activity for simple polyaryl analogues **18**−**24** and **25**−**34**, in which intramolecular flexibility and hydrogen bonding (which exist in **1**'s diphenylmethane moiety) have been eliminated. In **1**, the last two phenyl groups exist almost perpendicular to each other (Chart 1, ref 3). The analogues in Table 2 are modified diphenyl ring derivatives of **1**, creating a planar configuration. The fluorene and xanthene analogues **18** and **20**, respectively, have significantly improved activity when compared to **1** and the other simple benzophenone analogues of Table 1. Analogue **18** is a dihydroxyfluorene derivative with fused diphenyl rings, while analogue **20** possesses fixation of the diphenyl rings through an −O− atom, with less planarity (coplanar) than exists in analogue **18**. The activities for **18** and **20** were <0.1 μg/mL, and the IC_{50} values are projected values (no SD presented). Blocking the −NH group in analogue **24** destroyed anticancer activity. Analogue **20** is a xanthone phenylhydrazone with significant anticancer activity in at least three tumor types. These data have been verified, and analogue **20** is being evaluated for anticancer activity in a mouse model.

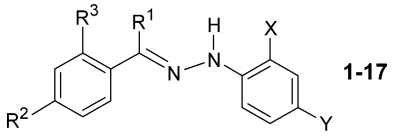
Table 3 reviews the biological activities for polyaryl analogues **25**−**34** (all possess the skeletal backbone of **1** plus fused/attached diphenyl rings) (Chart 4). The improved biological properties of the anthraquinone analogues **25**−**29** are not surprising. This supports the concept that the introduction of coplanarity not only improves **1**'s ability to penetrate cell membranes but also possibly allows the arylhydrazone system to intercalate with DNA and other biopolymers.[20] Unfortunately, the presence of polycyclic quinone rings would be expected to introduce new toxic properties (cardiac and myelosuppression), which **1** does not demonstrate.[5,10] Analogue **33** is an anthrapyrazole derivative and has no improved activities over other analogues tested. Analogue **34** is a four-ring polyaromatic system, which also had some improved activity in two tumor types. However, the potential toxicities of this ring system limit the appeal for extended studies.

Table 4 describes activities for two analogues, a phenylanthracen-1,9-pyridazinone, **35**, and a phenylfluoren-1,9-pyridazinone, **36**. Both analogues were designed and tested because they contained a more rigid configuration of **1**'s basic skeleton. Activity observed with both analogues was poor. Since the pyridazinone ring would not be expected to survive more than one pass through the liver during in vivo testing, interest in this ring system has been minimal.

Myelotoxicity and therapeutic indices (TI) for **1** vs doxorubicin (adriamycin) and three analogues **22**, **26**, and **27** are available using FDCP-1 murine GM-CFC cells vs eight tumor cell lines (Table 5 and Figure 1). The numbers are small (n = 1−3); however, the data indicate that **26** and **27** were more toxic than **1** and the results of the in vitro TI myelotoxicity studies were poor. This is in contrast to **1**, which has minimal toxicity (Table 5 and Figure 1). In clinical trials, **1** also had minimal toxicity.[1,2] Analogues **26** and **27** demonstrated excellent responses vs KM12 SM (colon carcinoma) and SN12 PM6 (renal cell carcinoma), respectively. Analogue **22**, on the other hand, demonstrated an excellent TI against MDA-MB-435 and B16 F10 cell lines. Favorable hematological responses in vivo would be predicted for **22**, compared to **1**. Of interest is that preliminary in vivo studies for 1,8-diacetoxyanthraquinone-10-2,4-DNP, **27**, when administered intraperitoneally (10 mg/(kg·day)) on days 1 and 5 to C57 mice bearing measurable B-16 F10 melanomas, have been encouraging.[8] Responses noted for analogue **27** vs B-16 mouse melanoma were 75% TGI (tumor growth inhibition) and 175% ILS (increased life span) vs 35% and 75% for **1**, respectively.[8] This analogue should also be evaluated in vivo vs SN12PM6 tumor to document therapeutic indices.[19]

Table 6 correlates IC_{50} values for **1** vs *cis*-DDP and DOX for melanoma, breast, colon, lung, and ovarian cancers. The values obtained are in agreement with

Table 1. Determination of in Vitro Cytotoxicity of Substituted Benzophenone Phenylhydrazones vs Various Human Tumors

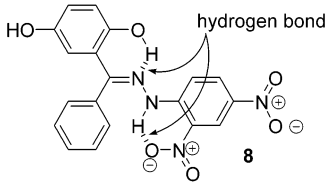


1-17

						averaage IC_{50}(SD), μg/mL				
compd	R^1	R^2	R^3	X	Y	breast	ovary	colon	lung	melanoma
1	4-HOPh	HO	H	NO_2	NO_2	*c*	*c*	*c*	*c*	*c*
2	Ph	H	H	NO_2	NO_2	>5	>10	NA[a]	>5	>10
3	Ph	HO	H	NO_2	NO_2	5(4)	0.3(1)	0.5(2)	NA[a]	>10
4	2,4-$(HO)_2$Ph	HO	HO	NO_2	NO_2	>10	0.03[b]	>5	>5	NA[a]
5	2-HOPh	H	HO	NO_2	NO_2	4.7(3)	5[b]	8(4)	6(1)	>5
6	4-HOPh	Cl	H	NO_2	NO_2	8.7(3)	NA[a]	>10	NA[a]	NA[a]
7	2-HO-4-MeOPh	MeO	HO	NO_2	NO_2	1.5(0.8)	>5	4.5(3)	NA[a]	NA[a]
8	Ph	HO	HO	NO_2	NO_2	0.5(4)	NA[a]	NA[a]	NA[a]	NA[a]
9	4-AcOPh	AcO	H	NO_2	NO_2	7[b]	>10	>10	>10	>10
10	4-HOPh	HO	H	Me	Me	10(2)	NA[a]	NA[a]	NA[a]	NA[a]
11	4-HOPh	HO	H	NO_2	H	3.9(3)	NA[a]	4.7(1)	NA[a]	NA[a]
12	4-HOPh	HO	H	H	H	>5	6.2	>5	>10	7[b]
13	Me	HO	H	H	NO_2	5(2)	6[b]	NA[a]	NA[a]	NA[a]
14	2-NH_2Ph	NH_2	H	NO_2	NO_2	0.7(2)	2(2)	8(5)	NA[a]	6[b]
15	4-Me_2NPh	Me_2N	H	NO_2	NO_2	4.2(4)	NA[a]	10	NA[a]	5[b]
16	4-Et_2NPh	Et_2N	H	NO_2	NO_2	>7	NA[a]	>10	NA[a]	5[b]
17	4-AcNHPh	AcNH	H	NO_2	NO_2	>10	>10	>10	NA	>10

[a] NA, not available. [b] One to two samples only. [c] See Table 6.

Chart 4. Two Possible Intermolecular Hydrogen Bonds for Analogue **8**



8

previously reported sensitivities for many of the cancers.[20] Drugs such gemcitabine, etc. were not available as standards when the project was initiated over 15 years ago; only DOX and *cis*-DDP were used as references.

Although **1** appears to be a simple molecule, it contains at least three active moieties: a *dinitrophenyl*, a *hydrazone*, and a *diphenylmethane*. At least one hydroxyl or an amino group appears to be necessary in the last moiety for cytotoxic activity. The 2-nitro group may also influence stability.[4,5,21] A free NH− group (in the hydrazone moiety) is needed for activity.[4,8,10] Fusion of the diphenyl rings produced coplanar systems that improved anticancer activities significantly in breast cancer [analogues **20**, **25**, and **27** vs **1**]. Fusion of the ═N−NH− moiety in **1** into an anthrapyrazole ring system (analogue **33**) did not improve activity. A single 2-nitro group on the phenylhydrazone ring slightly improved activity (analogue **11**). 2-Hydroxyl or 2,2′-dihydroxyl groups on **1**'s diphenylmethane moiety maintained the activity of **1**. Other isosteric/functional group substitutions (Cl, F, CH_3, OCH_3, $COCH_3$) in the aryl rings (of the diphenylmethane moiety) reduced activity to >5 μg/mL. Blocking the −NH, as in analogue **24** (*N*-benzylxanathone-2,4-DNP), also reduced activity (IC_{50} > 5 μg/mL).[4]

Since the polyaryl quinines in Tables 2 and 3 possess potential cardiotoxic moieties, the specific IC_{50} values were not determined. Analogues **18**, **20**, and **25**−**28** are probably inducing cell death by a mechanism different from that for the simple benzophenone analogues. **25**−**28** produced death within hours of cell contact; the analogues in Table 1 seemed to produce their changes over days.

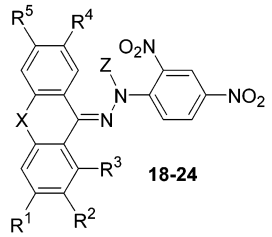
Cytotoxicity was measured by the microculture CYTOTEC assay, as previously described.[14] DOX demonstrated an IC_{50} from 0.3 to 0.75 μg/mL for breast cancer and from <0.1 to 0.4 μg/mL for ovarian cancers. *cis*-DDP had IC_{50} of >10 μg/mL for melanoma, colon, lung, and ovary cancers. The histology of the cancers were as follows: breast, infiltrating ductal adenocarcinoma (ER/PgR +/+, −/+, and −/−); ovarian, adenocarcinoma; lung, non-small-cell carcinoma; colon/rectal, adenocarcinoma; melanoma, pigmented and nonpigmented. Each value in Tables 1−4 represents 5−20 individual specimens from different patients. Breast cancer tissue was the most sensitive of the human tumor explants tested and was the most plentiful for assay (Table 6). The presence or absence of the ER/PgR (estrogen/progesterone receptors) network may have had an influence, at least for the activity of **1**. When the clinical courses for the patients with breast cancer are reviewed, those patients whose tumor tissues were sensitive to **1** at <3 μg/mL also demonstrated the highest values for their tumor tissue ER+/PgR+.[14,22]

Selected analogues from this series are currently being evaluated in vivo in human xenograft tumor models.[19]

Conclusion

In summary, we have described modifications to three functional moieties present in **1** that could influence biological activity. Analogues of **1** were prepared to introduce rigidity in the diphenylmethane ring system, as well as to influence electrostatic properties. Compounds that have evolved from this study demonstrated that fusing the diphenyl ring system into polyaryl

Table 2. Determination of in Vitro Cytotoxicity of Aryl-2,4-dinitrophenylhydrazones vs Various Human Tumors

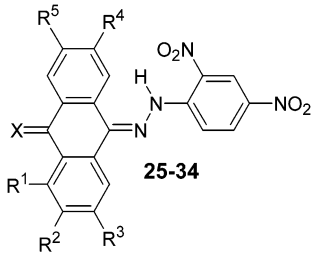


18-24

								averaage IC_{50}(SD), μg/mL				
compd	R^1	R^2	R^3	R^4	R^5	X	Z	breast	ovary	colon	lung	melanoma
18	H	HO	H	HO	H	*a*	H	0.05[b]	0.08[b]	0.06[b]	>10	4.1
19	H	H	HO	H	H	*a*	H	7.5(10)	NA[c]	>5	NA[c]	NA[c]
20	AcO	H	H	AcO	H	*a*	H	>5	0.9(2)	0.7(4)	>5	0.5
21	H	H	H	H	H	O	H	0.04[b]	0.05[b]	0.05[b]	>10	>10
22	MeO	H	H	H	MeO	O	H	>5	NA[c]	NA[c]	NA[c]	NA[c]
23	H	H	H	H	H	O	Bz[d]	>10	>10	NA[c]	NA[c]	>10
24	H	H	H	H	H	SO	H	>10	>10	>10	>10	>10

[a] Is a straight line between the rings, i.e., fluorene ring. [b] Projected value: activities are <0.1 μg/mL.[20,23] [c] NA, not available. [d] Bz = $C_6H_5CH_2$.

Table 3. Determination of in Vitro Cytotoxicity of Polyaryl-2,4-dinitrophenylhydrazones vs Various Human Tumors

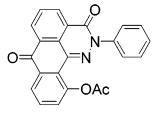
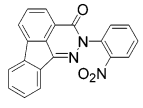


25-34

							averaage IC_{50}(SD), μg/mL				
compd	R^1	R^2	R^3	R^4	R^5	X	breast	ovary	colon	lung	melanoma
25	HO	HO	H	H	H	O	0.007[f]	>10	0.25(2)	>10	>5
26	HO	H	H	H	HO	O	0.03[f]	NA[a]	0.03[f]	0.12[f]	NA[a]
27	AcO	H	H	H	AcO	O	0.04[f]	0.3(0.1)	0.16	0.1(3)	0.07[f]
28	AcO	H	H	H	HO	O	0.05[f]	0.4(0.3)	0.03[f]	NA[a]	0.097
29	AcO	H	H	H	allylO[b]	O	0.1[f]	NA[a]	0.08	0.07	NA[a]
30	H	HO	H	HO	H	DNP[c]	2.5(2)	NA[a]	3.9(5)	1(2)	NA[a]
31	NH_2	NH_2	H	H	H	O	5(1)	6(7)	5(4)	NA[a]	NA[a]
32	H	NH_2	H	NH_2	H	DNP[c]	>5	>5	>5	NA[a]	NA[a]
33[d]	NH	H	H	H	H	N	>5	>5	>5	NA[a]	NA[a]
34[e]	HO	benzene ring		H	H	O	0.3(1)	NA[a]	NA[a]	0.1[f]	NA[a]

[a] NA, not available. [b] allylO = allyloxy (CH_2=CH−CH_2O). [c] DNP = 2,4-dinitrophenylhydrazone moiety [2,4-$(NO_2)_2C_6H_4$NHN]. [d] For **33**, R^1 and X are connected by a single bond (R^1−X = NH−N). [e] For **34**, R^2 and R^3 are connected through CH=CH−CH=CH for an additional benzene ring. [f] Projected value: activities are <0.1 μg/mL.[20,23]

Table 4. Determination of in Vitro Cytotoxicity of **35** and **36** vs Various Human Tumors

	averaage IC_{50}(SD), μg/mL				
compd	breast	ovary	colon	lung	melanoma
 **35**	>5	>10	NA[a]	NA[a]	NA[a]
 **36**	>5	>5	>5	>5	0.4(1)

[a] NA, not available.

Table 5. Determination of in Vitro Cytotoxicity of Analogues **1**, **22**, **26**, and **27** vs Various Tumor Cell Lines and Therapeutic Indices (vs FDCP-1 Murine GM-CFC Cells)

	average IC_{50} [a] (TI vs FDCP-1) (SD), μg/mL			
cell lines	**1**	**22**	**26**	**27**
FDCP-1	9.1(1.0)	0.95(1.00)	0.020(1.0)	0.045(1.00)
MDA-MB-435	2.1(3.5)	0.41(2.3)	0.020(1.0)	0.041(1.09)
MDA-MB-435/ lung met.	NA[b]	NA[b]	0.03(0.6)	NA[b]
KM12 C	NA[b]	NA[b]	0.03(0.6)	NA[b]
KM12 SM	5.4(1.9)	NA[b]	0.05(0.4)	NA[b]
KM12 L4	NA[b]	1.57(0.61)	0.02(0.8)	0.070(0.64)
B16 F10	1.0(9.1)	0.44(2.16)	NA[b]	0.042(1.07)
K1735 M2	NA[b]	0.64(1.48)	0.02(1.2)	0.042(1.07)
SN12 PM6	4.0(1.9)	0.62(1.53)	0.03(0.6)	0.021(2.14)

[a] IC_{50} values are derived from MTT assays. [b] NA, not available.

coplanar rings not only improved anticancer activity but also introduced potential problems from cardiac and bone marrow toxicities. Substitutions of electron-withdrawing groups into the dinitrophenyl ring system influenced activity. **1** and some of the simple analogues have intramolecular hydrogen bonding between the dinitrophenyl ring system and the N−NH= of the

Table 6. Comparative IC_{50} for Primary Human Cancer Culture Sensitivities: **1** vs Standards

		averaage IC_{50}(SD), μg/mL		
tissue	*N*	**1**	*cis*-DDP	DOX
breast cancer (ER+/PgR+)	28	3.5(2.7)	2.5(0.9)	0.5(1)
breast cancer (ER+/PgR−)	1	>10	>5	0.5
breast cancer (ER−/PgR+)	1	>10	3(2)	0.4(0.1)
breast cancer (ER−/PgR−)	23	12.6(5.5)	5(2)	0.2(0.3)
colon cancer	11	15.2(6.1)	>12(4)	>12(5)
ovarian cancer	12	12.3(4.5)	3(3)	4.2(4)
lung cancer	10	4.9(4)	5(5)	6(5)
melanoma	32	7(4.1)	5(4)	>10

hydrazone group, as well as between the hydrazone moiety and the hydroxyls of the diphenylmethane ring, all of which had an impact on biological activity. Fresh human cancer tissue was selected as a test system because of the natural heterogeneity that exists in a tumor nodule or mass, i.e., cancer cells, lymphocytes, fibroblasts, endothelial cells, and natural extracellular connective tissue matrices.

Experimental Section

Melting points were performed on a Mel-Temp II electrothermal melting point apparatus and are reported as uncorrected. The infrared spectra were recorded on a Perkin-Elmer PE 2000 FT-IR spectrometer as Nujol mulls. The UV spectra were recorded on a Cary 550 Scan UV−vis−NIR spectrophotometer in methanol solvent from 800 to 200 nm. The ^{1}H NMR spectra were recorded on a Varian Unity NMR spectrometer at 400 MHz or Gemini2000 spectrometer at 300 MHz in dimethyl sulfoxide (DMSO-d_6) solvent or $CDCl_3$ solvent as indicated. The ^{13}C NMR spectra were recorded at 125 MHz. As indicated, Me_4Si was used as a reference. The mass spectra (ESI) were obtained from a Micromass Quatro II triple quadrapole mass spectrometer with absolute methanol as solvent. Midwest Micro Lab, Indianapolis, IN, performed the elemental analyses. The chemicals and tissue culture supplies were purchased from Aldrich-Sigma-Fluka Chemical Co., St. Louis, MO.

1. Chemistry. The majority of hydrazones were prepared from corresponding carbonyl compounds (substituted benzophenones, acetophenones, anthraquinones, fluorenones, etc.) and substituted phenylhydrazones by one of two synthetic procedures (method A and method B).

Method A. MeOH suspensions (300 mL) of substituted phenylhenylhydrazines (0.148 mol) and concentrated sulfuric acid (20 mL) were stirred at 50 °C. After the hydrazine dissolved, a MeOH solution (300 mL) of the corresponding carbonyl compound (0.1 mol) was added to the hydrazine and the resulting reaction mixture was stirred at 50 °C for additional 30 min. The reaction mixture was concentrated to $^1/_4$ of its original volume under vacuum and diluted with water (500 mL). The precipitates were separated by filtration and washed with 3% aqueous $NaHCO_3$ (3 × 100 mL) and water (3 × 50 mL). Products were recrystallized from MeOH, EtOH, or glacial AcOH.

Method B. MeOH suspensions (15 mL) of the corresponding phenylhydrazine (2.7 mmol), ketones (2.1 mmol), and Dowex 50W-50X2-100 cation-exchange resin (0.5 g) [Dow Corp., Baton Rouge, LA] were refluxed for 1 h. The hot reaction mixture was separated by filtration and washed with methanol (3 × 0.5 mL). The filtrate was cooled to room temperature and diluted with water (20 mL). Resulting precipitates were filtered, washed with water (3 × 1 mL), and recrystallized from MeOH, EtOH, or glacial AcOH.

***N*-(4,4′-Dihydroxybenzhydrylidene)-*N*′-(2,4-dinitrophenyl)hydrazine (1)** was prepared by method A in 78% yield from 4,4′-dihydroxybenzophenone and 2,4-dinitrophenylhydrazine as deep-red crystals. The product was recrystallized from MeOH, mp 270−272 °C. IR: 3506 (N−H), 3288 (N−H), 2921 (Ar−H), 1614 (C═N), 1592, 1503, 1417, 1336, 1311, and 1139 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.13 (1H, s, NH), 10.05 (1H, broad singlet, OH), 9.99 (1H, broad singlet, OH), 8.78 (1H, d, J = 2.7 Hz, 3-H of 2,4-dinitrophenyl ring), 8.36 (1H, dd, J_1 = 9.6 Hz, J_2 = 2.7 Hz, 5-H of 2,4-dinitrophenyl ring), 8.12 (1H, d, J = 9.6 Hz, 6-H of 2,4-dinitrophenyl ring), 7.47 (2H, d, J = 8.7 Hz, *o*-H of benzophenone ring), 7.22 (2H, d, J = 8.4 Hz, *o*-H of benzophenone ring), 7.01 (2H, d, J = 8.1 Hz, *m*-H of benzophenone ring), and 6.81 (2H, d, J = 8.4 Hz, *m*-H of benzophenone ring) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz): δ 156.19, 155.31, 152.33, 140.47, 133.27, 126.50, 126.35, 126.02, 125.50, 124.07, 119.46, 118.19, 112.97, 112.90, and 111.91 ppm (15 signals for 15 different carbon atoms in **1**). Anal. Calcd for $C_{19}H_{14}N_4O_6$: C, 57.87; H, 3.58; N, 14.21. Found: C, 57.42; H, 3.81; N, 13.88.

***N*-Benzhydrylidene-*N*′-(2,4-dinitrophenyl)hydrazine (2)** was prepared by method A from benzophenone and 2,4-dinitrophenylhydrazine in 82% yield and purified by crystallization from EtOH or MeOH, mp 232−234 °C. IR: 3295 (N−H), 2928 (Ar−H), 1612 (C═N), 1590, 1504, 1416, 1334, 1127, 1091, 834, 777, 698, and 608 cm^{-1}. ^{1}H NMR (DMSO, 400 MHz): δ 11.25 (1 H, s, N−H), 9.07 (1 H, s), 8.37 (1 H, d, J = 8 Hz), 8.22 (1 H, d, J = 8 Hz), 7.67 (4 H, m, J = 8 Hz), 7.41 (6 H, m, J = 8 Hz). Anal. Calcd for $C_{19}H_{14}N_4O_4$: C, 62.98; H, 3.86; N, 15.46. Found: C, 63.17; H, 4.06; N, 15.49.

4-Hydroxybenzophenone-2,4-dinitrophenylhydrazone (3) was prepared by method B from 4-hydroxybenzophenone and 2,4-dihydroxyphenylhydrazine as red needles that crystallized from EtOH in 80% yield, mp 224−225 °C. IR: 3541 (N−H), 3271 (N−H), 3098 (O−H), 2905 (Ar−H), 1621 (C═N), 1591, 1511, 1418, 1329, 1276, 1130, 1083, 829, and 698 cm^{-1}. ^{1}H NMR (DMSO, 400 MHz): δ 11.23 (1/2 H, s, N−H), 10.99 (1/2 H, s, N−H), 10.1 (1 H, s, "broad" OH), 8.8 (1 H, s), 8.41 (1 H, d, J = 8 Hz), 8.19 (1 H, d, J = 8 Hz), 7.67 (2 H, m, J = 8 Hz), 7.48 (3 H, m, J = 8 Hz), 7.28 (1 H, dd, J = 8 Hz), 7.04 (1 H, dd, J = 8 Hz), 6.84 (1 H, dd, J = 8 Hz). Anal. Calcd for $C_{19}H_{14}N_4O_5$: C, 59.07; H, 3.62. Found: C, 59.71; H, 3.84.

2,2′,4,4′-Tetrahydroxybenzophenone-2, 4-dinitrophenylhydrazone (4) was prepared by method A from 2,2′,4,4′-tetrahydroxybenzophenone and 2,4-dinitrophenylhydrazine as a deep-red crystalline product that crystallized from EtOH in 75% yield, mp 290−291 °C. IR: 3631 (N−H), 3491 (N−H), 3272 (O−H), 2905 (Ar−H), 1614 (C═N), 1518, 1415, 1322, 1206, 1143, 1107, 974, 864, and 827 cm^{-1}. ^{1}H NMR (DMSO, 400 MHz): δ 11.59 (1 H, s, O−H), 11.15 (1 H, s, N−H), 10.12 (2 H, s, O−H), 9.91 (1 H, s, O−H), 8.85 (1 H, s), 8.48 (1 H, d, J = 8 Hz), 8.19 (1 H, d, J = 8 Hz), 7.61 (1 H, d, J = 8 Hz), 6.95 (1 H, dd, J = 8 Hz), 6.8 (1 H, dd, J = 8 Hz), 6.57 (1 H, s), 6.46 (1 H, dd, J = 8 Hz), 6.38 (1 H, s), 6.31 (1 H, dd, J = 8 Hz). Anal. Calcd for $C_{19}H_{14}N_4O_8$: C, 53.52; H, 3.28; N, 13.14. Found: C, 51.79; H, 3.19; N, 13.36.

2,2′-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (5) was prepared by method B from 2,2′-dihydroxybenzophenone and 2,4-dinitrophenylhydrazine as deep-red crystals in 85% yield. The product recrystallized from EtOH, mp 270−271 °C. IR: 3454 (N−H), 3279 (O−H), 2921 (Ar−H), 1618 (C═N), 1587, 1415, 1330, 1147, 1096, 750, 587, 441, and 415 cm^{-1}. ^{1}H NMR (DMSO, 400 MHz): δ 11.13 (1/2 H, s, O−H), 10.97 (1/2 H, s, N−H), 10.34 (1 H, s, "broad" O−H), 8.85 (1 H, s), 8.51 (1 H, d, J = 8 Hz), 7.79 (1 H, d, J = 8 Hz), 7.49 (1 H, t, J = 8 Hz), 7.35 (1 H, t, J = 8 Hz), 7.2 (1 H, dd, J = 8 Hz), 7.15 (1 H, dd, J = 8 Hz), 7.15 (1 H, dd, J = 8 Hz), 7.03 (1 H, m, J = 8 Hz), 6.87 (1 H, t, J = 8 Hz). Anal. Calcd for $C_{19}H_{14}N_4O_6$: C, 57.86; H, 3.55; N, 14.21. Found: C, 57.86; H, 3.54; N, 14.34.

4-Chloro-4′-hydroxybenzophenone-2,4-dinitrophenylhydrazone (6) was prepared by method B from 4-chloro-4′-hydroxybenzophenone and 2,4-dinitrophenylhydrazine as deep-red crystals (87% yield) that crystallized from EtOH, mp 195−197 °C. IR: 3567 (N−H), 3453 (N−H), 3290 (O−H), 3111 (O−H), 1621 (C═N), 1585, 1500, 1419, 1337, 1306, 1264, 1219, 1134, and 840 cm^{-1}. ^{1}H NMR (DMSO, 400 MHz): δ 11.21 (1/2 H, s, N−H), 10.94 (1/2 H, s, N−H), 10.13 (1 H, d, "broad" O−H), 8.81 (1 H, d, J = 8 Hz), 8.41 (1 H, t, J = 8 Hz), 8.18 (1 H, t, J = 8 Hz), 7.76 (1 H, d, J = 8 Hz), 7.65 (1 H, d, J = 8 Hz),

7.5 (4 H, m, $J = 8$ Hz), 7.28 (1 H, dd, $J = 8$ Hz), 7.05 (1 H, dd, $J = 8$ Hz), 6.84 (1 H, dd, $J = 8$ Hz).

2,2′-Dihydroxy-4,4′-dimethoxybenzophenone-2,4-dinitrophenylhydrazone (7) was prepared by method B from 2,2′-dihydroxy-4,4′-dimethoxybenzophenone and 2,4-dinitrophenylhydrazine (in 90% yield) as red crystals that crystallized from EtOH, mp 218−220 °C. IR: 3444 (N−H), 3269 (N−H), 3290 (O−H), 2981 (Ar−H), 1615 (C═N), 1521, 1335, 1209, 1132, 1607, 843, and 814 cm^{-1}. ^1H NMR (DMSO, 400 MHz): δ 11.51 (1 H, s, O−H), 11.14 (1 H, s, N−H), 10.38 (1 H, s, O−H), 8.85 (1 H, s), 8.49 (1 H, dd, $J = 8$ Hz), 7.67 (1 H, dd, $J = 8$ Hz), 7.1 (1 H, dd, $J = 8$ Hz), 6.88 (1 H, dd, $J = 8$ Hz), 6.67 (1 H, s), 6.56 (1 H, s), 6.47 (1 H, d, $J = 8$ Hz), 3.82 (3 H, s "methyl"), 3.79 (3 H, s "methyl"). Anal. Calcd for $C_{21}H_{18}N_4O_8$: C, 55.50; H, 3.96; N, 12.33. Found: C, 55.43; H, 3.95; N, 12.28.

2,4-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (8) was prepared by method A in 90% yield from 2,4-dihydroxybenzophenone and 2,4-dinitrophenylhydrazine as red crystals that crystallized from MeOH, mp 304−305 °C. IR: 3271 (N−H), 2929 (Ar−H), 2855 (Ar−H), 1619 (C═N), 1518, 1421, 1323, 1203, 1112, 868, and 702 cm^{-1}. ^1H NMR (DMSO, 400 MHz): δ 11.14 (1 H, s, N−H), 10.96 (1 H, s, O−H), 10.15 (1 H, s, O−H), 8.80 (1 H, s), 8.48 (1 H, d, $J = 8$ Hz), 7.69 (4 H, m, $J = 8$ Hz), 6.74 (1 H, dd, $J = 8$ Hz), 6.39 (1 H, s), 6.31 (1 H, dd, $J = 8$ Hz). Anal. Calcd for $C_{19}H_{14}N_4O_6$: C, 57.86; H, 3.55; N, 14.21. Found: C, 57.78; H, 3.51; N, 14.19.

4,4′-Diacetoxybenzophenone-2,4-dinitrophenylhydrazone (9) was prepared by dissolving **1** (0.01 mol) in 5 mL of 3 M NaOH and 15 g of crushed ice followed by 4.5 g (0.03 mol) of Ac_2O. The mixture was vigorously shaken and allowed to stand at room temperature. The acetate separated in a practically pure state as orange crystals that crystallized from MeOH; yield 95%, mp 205−207 °C. IR: 3287 (N−H), 3119 (N−H), 2929 (Ar−H), 1768 (C═O), 1623 (C═N), 1591, 1501, 1263, 1198, 916, and 600 cm^{-1}. ^1H NMR (DMSO, 400 MHz): δ 11.07 (1 H, s, N−H), 8.82 (1 H, s), 8.44 (1 H, d, $J = 8$ Hz), 8.25 (1 H, d, $J = 8$ Hz), 7.69 (2 H, dd, $J = 8$ Hz), 7.58 (2 H, dd, $J = 8$ Hz), 7.49 (2 H, dd, $J = 8$ Hz), 7.26 (2 H, dd, $J = 8$ Hz), 2.35 (3H,s), 2.30 (3H,s). Anal. Calcd for $C_{23}H_{18}N_4O_8$: C, 57.70; H, 3.80; N, 11.70. Found: C, 57.76; H, 3.77; N, 11.55.

4,4-Dihydroxybenzophenone-2,4-dimethylphenylhydrazone (10) was prepared by method A from 4,4-dihydroxybenzophenone and 2,4-dimethylphenylhydrazine as cream-colored crystals in 75% yield that crystallized from EtOH, mp 140−142 °C. IR (KBr): 3356 (N−H), 2885 (Ar−H), 1663 (C═N), 1509, 1256, 1228, 1170, 838, and 809 cm^{-1}. ^1H NMR (DMSO, 400 MHz): δ 9.87 (1/2 H, s, N−H), 9.6 (1/2 H, s, N−H), 7.36 (4 H, m), 7.16 (2 H, dd, $J = 8$ Hz), 6.99 (2 H, dd, $J = 8$ Hz), 6.95 (1 H, d, $J = 8$ Hz), 6.74 (2 H, dd, $J = 8$ Hz), 2.18 (3 H, s), 1.8 (3 H, s). Anal. Calcd for $C_{21}H_{20}N_2O_2$: C, 75.90; H, 6.02; N, 8.43. Found: C, 75.74; H, 6.02; N, 8.25.

4,4′-Dihydroxybenzophenone-2-nitrophenylhydrazone (11) was prepared from 4,4′-dihydroxybenzophenone and 2-nitrophenylhydrazine using method A. H_3PO_4 was substituted for H_2SO_4. The yield was 67%, and the product was recrystallized from aqueous EtOH as red crystals, mp 239−241 °C. ^1H NMR (DMSO-d_6): δ 10.806 (1H, NH), 9.930 (2H, broad, OH), 7.975 (2H, d + d, $J_1 = 6.3$, $J_2 = 6.3$, two hydrogens in 3- and 6-position of 2-nitrophenylhydazine moiety), 7.571 (1H, t, $J = 6.0$ Hz, hydrogen in 4-position of 2-nitrophenylhyrazine moiety), 7.427 (2H, d, $J = 6.6$ Hz, two hydrogens in *o*-position of the 4,4′-dihydroxybenzophenone moiety), 7.160 (2H, $J = 6.3$ Hz, two hydrogens in *o*-position of the 4,4′-dihydroxybenzophenone moiety), 7.005 (2H, $J = 6.6$ Hz, two hydrogens in *o*-position of the 4,4′-dihydroxybenzophenone moiety), 6.797 (2H, $J = 6.3$ Hz, two hydrogens in *m*-position of the 4,4′-dihydroxybenzophenone moiety), and 6.766 (1H, t, $J = 6.3$ Hz, hydrogen in 6-position of the 2-nitrophenylhydrazine moiety) ppm. ^{13}C NMR (DMSO-d_6): δ 155.529, 155.036, 148.315, 137.906, 133.081, 126.679, 126.352, 125.480, 125.063, 122.150, 119.153, 114.328, 113.107, 112.196, and 111.916 ppm (15 carbon signals for 15 different carbon atoms in **11**). Anal. Calcd for $C_{19}H_{15}N_4O_6$: C, 65.33; H, 4.30; N, 12.03. Found: C, 65.06; H, 4.49; N, 11.92.

4,4′-Dihydroxybenzophenonephenylhydrazone (12) was prepared by method A from 4,4′-dihydroxybenzophenone and phenylhydrazine·HCl by substituting glacial AcOH for H_2SO_4. The yield was 65%, and the product was crystallized from glacial AcOH as deep-red crystals, mp 185−187 °C. ^1H NMR (DMSO-d_6): δ 9.762 (1H, broad singlet, one of the OH groups), 9.566 (1H, broad singlet, other of two OH groups), 8.440 (1H, s, NH), 7.299 (2H, d, $J = 8.7$ Hz, 2H), 7.159 (4H, d, $J = 4.2$ Hz), 7.097 (2H, d, $J = 8.4$ Hz), 6.950 (2H, d, $J = 9$ Hz), 6.731 (2H, d, $J = 9$ Hz), and 6.683 (1H, t, $J = 4.2$ Hz) ppm. ^{13}C NMR (DMSO-d_6): δ 154.193, 153.924, 142.078, 140.738, 126.748, 126.668, 125.218, 124.091, 119.853, 115.092, 112.636, 111.482, and 109.171 ppm (13 signals for 13 different carbons of **12**). Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.26; N, 9.20. Found: C, 74.52; H, 5.37; N, 9.02.

4-Hydroxyacetophenone-4-nitrophenylhydrazone (13) was prepared by method A from 4-hydroxyacetophenone and 4-nitrophenylhydrazine in 82% yield. Deep-red crystals were crystallized from EtOH, mp 184−185 °C. ^1H NMR (DMSO-d_6): δ 10.062 (1H, s, NH), 9.750 (1H, broad singlet, OH), 8.094 (2H, d, $J = 6.6$ Hz), 7.678 (2H, d, $J = 6.6$ Hz), 7.291 (2H, d, $J = 6.6$ Hz), 6.820 (2H, d, $J = 6.6$ Hz), and 2.253 (3H, s, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 154.846, 148.155, 143.186, 134.667, 125.950, 123.910, 122.483, 111.794, 108.312, and 9.896 ppm (10 signals for 10 different carbons of **13**). Anal. Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 59.57; H, 5.02; N, 14.73.

4,4′-Diaminobenzophenone-2,4-dinitrophenylhydrazone (14) was prepared by method A from 4,4′-diaminobenzophenone and 2,4-dinitrophenylhydrazine in MeOH with 10 mL of concentrated H_2SO_4. The mixture was refluxed for 3 h and allowed to cool. The MeOH was flash-evaporated under reduced pressure, resulting in an orange solid. The solid was added to 100 mL of cold water, and the mixture was stirred in an ice/water bath. A solution of KOH (15 g in 50 mL of water) was added until pH ∼3.5 was attained. The fluffy, purple precipitate was filtered, washed with 100 mL of cold water, and allowed to air-dry. The yield of **14** was 98%, mp 268−270 °C. ^1H NMR (DMSO-d_6): δ 11.29 (s, 1H, N−H), 8.81 (s, 1H), 8.35 (d, 1H, $J = 8.8$ Hz), 8.12 (d, 1H, $J = 9.6$ Hz), 7.36 (dd, 2H, $J = 8.4$ Hz), 7.05 (dd, 2H, $J = 8.4$ Hz), 6.78 (dd, 2H, $J = 8.4$ Hz), 6.59 (dd, 2H, $J = 8.4$ Hz). ^{13}C NMR (DMSO-d_6): δ 153.4, 146.2, 145.2, 139.9, 132.4, 126.0, 125.7, 124.7, 120.9, 119.3, 114.9, 112.6, 111.0, and 109.9 ppm. Anal. Calcd for $C_{19}H_{16}N_6O_4$: C, 58.16; H, 4.11; N, 21.42. Found: C, 58.19; H, 4.17; N, 21.51.

4,4′-Dimethylaminobenzophenone-2,4-dinitrophenylhydrazone (15) was synthesized from the respective hydrazine and ketone using the procedure for the synthesis of **14**. The yield of **15** was 90%, mp 263−264 °C. ^1H NMR (DMSO-d_6): δ 11.29 (s, 1H, N−H), 8.81 (s, 1H), 8.35 (d, 1H, $J = 8.8$ Hz), 8.12 (d, 1H, $J = 9.6$ Hz), 7.36 (dd, 2H, $J = 8.4$ Hz), 7.05 (dd, 2H, $J = 8.4$ Hz), 6.78 (dd, 2H, $J = 8.4$ Hz), 6.59 (dd, 2H, $J = 8.4$ Hz) ppm. ^{13}C NMR (DMSO-d_6): δ 153.4, 146.2, 145.2, 139.9, 132.4, 126, 125.7, 124.7, 120.9, 119.3, 114.9, 112.6, 111.0, and 109.9 ppm. MS (electrospray), *m*/*z*: 449 $(M + H)^+$. Anal. Calcd for $C_{23}H_{24}N_6O_4$: C, 61.60; H, 5.39; N, 18.74. Found: C, 61.65; H, 5.43; N, 18.77.

4,4′-Diethylaminobenzophenone-2,4-dinitrophenylhydrazone (16) was synthesized from the respective hydrazine and ketone using the procedure for the synthesis of **14.** The yield was 98%, mp 268−270 °C. ^1H NMR (DMSO-d_6): δ 11.51 (s, 1H, N−H), 9.07 (s, 1H), 8.27 (dd, 1H, $J = 8$ Hz), 8.16 (dd, 1H, $J = 8$ Hz), 7.58 (dd, 2H, $J = 8.8$ Hz), 7.20 (dd, 2H, $J = 8.0$ Hz), 6.81 (dd, 2H, $J = 8.4$ Hz), 6.65 (dd, 2H, $J = 8.8$ Hz), 3.44 (m, 8H, $J = 7.2$ Hz), 1.23 (12H, m, $J = 6.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 158.1, 149.4, 149, 144.6, 136.9, 130.3, 130.2, 129.7, 128.7, 124.2, 117.8, 118.8, 111.9, 110.9, 44.6, and 12.8 ppm. MS (electrospray), *m*/*z*: 506.7 $(MH)^+$. Anal. Calcd for $C_{27}H_{32}N_6O_4$: C, 64.27; H, 6.39; N, 16.66. Found: C, 64.22; H, 6.40; N, 16.59.

4,4′-Diacetamidobenzophenone-2,4-dinitrophenylhydrazone (17) was prepared from **14** (0.522 g, 1.33 mmol) and 25 mL of Ac_2O. The mixture was refluxed for 1.5 h and cooled

to room temperature. The resulting orange crystals were washed with cold water (5 × 50 mL) and allowed to air-dry. The yield was 98%, mp 276−278 °C. ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H, N−H), 10.29 (s, 1H, N−H), 10.17 (s, 1H, N−H), 8.77 (s, 1H), 8.37 (d, 1H, J = 9.6 Hz), 8.16 (d, 1H, J = 9.6 Hz), 7.87 (dd, 2H, J = 8 Hz), 7.63 (dd, 2H, J = 8.4 Hz), 7.55 (dd, 2H, J = 8.8 Hz), 7.36 (dd, 2H, J = 8.4 Hz), 2.12 (s, 3H, CH_3), 2.05 (s, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 169.5, 169.2, 155.2, 144.6, 141.8, 141.5, 137.7, 131.4, 130.6, 129.9, 129.6, 128.9, 126.0, 123.4, 120.2, 119.1, 117.1, 24.6, and 24.5 ppm. MS (electrospray), *m/z*: 476.8 $(MH)^+$. Anal. Calcd for $C_{23}H_{20}N_6O_6$: C, 57.98; H, 4.23; N, 17.64. Found: C, 58.01; H, 4.31; N, 17.70.

2,7-Dihydroxy-9-fluorenone-2,4-dinitrophenylhydrazone (18) was prepared from 2,7-dihydroxy-9-fluorenone and 2,4-dinitrophenylhydrazine using method A. A reddish powdery product was purified by crystallization from glacial AcOH; yield 78%, mp >325 °C (dec). IR (KBr): 3445 (N−H), 2924 (Ar−H), 2854 (Ar−H), 1615 (C═N), 1585, 1507, 1424, 1335, 1308, 1269, 1144, 1117, 1081, 834, and 738 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.64 (1 H, s, N−H), 9.87 (1 H, s, O−H), 9.57 (1 H, s, O−H), 8.77 (1 H, s), 8.36 (1 H, dd, J = 8 Hz), 8.11 (1 H, dd, J = 8 Hz), 7.45 (1 H, dd, J = 8 Hz), 7.40 (1 H, s), 7.35 (1 H, dd, J = 8 Hz), and 7.12 (1H, s). Anal. Calcd for $C_{19}H_{12}N_4O_6$: C, 58.17; H, 3.08; N, 14.28. Found: C, 56.34; H, 2.82; N, 13.76.

1-Hydroxy-9-fluorenone-2,4-dinitrophenylhydrazone (19) was prepared from 1-hydroxy-9-fluorenone and 2,4-dinitrophenylhydrazine using method A. A reddish powdery product (90% yield) was crystallized from glacial AcOH, mp >270 °C (dec). IR (KBr): 3338 (N−H), 2927 (Ar−H), 2855 (Ar−H), 1617 (C═N), 1508, 1427, 1336, 1318, 1142, 1116, 835, and 739 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.69 (1 H, s, N−H), 10.1 (1 H, s, O−H), 9.77 (1 H, s, O−H), 8.75 (1 H, s), 8.34 (1 H, dd, J = 8 Hz), 8.12 (1 H, dd, J = 8 Hz), 7.92 (1 H, s), 7.69 (3 H, m), 7.59 (1 H, dd, J = 8 Hz), 7.52 (1 H, dd, J = 8 Hz), 7.47 (1 H, s), 7.33 (2 H, m), 7.2 (1 H, t, J = 8 Hz), 7.14 (1 H, s), 6.95 (1 H, dd, J = 8 Hz), 6.78 (1H, dd, J = 8 Hz) ppm. Anal. Calcd for $C_{19}H_{12}N_4O_5$: C, 60.64; H, 3.21; N, 14.89. Found: C, 60.64; H, 3.10; N, 14.64.

2,7-Diacetyl-9-fluorenone-2,4-dinitrophenylhyrazone (20) was prepared through acetylation of **18** with Ac_2O/NaOAc in 75% yield. A solid yellow product was recrystallized from glacial AcOH, mp 295−296 °C. IR (KBr): 3324 (N−H), 3097 (O−H), 2953 (Ar−H), 2825 (Ar−H), 2854 (Ar−H), 1760 (C═O), 1614 (C═N), 1583, 1507, 1422, 1338, 1207, 1123, 914, 834, and 527 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.97 (1 H, s, N−H), 8.86 (1 H, s), 8.47 (1 H, d, J = 6 Hz), 8.38 (1 H, s), 8.24 (1 H, d, J = 12 Hz), 8.17 (2 H, m), 8.12 (2 H, m), 7.89 (4 H, m), and 7.75 (3 H, m) ppm. Anal. Calcd for $C_{23}H_{16}N_4O_8$: C, 57.99; H, 3.39; N, 11.76. Found: C, 58.02; H, 3.26; N, 11.80.

9-Xanthenone-2,4-dinitrophenylhydrazone (21) was prepared from 9-xanthenone and 2,4-dinitrophenylhydrazine using method A**.** A deep-yellow product was crystallized from EtOH (yield 85%), mp 276−277 °C. IR (KBr): 3266 (N−H), 2926 (Ar−H), 2854 (Ar−H), 1619 (C═N), 1505, 1454, 1425, 1334, 1311, 1127, 1082, 907, 772, 752, and 620 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.51 (1 H, s, N−H), 8.89 (1 H, s), 8.53 (1 H, d, J = 6 Hz), 8.42 (1 H, d, J = 6 Hz), 8.29 (2 H, d, J = 6 Hz), 8.24 (2 H, d, J = 6 Hz), 7.72 (2 H, d, J = 6 Hz), 7.58 (2 H, d, J = 6 Hz), 7.48 (2 H, d, J = 6 Hz), and 7.41 (2 H, t, J = 6 Hz) ppm.[4]

***N*-(3,6-Dimethoxyxanthen-9-ylidene)-*N*′-(2,4-dinitrophenyl)hydrazone (22).** 3,6-Dimethoxyxanthenone was prepared by refluxing an acetone solution of 3,6-dihyroxy-9-xanthenone (7.4 mmol), dimethyl sulfate (28 mmol), and potassium carbonate (1.6 mmol) for 6 h. The mixture was evaporated to dryness, and the resulting reddish brown powder of 3,6-dimethoxy-9-xanthenone was crystallized from dioxane. The 3,6-dimethoxy-9-xanthenone (0.5 mmol) and 2,4-dinitrophenylhydrazine (0.5 mmol) in DEP (16 mL) were reacted using method A with a 78% yield of analogue **22**, mp 288−289 °C. IR (KBr): 3318 (N−H), 3105 (O−H), 2925 (Ar−H), 2853 (Ar−H), 1617 (C═N), 1586, 1503, 1462, 1414, 1345, 1294, 1256, 1180, 1132, 850, and 740 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.97 (1 H, s, N−H), 8.86 (1 H, s), 8.47 (1 H, d, J = 6), 8.38 (1 H, s), 8.24 (1 H, d, J = 12 Hz), 8.17 (2 H, m), 8.12 (2 H, m), 7.89 (4 H, m), and 7.75 (3 H, m) ppm. Anal. Calcd for $C_{21}H_{16}N_4O_7$: C, 57.80; H, 3.70; N, 12.84. Found: C, 57.92; H, 3.68; N, 12.83.

***N*-Benzyl-*N*-(2,4-dinitrophenyl)-*N*′-xanthen-9-ylidenehydrazone (23)** was synthesized by our reported procedure[4] with a yield of 50%, mp 219−220 °C. ^{1}H NMR ($CDCl_3$): δ 4.9 (s, 2H, N−CH_2−). Anal. Calcd for $C_{26}H_{18}N_4O_5$: C, 66.95; H, 3.89; N, 12.01. Found: C, 67.05; H, 3.87; N, 12.03.

9-Sulfoxyxanthenone-2,4-dinitrophenylhydrazone (24) was synthesized from 9-sulfoxyxanthenone and 2,4-dinitrophenylhydrazine in 90% yield using method A. The yellow crystalline product was crystallized from EtOH, mp 248−250 °C. IR (KBr): 3290 (N−H), 3286 (N−H), 2926 (Ar−H), 2855 (Ar−H), 1615 (C═N), 1590, 1506, 1334, 1101, 1037, 787, and 551 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.97 (1 H, s, N−H), 8.86 (1 H, s), 8.47 (1 H, d, J = 6 Hz), 8.38 (1 H, s), 8.24 (1 H, d, J = 12 Hz), 8.17 (2 H, m), 8.12 (2 H, m), 7.89 (4 H, m), and 7.75 (3 H, m) ppm.[4]

10-[(2,4-Dinitrophenyl)hydrazono]-1,2-dihydroxy-10*H*-anthracen-9-one (25) was prepared from 1,2-dihydroxyanthraquinone and 2,4-dinitrophenylhydrazine by method A, with the exception that the reaction mixture was refluxed for 48 h. After evaporation of the solvent, the solid residue was crystallized from dioxine/hexane to afford a pure product in 92% isolated yield, mp 240−242 °C. IR (KBr): 3502 (N−H), 3287 (N−H), 3113 (O−H), 2925 (Ar−H), 2559 (Ar−H), 1618 (C═N), 1595, 1501, 1441, 1335, 1298, 1135, 1091, and 781 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.97 (1 H, s, N−H), 8.86 (1 H, s), 8.47 (1 H, d, J = 6 Hz), 8.38 (1 H, s), 8.24 (1 H, d, J = 12 Hz), 8.17 (2 H, m), 8.12 (2 H, m), 7.89 (4 H, m), and 7.75 (3 H, m) ppm. Anal. Calcd for $C_{20}H_{12}N_4O_7$: C, 57.15; H, 2.88; N, 13.33. Found: C, 56.58; H, 2.99; N, 12.81.

10-[(2,4-Dinitrophenyl)hydrazono]-1,8-dihydroxy-10*H*-anthracen-9-one (26) was prepared in 90% yield from 1,8-dihydroxyanthraquinone (0.5 mmol) and 2,4-dinitrophenylhydrazine (0.5 mmol) by method A (using H_2SO_4 as a catalyst). The orange crystalline product was purified by recrystallization from MeOH, mp 284−285 °C (dec). IR (KBr): 3326 (N−H), 3275 (N−H), 3064 (O−H), 2951 (Ar−H), 2921 (Ar−H), 2852 (Ar−H), 1634 (C═N), 1618 (C═N), 1596, 1504, 1418, 1342, 1320, 1219, 1168, 1102, 985, 830, 741, and 589 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.93 (1 H, s, N−H), 7.82 (2 H, t, J = 6 Hz), 7.72 (2 H, d, J = 6 Hz), and 7.39 (2 H, d, J = 6 Hz) ppm. Anal. Calcd for $C_{20}H_{13}N_3O_5$: C, 64.00; H, 3.46; N, 11.20. Found: C, 63.98; H, 3.46; N, 11.13.

10-[2,4-Dinitrophenyl)hydrazono]-1,8-acetoxy-10*H*-anthracen-9-one (27) was prepared from analogue **26** with Ac_2O/NaOAc and recrystallized from EtOH or AcOH as a yellow-orange powder; yield 85%, mp 278−279 °C. IR (KBr): 3219 (N−H), 3102 (N−H), 2928 (Ar−H), 2860 (Ar−H), 1775 (C═O), 1666 (C═N), 1599, 1501, 1421, 1329, 1201, 1014, 883, 839, 583, and 589 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.05 (1 H, s, N−H), 8.88 (1 H, s), 8.45 (2 H, q, J = 6 Hz), 8.31 (1 H, d, J = 6 Hz), 8.23 (1 H, d, J = 6 Hz), 7.96 (1 H, t, J = 6 Hz), 7.82 (1 H, t, J = 6 Hz), 7.35 (1 H, d, J = 6 Hz), 2.36 (3 H, s-CH_3), and 2.34 (3 H, s-CH_3) ppm. Anal. Calcd for $C_{24}H_{16}N_4O_9$: C, 57.15; H, 3.20; N, 11.11. Found: C, 57.13; H, 3.09; N, 11.08.

10-[(2,4-Dinitrophenyl)hydrazono]-1-acetoxy-8-hydroxy-10*H*-anthracen-9-one (28) was synthesized from analogue **27**. To a solution of acetobromoglucose, 1.7 g (4.2 mmol) and TMSOTf (1.8 g, 8 mmol) in 100 mL of CH_2Cl_2, under a nitrogen atmosphere at 0 °C, **27** (1 g, 2 mmol) in 50 mL of CH_2Cl_2 was slowly added (over 15 min). Stirring continued for another 90 min, and the reaction was quenched with saturated $NaHCO_3$ (14 g and 140 mL of water) at 5 °C. The solid material was filtered, and the CH_2Cl_2 layer was separated, washed with a saturated $NaHCO_3$ solution, and dried. Analogue **28** was purified by Al_2O_3 column chromatography with CH_2Cl_2 as a solvent. The above reaction does not require acetobromoglucose; however, an improved yield results; 82%, mp 224−225

°C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.93 (1 H, s, N−H), 7.82 (2 H, t, J = 6 Hz), 7.72(2 H, d, J = 6 Hz), and 7.39 (2 H, d, J = 6 Hz) ppm. Anal. Calcd for $C_{22}H_{14}N_4O_8$: C, 57.15; H, 3.05; N, 12.12. Found: C, 57.32; H, 3.18; N, 11.93.

10-[(2,4-Dinitrophenyl)hydrazono]-1-acetoxy-8-allyloxy-10*H*-anthracen-9-one (29). A solution of CH_2Cl_2 (50 mL) containing analogue **28** (0.89 g, 2 mmol), allyl chloride (0.39 g, 2 mmol), and TEA (0.6 g, 6 mmol) was stirred at room temperature overnight. The solvent was evaporated, and the solid residue was crystallized from dioxane/hexane to afford pure **29** in 62% yield, mp 245−247 °C. IR (KBr): 3300 (N−H), 2924 (Ar−H), 767 (C═O), 1744 (C═O), 1669 (C═N), 1612 (C═N), 1601, 1506, 1410, 1339, 1199, 1138, 966, 833, and 792 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.05 (1H, s, N−H), 8.88 (1H, s), 8.47 (2H, m, J = 6 Hz), 8.32 (1H, d, J = 6 Hz), 8.24 (1H, t, J = 6 Hz), 7.97 (1H, q, J = 6 Hz), 7.82 (1H, q, J = 6 Hz), 7.55 (1H, dd, J = 6 Hz), 7.38 (1H, dd, J = 6 Hz), 6.5 (3H, m), 6.23 (1H, m), 2.26 (3H, s), and 2.25 (3H, s) ppm. Anal. Calcd for $C_{25}H_{16}N_4O_9$: C, 58.13; H, 3.10; N, 10.85. Found: C, 58.00; H, 3.10; N, 10.91.

2,6-Dihydroxy-9,10-[di-(2,4-dinitrophenylhydrazono)]-9*H*,10*H*-anthracenene (30) was prepared as red crystals from 2,6-dihydroxyanthraquinone and 2,4-dinitrophenylhydrazine (2 equiv) in 75% yield following method A. The product was purified by crystallization from MeOH, mp >348 °C (dec). IR (KBr): 3441 (N−H), 3290 (N−H), 2949 (Ar−H), 2922 (Ar−H), 2858 (Ar−H), 1672 (C═N), 1611 (C═N), 1517, 1422, 1332, 1128, 828, and 575 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.99 (1H, s, N−H), 11.94 (1H, s), 10.81 (1H, s-OH), 10.58 (2H, s), 0.22 (1H, s-OH), 8.88 (2H, s), 8.47 (2H, d, J = 8 Hz), 8.18 (2H, d, J = 8 Hz), 7.62 (2H, d, J = 8 Hz), 7.06 (2H, d, J = 8 Hz), 2.89 (3H, s), and 2.73 (3H, s) ppm. Anal. Calcd for $C_{26}H_{16}N_8O_{10}$: C, 52.00; H, 2.66; N, 18.66. Found: C, 51.25; H, 2.59; N, 18.05.

1,2-Diamino-10-[(2,4-dinitrophenyl)hydrazono]-10*H*-anthracen-9-one (31) was prepared in 80% yield from 1,2-diaminoanthraquinone (2 equiv) and 1 equiv of 2,4-dinitrophenylhydrazine by a slight modification of method A. EtOH, instead of MeOH, was the solvent, and the catalyst used was phosphoric acid instead of sulfuric acid. The reaction mixture was refluxed for 10 h. Red crystals formed from EtOH, mp >250 °C. IR (KBr): 3479 (N−H), 3389 (N−H), 3298 (N−H), 3104 (N−H), 2957 (Ar−H), 2930 (Ar−H), 2858 (Ar−H), 1615 (C═N), 1585, 1503, 1421, 1333, 1312, 1128, 826, and 570 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.93 (2H, s), 8.87 (2H, s), 8.43 (2H, d, J = 9 Hz), 8.13 (2H, d, J = 9 Hz), 7.98 (2H, d, J = 9 Hz), 7.39 (1H, s), 6.79 (2H, d, J = 9 Hz), and 6.14 (1H, s) ppm. Anal. Calcd for $C_{20}H_{14}N_6O_5$: C, 57.41; H, 3.34; N, 20.09. Found: C, 57.51; H, 3.29; N, 19.79.

2,6-Diamino-9,10-[di-(2,4-dinitrophenyl)hydrazono)]-9*H*,10*H*-anthracene (32) was prepared in 70% yield from 2,6-diaminoanthraquinone (1 equiv) and 2,4-dinitrophenylhydrazine (2 equiv) by slight modification of method A. EtOH was used as solvent, and phosphoric acid was used as an acid catalyst. The reaction mixture was refluxed for 24 h. The resulting product upon cooling was purified by crystallization from EtOH, mp 328−330 °C. IR (KBr): 3479 (N−H), 3389 (N−H), 3298 (N−H), 3104 (N−H), 2957 (Ar−H), 2930 (Ar−H), 2858 (Ar−H), 1615 (C═N), 1585, 1503, 1421, 1333, 1312, 1128, 826, and 570 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.93 (2H, s), 8.87 (2H, s), 8.43 (2H, d, J = 9 Hz), 8.13 (2H, d, J = 9 Hz), 7.98 (2H, d, J = 9 Hz), 7.39 (1H, s), 6.79 (2H, d, J = 9 Hz), 6.14 (1H, s) ppm. Anal. Calcd for $C_{26}H_{18}N_{10}O_8$: C, 52.17; H, 3.01; N, 23.40. Found: C, 51.88; H, 3.03; N, 22.66.

***N*-(2*H*-Dibenzo[*cd*,*g*]indazol-6-ylidene)-*N*′-(2,4-dinitrophenyl)hydrazone (33).** 2*H*-Dibenzo[*cd*,*g*]indazol-6-one was prepared from a pyridine (150 mL) solution of 1-chloroanthraquinone (50 mmol) and 1 equiv of hydrazine monohydrate by refluxing for 6 h. The solution was poured into water, and the yellow product was filtered and crystallized from chlorobenzene, mp 281−282 °C. Analogue **33** was prepared from 2*H*-dibenzo[*cd*,*g*]indazol-6-one and 2,4-dinitrophenylhydrazine in 62% yield by a slight modification of method A. The solvent used was diethyl phosphite, and the reactants were heated at 100 °C for 3 h, mp 330−331 °C. IR (KBr): 3341 (N−H), 3260 (N−H), 3110 (N−H), 2926 (Ar−H), 2851 (Ar−H), 1611 (C═N), 1582, 1503, 1414, 1332, 1254, 1125, 1060, 864, 767, and 667 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.56 (1H, NH), 12.06 (1H, NH), 8.88 (1H, s), 8.62 (1H, d, J = 8 Hz), 8.45 (1H, d, J = 8 Hz), 8.32 (2H, m), 8.14 (1H, d, J = 8 Hz), 7.85 (1H, d, J = 8 Hz), 7.76 (1H, t, J = 8 Hz), 7.64 (1H, t, J = 8 Hz), 7.56 (1H, t, J = 8 Hz). Anal. Calcd for $C_{20}H_{12}N_6O_4$: C, 60.00; H, 3.02; N, 21.00. Found: C, 60.10; H, 2.97; N, 20.99.

12-[2,4-Dinitrophenylhydrazono]-6-hydroxy-12*H*-naphthacen-5-one (34). Analogue **34** was synthesized in 71% yield from 6-hydroxynaphthacene-5,12-dione and 2,4-dinitrophenylhydrazine (1:1 ratio) by a slight modification of method A. The reaction mixture was refluxed for 48 h, and the red precipitate was washed with $CHCl_3$, mp 261−262 °C (dec). IR (KBr): 3307 (N−H), 3265 (N−H), 3099 (N−H), 2920 (Ar−H), 2855 (Ar−H), 1617 (C═N), 1584, 1506, 1420, 1338, 1282, 1137, 1074, 1040, 872, 757, and 601 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.81 (1H, s), 8.45 (1H, d, J = 8 Hz), 8.37 (1H, d, J = 8 Hz), 8.29 (2H, t, J = 8 Hz), 8.26 (1H, s), 8.13 (1H, s), 8.06 (2H, d, J = 8 Hz), 7.98 (2H, t, J = 8 Hz), 7.86 (4H). Anal. Calcd for $C_{24}H_{14}N_4O_6$: C, 63.44; H, 3.11; N, 12.33. Found: C, 63.56; H, 3.11; N, 12.14.

11-Acetoxy-2-phenyl-2*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (35). 11-Hydroxy-2-phenyl-2*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione was prepared from a pyridine solution (10 mL) of 8-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-carboxylic acid (1.5 mmol) and phenylhydrazine (1.5 mmol) by was refluxing for 30 min. The reaction mixture was cooled to room temperature, and the precipitate was separated by filtration and washed with 50 mL of water and 25 mL of EtOH. The IR spectral analysis of the solid product indicated the absence of −C═O vibration at 1705 cm^{-1} present in starting material. The yield of the crystalline product was 92%, mp 288−290 °C. The hydroxycinnolinedione was dissolved in a mixture of Ac_2O (4 mL) and NaOAc (0.16 g) and stirred at room temperature for several hours. The solvent was evaporated at reduced pressure, and the solid residue was washed with water, 10% $NaHCO_3$, water, and EtOH to afford analogue **35** as a dark powder in 55% yield, mp 254−255 °C. IR (KBr): 3073 (N−H), 2951 (Ar−H), 2923 (Ar−H), 2855 (Ar−H), 1744 (C═O), 1677 (C═N), 1667 (C═N), 1590, 1460, 1333, 1227, 1133, 783, and 699 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73 (2H, q, J = 8 Hz), 8.33 (1H, d, J = 8 Hz), 8.19 (2H, t, J = 8 Hz), 7.79 (2H, t, J = 8 Hz), 7.62 (1H, m), and 1.6 (3H, s) ppm. Anal. Calcd for $C_{23}H_{14}N_2O_4$: C, 72.25; H, 3.69; N, 7.33. Found: C, 72.30; H, 3.61; N, 7.34.

2-(2-Nitrophenyl)-2*H*-indeno[1,2,3-*de*]phthalazin-3-one (36) was prepared by a modified procedure of Campbell et al.[12] 9-[(2-Nitrophenyl)hydrazono]-9*H*-fluorene-1-carboxylic acid was prepared from a pyridine solution (20 mL) of 9-oxo-9*H*-fluorene-1-carboxylic acid (0.02 mol) and 2-nitrophenylhydrazine (0.02 mol) by refluxing for 4 h. The reaction mixture was poured into ice/water, and the precipitate was filtered, washed with water (50 mL), and EtOH (50 mL). 9-[(2-Nitrophenyl)hydrazono]-9*H*-fluorene-1-carboxylic acid was obtained in 85% yield, mp >220 °C (dec). Thionyl chloride (0.13 mmol) and the carboxylic acid (0.01 mol) were refluxed for 1.5 h. Excess thionyl chloride was removed at reduced pressure, and the oily residue was mixed with ice/water (50 mL). A precipitate formed which was collected by filtration, washed with water (50 mL) and 20% $NaHCO_3$ (10 mL), and dried to afford analogue **36** in 85% yield, mp 220−222 °C. IR (KBr): 3071 (N−H), 3041 (N−H), 2919 (Ar−H), 2886 (Ar−H), 1689 (C═N), 1645 (C═N), 1509, 1359, 1094, 974, 852, 777, and 716 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.32 (1H, d, J = 8 Hz), 8.20 (1H, d, J = 8 Hz), 8.05 (1H, d, J = 8 Hz), 7.99 (2H, m, J = 4 Hz), 7.95 (1H, d, J = 8 Hz), 7.92 (3H, m), 7.86 (1H, d, J = 8 Hz), 7.78 (1H, t, J = 12 Hz), 7.57 (1H, t, J = 8 Hz), 7.47 (1H, t, J = 8 Hz). Anal. Calcd for $C_{20}H_{11}N_3O_3$: C, 70.58; H, 2.94; N, 12.35. Found: C, 70.58; H, 3.10; N, 12.43.

2. Biological Studies. In Vitro Therapeutic Index Data. Cytotoxicity for the synthesized hydrazones was evaluated in a human explant system (CYTOTAC).[14] Selected

analogues were also evaluated in human and murine xenograft cell lines: FDCP-1, MDA-MB-435, MDA-MB-435/lung met, KM12 C, KM12 SM, KM12 L4, SN12 PM6, and B16 F10 (ATCC, Manassas, VA).

a. Permanent Murine Hematopoietic Progenitor (PMHP) Cell Lines. The FDCP-1 cell line was available in the laboratory of one of the authors (Dr. Dominic Fan). IL-3, necessary for cell growth, was obtained from conditioned medium of murine WEHI-3 myelomonocytic leukemia cell lines.[16,18]

b. Cell Lines and Culture Conditions. The following cell lines were purchased from ATCC (American Tissue Culture Collection, Gathersburg, MD) or made available by one of the authors (D.F.): MDA-MB-435 human breast carcinoma (also lung metastasis variant), SN12 M6 renal cell cancer, KM12 SM/L4 colon cancers, K1735 M2 human melanoma, and B16 F10 murine melanoma. They were maintained in serial culture in RPMI-1640 media (BioCell) supplemented with 10% fetal calf serum, 100 mcg/mL streptomycin, and 100 units/mL penicillin at 36 °C and 10% CO_2 (moist).

c. Assay for in Vitro Antiproliferative Effects in Human Tumor Cell Lines. The cancer cell lines were suspended in medium and seeded at 2000−3000 cells/well in 96-well tissue culture plates. The cells were fed with fresh medium (controls) or with medium containing different concentrations of the hydrazones after 18 h. After an additional 36 h, antiproliferative activity was determined by monitoring the number of viable cells. This was accomplished using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue)) assay.[15−17] Growth inhibition was calculated from the formula

$$\text{cytostasis (\%)} = \left(1 - \frac{A}{B}\right) \times 100$$

where A is the absorbance at 570 nm of treated cells and B is the absorbance at 570 nm of control cells.

d. Assay for in Vitro Myelotoxicity.[5,15] FDCP-1 cells were harvested during exponential growth. The hydrazone analogues and controls were each tested at 5−10 concentrations, covering a 1 log to 3 log concentration range (to determine IC_{50} data for hydrazones compared to doxorubicin (DOX) and/or cisplatinum (*cis*-DDP)). DOX and *cis*-DDP dose ranges were 0.3−0.75 mcg/mL. After 36−48 h of incubation, an amount of 0.2 mg (40 μL of 5 mg/mL) of MTT was added to each well, and cells were incubated at 37 °C for an additional 2 h. By use of the MTT assay, absorbance levels from drug-tested cells were compared with untreated control absorbance values.[5,15] The data were fitted to a second-order quadratic equation, and the IC_{50} value was calculated using the coefficients of a fitted curve.[5]

e. Human Tumor Explants.[14] Under sterile conditions, specimens of viable cancer tissue were transferred (in RPMI from Sigma Chemical Co.) from surgery to DEKK-TEC where they were minced into fragments no larger than 0.1 mm. The fragments were suspended in 16 mL of RPMI-1640 medium containing 10% fetal bovine serum (Biocell Co.), 100 mcg/mL streptomycin (Sigma Chemical Co.), and 100 U/mL penicillin (Sigma Chemical Company). The cancer tissue suspension density was 10^6−10^8 cells/mL. Cell counts and viability were determined with a density cytometer or MTT exclusion.[5]

An amount of 1 mL of the above suspensions was added to each of up to nine pairs of NUNC Slide Flasks (Nunc, Inc.) containing 5 mL of the above growth medium and control fluid or test agent at concentrations that will dilute to a concentration of one of the following test items: (a) control; (b) test compound (0.1−20 μg/mL), at least five doses (over 3 logs); (c) doxorubicin (0.1−5 μg/mL); (d) *cis*-DDP (1−10 μg/mL). Each was done in duplicate.

The flasks were incubated for 24 h in a 5% CO_2 incubator at 37 °C. The cells were washed with RPMI media and recultured in the above flasks. Sufficient cell growth (after 7−14 days) was present when there were at least 10 cells (or clumps of cells per HPF in the control). The medium was decanted, and the bottom slide was peeled from the flask. The slides were air-dried for 10 min and sprayed with a cytofixative (Pro-Fixx, Lerner Labs). The slides were examined and counted. The results were interpreted in the following fashion. (i) Scan high-power fields and count. For controls, 5−10 cells or clumps of cells per HPF in 5 HPF is considered as 100% growth. (ii) Average the growth results from duplicate flasks, and (iii) tabulate the percent growth vs test agents. (iii) Determine the IC_{50} (and SD) for A-007 and other agents vs control cancer cultures as per USP (111) or equivalent algorithm.[22]

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