mL) was added. The mixture was heated at reflux for 5 min, cooled to room temperature, and filtered. The filtrate was washed with water and brine, dried (Na_2SO_4), and evaporated. The residual material was chromatographed on silica gel with benzene–AcOEt (49:1) as eluent to give 368 mg (86%) of **2p** as yellow crystals. Recrystallization from ether–*n*-hexane gave pale yellow prisms: mp 132–133 °C; IR (KBr) 1616, 1591, 1560, 1481, 1414 cm^{-1}; NMR ($CDCl_3$) δ 2.10 (s, 3 H, CH_3), 2.28 (s, 3 H, CH_3), 5.07 and 3.94 (AB q, 2 H, J = 13.0 Hz, CH_2N), 5.97 (s, 1 H, H-2), 7.06 (m, 1 H, aromatic), 7.23–7.62 (m, 6 H, aromatic).

Pharmacology. Methods. CNS Actions. Male ddY mice weighing 18–24 g were used for all studies reported here. The test compounds were suspended in 5% arabic gum solution and administered orally to a group of six mice per dose. The compounds were examined suitably at three or four dosage levels selected from 100, 30, 10, 3, 1, 0.3, and 0.1 mg/kg. The ED_{50} values were calculated by the method of Litchfield–Wilcoxon.[21] Procedures for measuring the activities of the test compounds—antipentylenetetrazole activity, antimaximal electroshock activity, taming activity in fighting mice induced by electrofootshock, muscle relaxant activity using an inclined wooden board, motor incoordinating activity, and potentiation of thiopental—have been described previously.[1]

Acute Toxicity. Male ddY mice weighing 18–24 g were used. The test compounds were suspended in 5% arabic gum solution and administered orally to a group of four mice at a dosage of 1000 and 2000 mg/kg and to a group of eight mice at a dosage of 3000 mg/kg.

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References and Notes

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(18) Although the pure isomer was not obtained, data assignment was done by analysis of the spectral change through recrystallization.
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Synthesis and Antitumor Activity of Halogen-Substituted 4-(3,3-Dimethyl-1-triazeno)quinolines

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Halogenated 4-(3,3-dimethyl-1-triazeno)quinolines were synthesized as potential antitumor agents on the basis of the biochemical pharmacological properties of existing triazenes, their structural–activity relationships, and the high melanin binding of chloroquine and iodoquine in vivo and in vitro. They were synthesized by diazotization of appropriate halogen-substituted 4-aminoquinolines in fluoboric acid at −5 °C followed by coupling with dimethylamine. Among these new compounds, 8-chloro-4-(3,3-dimethyl-1-triazeno)quinoline produces significant antitumor activity against both P-388 and L1210 murine leukemias. Although only marginally active or inactive against P-388, the other chloro, bromo, or iodo analogues show activity against L1210 comparable to that of dacarbazine (DIC). However, none of these compounds is active against B-16 melanoma. Compared with DIC these new agents demonstrate a higher in vitro affinity for melanin; however, this affinity is apparently not correlated with their antitumor activity.

Although metastatic melanoma is only one of the many varieties of malignant tumors, its virulence and high mortality have stimulated studies over the ages. It comprises about 2% of all cancers, and about 80–90% of them arise in the skin.[1] 5-(3,3-Dimethyl-1-triazeno)-imidazole-4-carboxamide (DIC) is by far the most useful single agent against melanoma in man. However, it has produced only temporary responses in 19% of the patients with this disease.[2] The lack of effective agents against melanoma justifies the continuous search for better new agents. A large number of triazenes with diverse structures have been synthesized and tested in experimental systems.[3]

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Among them 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (BIC)[4-6] was able to cure mouse leukemia L1210. However, in the treatment of brain tumors,[7] childhood malignancy,[8] gastrointestinal cancer,[9] and metastatic malignant melanoma,[10] the results were disappointing. Though puzzling, the discrepancy in the antitumor effect of BIC between man and the mouse is not unique, since no experimental animal system reliably predicts clinical results. However, in the case of BIC, the absence of significant clinical antitumor activity is at least partly attributable to its extreme instability and susceptibility to internal alkylation in aqueous media at room temperature with the formation of an isomeric triazolinium salt.[5]

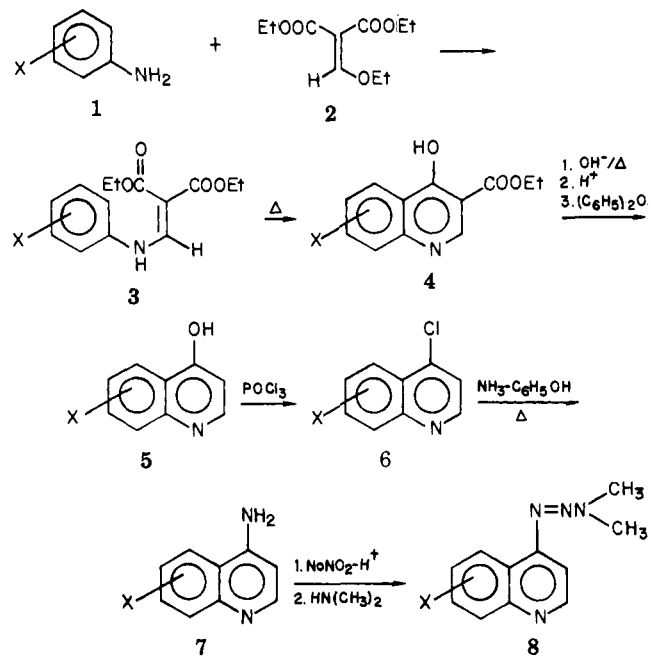
An understanding of the biochemical basis for the carcinostatic activity of this class of compounds is necessary for the rational design of clinically more effective agents. Although many questions remain unanswered at present, a general consensus regarding the possible mechanism of action of DIC has gradually emerged.[11-16] Two possible mechanisms of action of DIC have been suggested. These involve (a) the oxidative N-demethylation and subsequent decomposition of the monomethyltriazene to generate an unstable methyl carbonium ion in vivo, and (b) light-induced decomposition of DIC to 5-diazoimidazole-4-carboxamide (DZC) and dimethylamine. Both the methyl carbonium ion and DZC are highly reactive species which inhibit cell growth by alkylating DNA.

Structure–activity relationship studies of a large number of triazenes have indicated that the *N,N*-dimethyl function of triazenes is essential,[3] but the imidazole ring of DIC can be replaced by a phenyl ring[17-19] or heterocyclic rings such as the pyrazole[20-22] or *v*-triazole[23] ring without loss of activity. Based on these findings, it is possible that *N,N*-dimethyltriazenes with a heterocyclic ring having high affinity for melanin may be particularly effective against melanotic melanoma.

Among heterocyclic compounds, chloroquines[24-28] and chlorpromazine[29] showed marked affinity for pigmented tissues. Potts[29] reported that several phenothiazine compounds localize in high concentrations in the uveal tract of pigmented but not albino animals; a reaction between these compounds and the uveal melanin was therefore postulated.[29] Subsequently, Potts[30] evaluated over 40 compounds for their ability to interact with melanin in aqueous suspension and found that polycyclic drugs such as quinolines, acridines, and phenothiazines were rapidly absorbed by melanin, whereas monocyclic compounds, such as pyridine, hydroquinone, and aliphatic compounds, were not. These studies have led Counsel[31,32] to synthesize radioiodinated quinoline derivatives as melanoma localizing agents. Among them, 4-(3-dimethylaminopropylamino)-7-[^{125}I]iodoquinoline was shown to concentrate in malignant melanoma of mice, Syrian hamsters, dogs, and man.[31,32] Recently, Adamson[33] has suggested that alkylating agents be designed utilizing carriers that are most likely to deliver the agents to specific target sites. He further suggested that by designing drugs with activity against specific types of tumors rather than with generalized cytotoxic agents, it should be possible to increase the therapeutic index of these agents. Therefore, *N,N*-dimethyltriazeno derivatives with a heterocyclic ring which has high affinity to melanin may be selectively active against the melanotic melanoma.

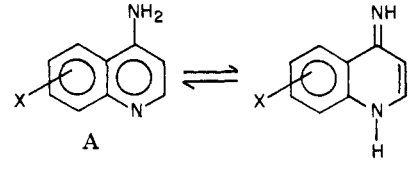
Accordingly, we have synthesized several halogen-substituted 4-(3,3-dimethyl-1-triazeno)quinolines and determined their antitumor activities against leukemias P-388, L1210, and B-16 melanoma. In addition, we determined the in vitro effect of melanin binding in relation to the efficacy of these drugs against experimental melanotic melanoma.

Scheme I



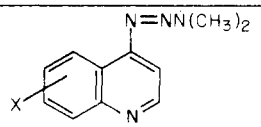
X = 6-, 7-, or 8-Cl, -Br, or -I

Chemistry. Procedures used by Price[34] or Surrey[35] for the preparation of 7-chloro-, 7-bromo-, or 7-iodo-4-chloroquinoline were adapted to prepare 6- or 8-halogeno-4-chloroquinoline. As shown in Scheme I, anilines **1** condensed smoothly with diethyl ethoxymethylenemalonate (**2**) to give ethyl α-carbethoxy-β-halogenated anilinoacrylates **3** in good yield. Heating of compound **3** in boiling diphenyl ether gave the cyclized products **4** with a yield of 60–80%. Hydrolysis of **4** with NaOH solution followed by decarboxylation in boiling diphenyl ether gave **5** in moderate yield. The conversion of the 4-hydroxyl to the 4-amino function was achieved by treatment of **5** with phosphorus oxychloride, followed by amination in phenol to give compound **7**. Compounds **7** showed absorption at 1670 cm^{-1} in the infrared region indicating that it was partly in the imino form B.



Although diazotization of aromatic amines is a common and routine reaction, the diazotization of 2- or 4-aminopyridines or 2- or 4-aminoquinolines is difficult.[36] For the diazotization of 4-aminoquinoline (**7**) the optimal procedure is to carry out the reaction in fluoboric acid at salted ice bath temperature. Since the diazonium salt is unstable, it was generated and used for coupling with dimethylamine in situ. The yield of the final step was 10–60%, depending upon the nature and position of the substituents. The NMR spectra indicate that the two methyl groups on N_3 of triazene are nonequivalent. They appeared as two broad singlets of equal intensity around δ 3.3, suggesting a restriction of free rotation between the N_2 and N_3 bond. This may be due to the partial double bond character

Table I. Antitumor Activity of Halogen-Substituted Triazenoquinolines against P-388 Leukemia



X	Dose, mg/kg[a]	% Δ wt[b]	% T/C[c]
6-Br	20	+18.8	123
	30	+10.8	127
	40	-3.5	111
	50	-8.9	131
7-Br	30	-2.4	133
	40	-8.9	133
	50	-15.1	125
8-Br	20	+16.0	109
	30	+4.8	113
	40	-3.3	125
	50	-5.0	117
6-Cl	10	-3.1	129
	20	+0.8	139
	30	-12.9	137
7-Cl	30	-16.3	137
	40	-22.6	119
8-Cl	20	-2.1	172
	30	-15.0	167
6-I	40	+10.5	117
	60	+2.6	133
	80	+7.1	123
7-I	20	+10.8	117
	40	+5.7	130
	60	-5.2	127
8-I	70	+8.2	120
	80	-2.4	120
	90	-1.6	116
DIC[d]	100		150

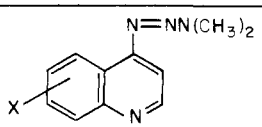
[a] Administered ip once daily for 9 days, beginning 24 h after tumor implantation. [b] The average body weight change at the end of drug treatment. [c] % T/C = (average survival of treated mice)/(average survival of control mice) × 100. [d] Data from Arthur D. Little.

between the N_2 and N_3 bond as a result of the extended conjugation of the triazene moiety and the quinoline ring.

Antineoplastic Activity. Agents synthesized in this study were tested for antineoplastic potency against P-388 and L1210 murine neoplasma in vivo. The NCI protocol was followed: groups of five to ten mice each were inoculated intraperitoneally with approximately 10^6 cells. Drugs were administered over a range of dose levels 24 h after tumor implantation and continued once daily for 9 consecutive days. The results (Table I) indicate that 8-chloro-4-(3,3-dimethyl-1-triazeno)quinoline produces significant antitumor activity against P-388 at its optimal dosage (20 mg/kg) with a % T/C of 172. The other chloro, bromo, or iodo analogues are marginally active or inactive under the same conditions. However, against L1210 these new triazenes showed antitumor activity (Table II) comparable to that of DIC, with % T/C ranging from 131 to 165. The 8-chloro analogue again showed significant activity against L1210. In view of the antitumor effect of 8-chloro-4-(3,3-dimethyl-1-triazeno)quinoline against both P-388 and L1210, the 8-chloro, 8-bromo, and 8-iodo derivatives were further screened against the B-16 melanoma together with DIC and BIC. The results in Table III indicate that they are inactive. However, DIC is also inactive, although BIC showed significant activity. Since DIC is clinically useful while BIC has little value in the treatment of human melanoma, the B-16 melanoma may not be an appropriate model for the human disease.

Melanin Binding. The affinity for melanin of these new triazines may be an important factor in their distribution and specificity against melanotic melanoma. We have therefore studied the relative in vitro melanin binding of these compounds. The results in Table IV show that the halogenated 4-(3,3-dimethyl-1-triazeno)quinolines synthesized by us were significantly bound to melanin after incubation for 15 min in vitro. In contrast, DIC was only

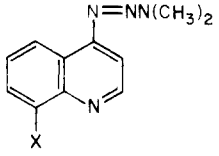
Table II. Antitumor Activity of Halogen-Substituted Triazenoquinolines against L1210 Leukemia



X	Dose, mg/kg[a]	% Δ wt[b]	% T/C[c]
6-Br	30	-1.0	150
	40	-5.8	154
7-Br	30	+10.0	129
	40	-2.8	141
8-Br	30	+6.1	137
	40	-5.9	144
6-Cl	20	+1.0	146
	30	-10.0	156
7-Cl	20	+5.1	154
	30	-1.0	152
8-Cl	30	0	165
	40	-4.9	155
6-I	70	-23.8	148
	80	-8.2	160
	90	-6.4	158
7-I	50	+4.9	107
	60	+2.9	131
8-I	70	+7.7	127
	80	-6.9	129
	90	-7.0	139
DIC[d]	100		167

[a] Administered ip once daily for 9 days, beginning 24 h after tumor implantation. [b] The average body weight change at the end of drug treatment. [c] % T/C = (average survival of treated mice)/(average survival of control mice) × 100. [d] Data from Arthur D. Little.

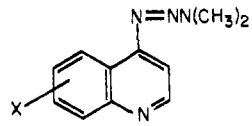
Table III. Antitumor Effects of Triazenes against B-16 Melanoma



Compd, X	Dose,[a] mg/kg	Δ wt[b]	Av survival, days	% T/C[c]
Control			21.0	100
Cl	50.0	-3.9	20.8	99
	30.0	-2.9	21.3	101
	18.0	-0.3	21.6	103
Br	66.7	-3.7	21.3	101
	40.0	-3.0	19.0	90
	24.0	-1.1	20.3	97
1	116.7	-4.6	19.3	92
	70.0	-1.5	20.8	99
	42.0	-1.3	20.0	97
DIC	360	-3.0	16.0	76
	216	-3.2	22.8	109
	130	-2.9	22.0	105
BIC	216	-3.7	15.3	73
	130	-2.9	32.8	156
	77.8	-2.3	25.0	119

[a] Administered ip once daily for 9 days, beginning 24 h after tumor implantation. [b] The average body weight change at the end of drug treatment. [c] % T/C = (average survival of treated mice)/(average survival of control mice) × 100.

Table IV. Melanin Binding Affinity of Halogen-Substituted Triazenoquinolines



X	% binding	X	% binding
6-Cl	41.0	7-Br	57.3
7-Cl	48.0	8-Br	48.1
8-Cl	56.4	6-I	60.6
6-Br	55.3	DIC	19.3

19% bound under similar conditions. No correlation, however, is apparent between their antitumor effect against B-16 and the melanin binding affinity.

Experimental Section

Synthesis. All melting points were taken on a calibrated Thomas-Hoover capillary melting point apparatus. Analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Spectral data were obtained using Perkin-Elmer Infrared Model 727B and Varian T-60A spectrometers. The latter used Me_4Si as an internal standard. The NMR and IR spectra were as expected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within 0.4% of the theoretical values.

(I) Preparation of 6-, 7-, or 8-Halogeno-3-carbethoxy-4-hydroxyquinolines (4). A mixture of 2-, 3-, or 4-halogenoaniline (0.1 mol) and ethyl ethoxymethylenemalonate (0.1 mol) was stirred at room temperature until homogeneous. The homogeneous solution was heated in an oil bath at 100 °C for 2.5 h. A stream of nitrogen gas was passed through the mixture to aid in the removal of ethanol during the reaction. The molten acrylate was poured slowly through the top of an air condenser into 100 mL of boiling diphenyl ether. The mixture was refluxed for 2 h and cooled to room temperature. Petroleum ether (100 mL) was added to the semisolid mass. The precipitated product was collected and washed twice with 50-mL portions of petroleum ether. The product was used for further reaction without purification.

(II) Preparation of 6-, 7-, or 8-Halogeno-4-hydroxyquinolines (5). 6-, 7-, or 8-halogeno-3-carbethoxy-4-hydroxyquinoline (0.05 mol) was refluxed in a 10% sodium hydroxide solution (100 mL) for 1–2 h until the solution became clear. The solution was allowed to cool to room temperature and acidified with 18% HCl solution. The precipitate was collected, washed with water, and dried. The decarboxylation was carried out by refluxing the carboxylic acid in diphenyl ether until effervescense ceased. After cooling, equal volumes of petroleum ether were added to the reaction mixture, and the white precipitate was collected after trituration. The product was washed several times with petroleum ether, dried, and subjected to chlorination without further purification. These halogenated 4-hydroxyquinolines are insoluble in $CHCl_3$ and water but soluble in hot aqueous sodium hydroxide solution.

(III) Preparation of 6-, 7-, or 8-Halogeno-4-chloroquinolines (6). 6-, 7-, or 8-halogeno-4-hydroxyquinoline (0.01 mol) was refluxed in 20 mL of $POCl_3$ for 2 h. The phosphorus oxychloride was removed under reduced pressure, and the residue was poured into ice water. The mixture was made alkaline with NH_4OH and extracted twice with methylene chloride. The extracts were combined, washed once with H_2O, dried over sodium sulfate, and evaporated to dryness. The product was recrystallized from either ethanol or a mixture of ethyl acetate and petroleum ether. The physical properties of these compounds are shown in Table V.

(IV) Preparation of 6-, 7-, or 8-Halogeno-4-aminoquinolines (7). 6-, 7-, or 8-halogeno-4-chloroquinoline (0.05 mol) and phenol (80 g) were heated in an oil bath to 170 °C in a three-neck flask equipped with a stirrer, a reflux condenser, and a thermometer with a gas inlet tube. Ammonia gas was bubbled through the mixture at a moderate rate. The temperature of the oil bath was raised to 200 °C and the passage of ammonia was allowed to continue for 2.5 h. After cooling to room temperature, a solution of 75 mL of glacial acetic acid in 150 mL of H_2O was added to dissolve the solidified mixture. Anhydrous ether (500 mL) was added to the solution to precipitate the aminoquinoline hydrochloride which was collected and washed with anhydrous ether. The filtrate and etheral washings were combined and extracted with H_2O (2 × 150 mL). The aqueous extracts were basicified with 20% NaOH solution to precipitate the halogenated 4-aminoquinoline. The hydrochlorides of 4-aminoquinolines were converted to free bases by dissolving in 300–400 mL of hot H_2O and basicified with an excess of NaOH. The precipitate was collected, washed with H_2O, and recrystallized from aqueous EtOH. The physical properties of these compounds are shown in Table VI.

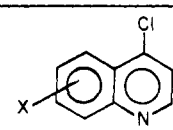
(V) Preparation of 6-, 7-, or 8-Halogeno-4-(3,3-dimethyl-1-triazeno)quinolines (8). 6-, 7-, or 8-halogeno-4-aminoquinoline (0.01 mol) in 30 mL of 48% fluoboric acid was cooled in a salted ice bath to −5 °C. To the suspension was added dropwise an aqueous solution of $NaNO_2$ (2 g in 4 mL of H_2O) over a period of 1 h. After the addition of $NaNO_2$, the reaction mixture was stirred at ice-cold temperature for another 1 h. Dimethylamine (40%, 6 mL) was added dropwise to the reaction mixture, which was alkalinized with 5 N NaOH solution. The reaction mixture was stirred for an additional 30 min, diluted with an equal volume of water, and extracted three times with EtOAc. The EtOAc extracts were combined and washed with H_2O twice, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel column chromatography. The physical properties of these compounds are listed in Table VII.

Determination of Antineoplastic Effect. Compounds prepared in this study were tested for the antineoplastic effect against P-388, L1210, and B-16. NCI protocol was followed during the study. The results are shown in Tables I–III.

Melanin-Binding Study in Vitro. 1. Preparation of Synthetic Melanin. Synthetic melanin was prepared from DL-β-3,4-dihydroxyphenylalanine (Dopa) by the action of tyrosinase as follows. Dopa (100 mg, Nutritional Biochemicals) and 4 mg of tyrosinase (Worthington Biochemical Co., 500 units/mg) were dissolved in 30 mL of 0.1 M phosphate buffer, pH 6.6, and shaken in a water bath at 37 °C for 4 h. The resultant black precipitate was collected by centrifugation and washed three times by resuspending in distilled water and centrifuging. The yield was 90–95%.

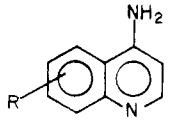
2. Binding. The method of Potts[30] was adapted and modified in this study. In a 25-mL Erlenmeyer flask was placed in 1 mL

Table V. Physical Properties of 4-Chloroquinolines



R	Formula	Recrystn solvent	Mp, °C	% yield	Analyses
6-I	C_9H_5ClIN	EtOH	138–139.5	72	C, H, N
6-Br	C_9H_5BrClN	EtOH	111–112	28	C, H, N
6-Cl	$C_9H_5Cl_2N$	EtOH and H_2O	104–105	50	C, H, N
8-Cl	$C_9H_5Cl_2N$	EtOH and H_2O	155–156	90	C, H, N
8-Br	C_9H_5BrClN	EtOH and H_2O	147–148	45	C, H, N
8-I	C_9H_5ClIN	EtOH	104–105	50	C, H, N

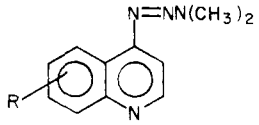
Table VI. Physical Properties of 4-Aminoquinolines



R	Formula	Mp, °C[a]	% yield	Analyses
6-Cl	$C_9H_7ClN_2$	232–233	75	C, H, N
7-Cl	$C_9H_7ClN_2$	146–147	67	C, H, N
8-Cl	$C_9H_7ClN_2$	195–196	63	C, H, N
6-Br	$C_9H_7BrN_2$	233–234	84	C, H, N
7-Br	$C_9H_7BrN_2$	166–168	92	C, H, N
8-Br	$C_9H_7BrN_2$	187–188	96	C, H, N
6-I	$C_9H_7IN_2$	220–222	85	C, H, N
7-I	$C_9H_7IN_2$	196–197	82	C, H, N
8-I	$C_9H_7IN_2$	168–169	93	C, H, N

[a] Solvents for recrystallization in all cases are EtOH + H_2O.

Table VII. Physical Properties of 4-(3,3-Dimethyl-1-triazeno)quinolines



R	Formula	Mp, °C[a]	% yield	Analyses
6-Cl	$C_{11}H_{11}N_4Cl$	106–108	34	C, H, N
7-Cl	$C_{11}H_{11}N_4Cl$	95–97	20	C, H, N
8-Cl	$C_{11}H_{11}N_4Cl$	115–117	56	C, H, N
6-Br	$C_{11}H_{11}N_4Br$	119–120	40	C, H, N
7-Br	$C_{11}H_{11}N_4Br$	102–104	10	C, H, N
8-Br	$C_{11}H_{11}N_4Br$	115–117	40	C, H, N
6-I	$C_{11}H_{11}N_4I$	126–127	14	C, H, N
7-I	$C_{11}H_{11}N_4I$	132–134	19	C, H, N
8-I	$C_{11}H_{11}N_4I$	131–133	40	C, H, N

[a] General solvents for recrystallization are petroleum ether and ethyl acetate.

of a melanin suspension containing 1.1 mg of melanin (by dry weight), 2 mL of phosphate buffer (0.05 M, pH 6.4, plus 5% Me_2SO), 2 mL of water, and 1 mL of a drug solution containing 5.8 mg of the drug in 100 mL of phosphate buffer (0.05 M, pH 6.4, plus 5% Me_2SO). The suspension was shaken at room temperature for 15 min and filtered to remove the melanin. A portion of the filtrate was removed for determination of unbound drug by the UV spectroscopic method. The reference assay sample was prepared by the same procedure without the drug but substituted with 1 mL of phosphate buffer. The results are shown in Table IV.

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