# Synthesis and Antitumor Activity of New Retinobenzoic Acids

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New retinobenzoic acid derivatives have been synthesized starting from 1,2,3,4-tetrahydro-1,1,2,4,4,6-hexamethylnaphthalene and 1,1,2,3,3-pentamethylindane. Four of the synthetic compounds displayed potent cytotoxic activities *in vitro* against human breast cancer and leukemic cell lines. Thus, these molecules can be further evaluated for the treatment of human leukemia and breast cancer.

**Introduction.** – Retinoic acid (**1** (all-*E*)) and its analogs (retinoids) modulate various biological functions such as cell differentiation, proliferation, and embryonic development in vertebrates. The most important activities of retinoids are certainly the effects on the differentiation and proliferation of many types of cells and include the treatment of the neoplastic disorders [1–4]. The (all-*E*)-retinoic acid (**1**) and *Isotretinoin*® (**2**; *Fig.*) have revolutionized the treatment of acute promyelocytic leukemia (APL) by causing terminal differentiation of the malignant cells [5]. Further, the inhibitory effect of retinoids on IL-6 production suggests their possible usefulness in various IL-6-associated diseases including psoriasis and rheumatoid arthritis. Some synthetic retinoids have been successfully used in the treatment of certain dermatological conditions such as acne, psoriasis, eczema, and photo aging of skin [6][7].

The molecular basis for the activity of retinoic acid has been the subject of considerable research. A major breakthrough was the discovery of the nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which have (all-*E*)- and (9*Z*)-retinoic acids as ligand molecules, respectively [8]. Retinoids bind to these proteins, then the ligand/protein complex binds to DNA, and the transcription of the retinoid responsive genes is activated (or depressed). Three forms of the RARs and RXRs have been termed as $RAR_\alpha$, $RAR_\beta$, and $RAR_\gamma$, and $RXR_\alpha$, $RXR_\beta$, and $RXR_\gamma$ which belong to the steroid/thyroid nuclear subfamily and differ in their tissue distribution. It is generally accepted that biological activities of retinoids are mediated by RAR-RXR heterodimers, whose subunits follow a defined hierarchy of ligand responsiveness. Various compounds with selective affinities for these receptors have been reported.

Despite several beneficial properties, the use of available retinoids is associated with a number of significant side-effects [9]. For example, systemic use of clinically available retinoids such as isotretinoin causes an elevation of triglyceride levels in *ca.* 1/3 of the patients treated. Therefore, synthesis of potent new compounds with retinoidal activities should facilitate the application of retinoids to medicinal and

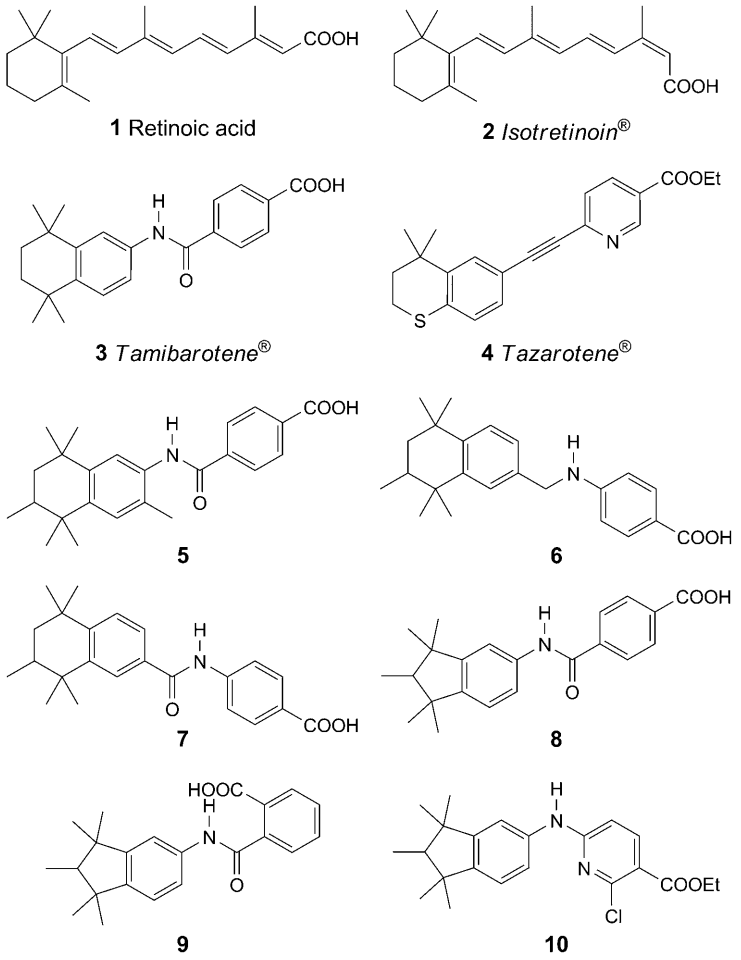



Figure. Tamibarotene® *analogs*

chemotherapeutic areas. *Tamibarotene*® (*AM 80*; **3**) [10–14] and *Tazarotene*® (**4**) [15] are synthetic retinobenzoic acid derivatives with considerable activity against acute promyelocytic leukemia (APL). *AM 80* has recently been approved for relapsed or refractory APL treatment in Japan. It is chemically more stable and several times more potent as an inducer of differentiation in promyelocytic leukemia cells. Furthermore, in clinical trials of *AM 80*, adverse side-effects were milder than those of (all-*E*)-retinoic acid (**1**). *AM 80* is being investigated for the treatment of multiple myeloma and *Crohn*'s disease in clinical trials [13]. The combination of *AM 80* and glucocorticoids was found to have synergistic growth inhibitory effect in human myeloma cells [16].

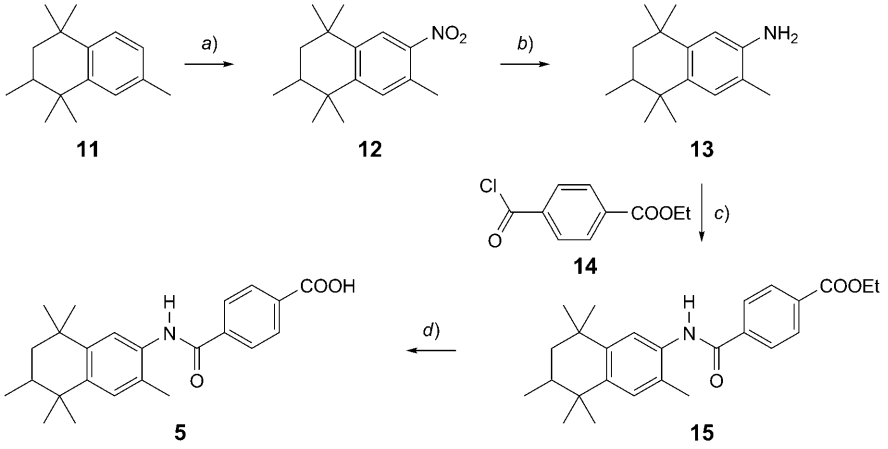
The present study involves the synthesis and evaluation of the antitumor activity of *AM 80* analogs, with the following structural modifications: *i*) additional Me group in ring *A*; *ii*) incorporation of 1,1,2,3,3-pentamethylindane moiety in place of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene; *iii*) incorporation of ethyl nicotinate in

place of benzoic acid; *iv*) replacement of –NH–CO– linkage between two aromatic residues with the $–CH_2–NH–$ group. We have achieved the synthesis of new retinobenzoic acid derivatives **5**–**10** starting from easily available 1,2,3,4-tetrahydro-1,1,2,4,4,6-hexamethylnaphthalene and 1,1,2,3,3-pentamethylindane. The antitumor activity has been studied against six important human breast cancer, leukemia, and T-lymphoblast cell lines.

**Results and Discussion.** – The retinobenzoic acids **5**–**10** investigated in the present study are shown in the *Figure*. The starting materials, namely 1,2,3,4-tetrahydro-1,1,2,4,4,6-hexamethylnaphthalene (**11**), and 1,1,2,3,3-pentamethylindane (**19**), were subjected to a series of reactions as described in *Schemes 1–3* to obtain the target retinobenzoic acids **5**–**10**.

The synthesis of 4-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphthalen-2-ylcarbamoyl)benzoic acid (**5**) was achieved through a parallel reaction sequence as reported for the synthesis of **3** and its analogs [3][10]. Thus, the nitration of 1,2,3,4-tetrahydro-1,1,2,4,4,6-hexamethylnaphthalene (**11**) yielded the mononitro derivative **12**, which was hydrogenated to the amine **13**. The latter was condensed with terephthalic acid monochloride monoester (**14**) leading to the ethyl ester **15**, which, on base hydrolysis with aqueous NaOH, gave the required acid **5** (*Scheme 1*).

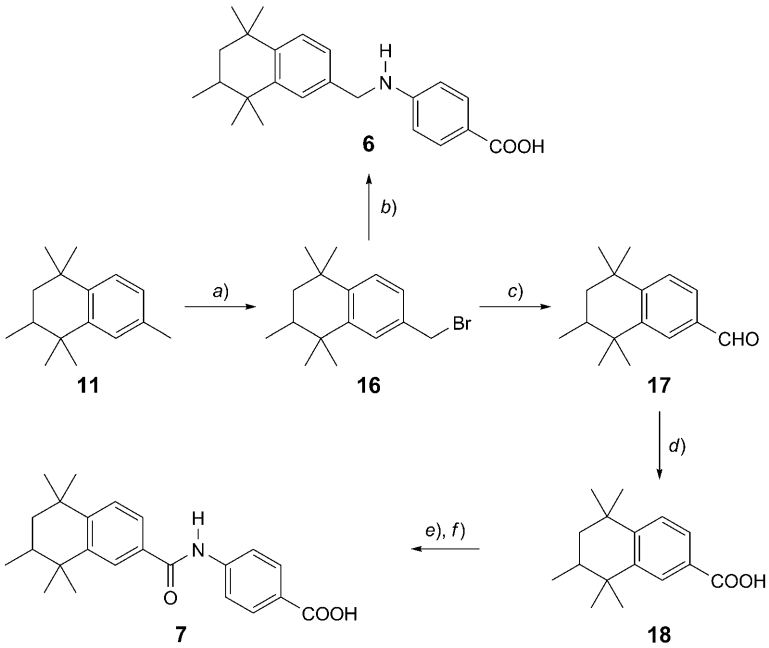
Scheme 1. *Synthesis of 4-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphthalen-2-ylcarbamoyl)benzoic Acid* (**5**)




*a*) $HNO_3$, $H_2SO_4$, 60–70°. *b*) $H_2$/Pd, EtOH, 50 psi, 40–50°. *c*) Pyridine. *d*) 10% NaOH, EtOH, 60–70°.

The benzylic bromination of **11** with *N*-bromosuccinimide (NBS) in $CCl_4$ gave the compound **16** [17], which was condensed with *p*-aminobenzoic acid (PABA) to afford the acid **6** (*Scheme 2*). The compound **16** was treated with hexamethylenetetramine (=1,3,5,7-tetraazatricyclo[3.3.1.1$^{3,7}$]decane; HMT) to yield the aldehyde **17** [18], which was further oxidized with $KMnO_4$ to give the acid **18**. The acid was converted to aminocarbonyl derivative **7** by the treatment with $SOCl_2$, followed by the condensation with PABA in the presence of pyridine.

Scheme 2. *Synthesis of 4-[(5,6,7,8-Tetrahydro-5,5,6,8,8-pentamethylnaphthalen-3-yl)methylamino]benzoic Acid* (**6**) *and 4-[(5,6,7,8-Tetrahydro-5,5,6,8,8-pentamethylnaphthalen-3-yl)carbonylamino]benzoic Acid* (**7**)



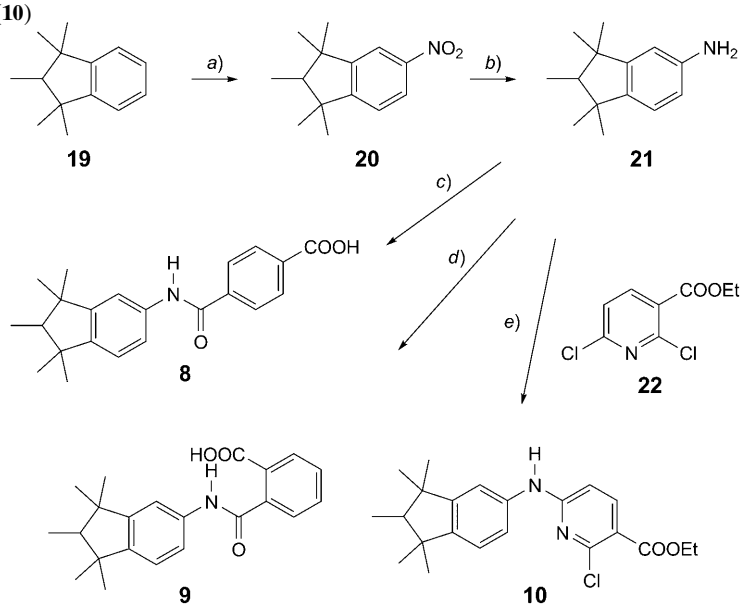

*a*) *N*-Bromosuccinimide (NBS), $CCl_4$, reflux. *b*) *p*-Aminobenzoic acid (PABA), acetone, anh. $K_2CO_3$, reflux. *c*) Hexamethylenetetramine 1,3,5,7-tetraazatricyclo[3.3.1.1$^{3,7}$]decane; HMT, $CHCl_3$, reflux, 50% gl. AcOH and HCl. *d*) $KMnO_4$, $H_2O$, 70–80°. *e*) $SOCl_2$, 45–50° *f*) PABA, pyridine.

1,1,2,3,3-Pentamethylindane (**19**) was nitrated to give the mononitro derivative **20**, which, on hydrogenation, gave amine **21** (*Scheme 3*). The latter was condensed with terephthalic acid monochloride monoester (**14**) to yield the amide, which was hydrolyzed with aqueous NaOH to 4-[(1,1,2,3,3-pentamethylindan-5-yl)carbamoyl]-benzoic acid (**8**). Similarly, the treatment of **21** with phthalic anhydride gave the 2-[(1,1,2,3,3-pentamethylindan-5-yl)carbamoyl]benzoic acid (**9**). Finally, ethyl 2-chloro-6-[(1,1,2,3,3-pentamethylindan-5-yl)amino]nicotinate (**10**) was obtained by the condensation of **21** with ethyl 2,6-dichloronicotinate (**22**) in the presence of NaH [19].

*Biology.* The retinobenzoic acid analogues **5**–**10** were evaluated *in vitro* against various tumor cell lines using SRB assay. The cell lines were grown in *RPMI 1640* medium containing 10% fetal bovine serum and 2 mM L-glutamine. The dose–response parameters were calculated for each test sample. Growth inhibition of 50%, $IC_{50}$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the incubation, was calculated for each sample in triplicate, and the mean values are given in *Tables 1* and *2*.

*Structure–Activity Studies.* Compound **7**, with an additional Me group at C(6) as compared to *AM 80* and isomeric amide linkage, showed appreciable activity against human erythroleukemia K562, T-lymphoblast-*Jurkat*, and human breast cancer Zr-75-1

Scheme 3. *Synthesis of 4- or 2-(2,3-Dihydro-1,1,2,3,3-pentamethyl-1*H*-inden-5-ylcarbamoyl)benzoic Acids* (**8** and **9**, resp.) *and Ethyl 2-Chloro-6-{[2,3-dihydro-1,1,2,3,3-pentamethyl-1*H*-inden-5-yl]amino}-nicotinate* (**10**)




*a*) $HNO_3$, $H_2SO_4$, 60–70°. *b*) $H_2$, Pd/C, 50 psi, EtOH, 40–50. *c*) *i*) **14**, pyridine; *ii*) NaOH, EtOH. *d*) NaH, phthalic anhydride, THF, reflux. *e*) NaH, THF, reflux.

and MC-F7 cell lines. Compound **5**, containing two additional Me groups at C(3) and C(6), compared to *AM 80*, displayed better activity against breast cancer MC-F7 cell line. Compound **8**, the 1,1,2,3,3-pentamethylindane analog, showed potent activity against breast cancer MC-F7 cell line, and finally, compound **10** showed appreciable inhibitory activity against human leukemia HL-60 and U937 cell lines.

Table 1. *Antitumor Activity of Compounds* **5**–**10** *against Leukemia Cell Lines*

| Compound[c]) | % Inhibition as compared to control[a])[b]) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Erythroleukemia K 562 | | | | | Leukemia U 937 | | | | | Leukemia HL-60 | | | | |
| | 10 | 20 | 40 | 80 | $IC_{50}$[d]) | 10 | 20 | 40 | 80 | $IC_{50}$ | 10 | 20 | 40 | 80 | $IC_{50}$ |
| **5** | 24 | 41 | 58 | 72 | 30.8 | 27 | 36 | 48 | 53 | 55 | 11 | 20 | 34 | 44 | >80 |
| **6** | 4 | 17 | 48 | 58 | 49.6 | 21 | 46 | 52 | 54 | 33.5 | 2 | 18 | 45 | 52 | 64.8 |
| **7** | 38 | 66 | 84 | 78 | 15.2 | 31 | 44 | 52 | 61 | 34.5 | 11 | 28 | 43 | 49 | >80 |
| **8** | 40 | 39 | 60 | 74 | 30.2 | 28 | 24 | 41 | 71 | 30 | 16 | 36 | 48 | 60 | 47.6 |
| **9** | 18 | 22 | 57 | 69 | 36 | 13 | 27 | 38 | 44 | >80 | 0.0 | 14 | 29 | 39 | >80 |
| **10** | 22 | 35 | 51 | 75 | 38.5 | 35 | 65 | 68 | 67 | 15.5 | 16 | 49 | 64 | 83 | 22.5 |
| AM-80 | 19 | 37 | 48 | 52 | 56.5 | 23 | 53 | 71 | 77 | 19.4 | 35 | 45 | 60 | 80 | 26.5 |
| Dox[e]) | 75 | 52 | 57 | 68 | – | 70 | 63 | 72 | 77 | – | 80 | 71 | 68 | 66 | – |

[a]) All values are averages of triplicate readings. [b]) *In vitro* antitumor activity was evaluated using SRB assay. [c]) Concentration μg/ml. [d]) Concentration of drug required in μg/ml to reduce cell growth to 50% of that obtained with control cells. [e]) Doxorubicin.

Table 2. *Antitumor Activity of Compounds* **5**–**10** *against Lymphoma and Breast Cancer Cell Lines*

| Compound[c]) | % Inhibition as compared to control[a])[b]) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T-Lymphoblast-*Jurkat* | | | | | Breast cancer Zr-75-1 | | | | | Breast cancer MC F 7 | | | |
| | 10 | 20 | 40 | 80 | $IC_{50}$[d]) | 10 | 20 | 40 | 80 | $IC_{50}$ | 10 | 20 | 40 | 80 | $IC_{50}$ |
| **5** | 8.2 | 17 | 26 | 51 | 79 | 5.9 | 11 | 47 | 42 | >80 | 36 | 41 | 70 | 73 | 26.2 |
| **6** | 9.3 | 25 | 65 | 85 | 32.5 | 0.0 | 8 | 21 | 67 | 65 | 15 | 23 | 37 | 75 | 54.5 |
| **7** | 24 | 49 | 69 | 63 | 22 | 15 | 50 | 64 | 69 | 20 | 37 | 58 | 72 | 67 | 16.4 |
| **8** | 13 | 24 | 41 | 70 | 52 | 15 | 31 | 48 | 62 | 47 | 41 | 60 | 69 | 77 | 17.4 |
| **9** | 02 | 7.4 | 15 | 15 | NA | 0.4 | 3.3 | 16 | 56 | 73 | 20 | 35 | 36 | 76 | 54.5 |
| **10** | 19 | 29 | 32 | 33 | NA | 17 | 26 | 78 | 41 | 29 | 15 | 22 | 41 | 86 | 48 |
| AM-80 | 70.3 | 62 | 63 | 82 | <10 | 17 | 26 | 36 | 64 | 59 | 13 | 26 | 63 | 66 | 33.5 |
| Dox[e]) | 64 | 73 | 73 | 76 | <10 | 72 | 76 | 80 | 84 | – | 4 | 68 | 77 | 80 | – |

[a]) All values are averages of triplicate readings. [b]) *In vitro* antitumor activity was evaluated using SRB assay. [c]) Concentration μg/ml. [d]) Concentration of drug required in μg/ml to reduce cell growth to 50% of that obtained with control cells. [e]) Doxorubicin.

In summary, we have disclosed appreciable antitumor activity of new retinobenzoic acids against human leukemia and breast cancer cell lines. Thus, these molecules can be further evaluated for the treatment of human leukemia and breast cancer.

## Experimental Part

*Chemistry. General. 1,2,3,4-Tetrahydro-1,1,3,4,4,6-hexamethylnaphthalene* (**11**) and *1,1,2,3,3-pentamethylindane* (**19**) were obtained from *S. H. Kelkar & Company*, Mumbai. Products were obtained as colorless to pale yellow needles from AcOEt/petroleum ether (unless mentioned otherwise). Column chromatography (CC): silica gel ($SiO_2$; 100–200 mesh) was activated by heating at 120° for 4 h. M.p.: *Thermonik* cap. instrument; are uncorrected. IR Spectra: *Perkin-Elmer Spectrum One* spectrometer. $^1$H- and $^{13}$C-NMR spectra: *Varian* spectrometers at 400 and 75 MHz, resp., in $CDCl_3$ with TMS as internal standard. Elemental analyses: *Carlo Erba Strumentazione 1106* analyzer.

*Synthesis of 4-[(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethylnaphthalen-2-yl)carbamoyl]benzoic Acid* (**5**). To a mixture of cold conc. $HNO_3$ and $H_2SO_4$ (1:1, 9 ml), **11** (10.0 g, 0.04 mol) was added dropwise, and the resulting mixture was stirred at 0° for 10–15 min. The mixture was allowed to warm to r.t. and was then heated at 60–70° for 30 min. The dark soln. was poured into ice and extracted with AcOEt. The org layer was successively washed with $H_2O$, $NaHCO_3$, and brine, and dried ($Na_2SO_4$). Removal of the solvent *in vacuo* and recrystallization of the residue from toluene gave *1,2,3,4-tetrahydro-1,1,2,4,4,7-hexamethyl-6-nitronaphthalene* (**12**; 6.20 g, 50%; m.p. 110–120°). Compound **12** (5.0 g) was subjected to hydrogenation under $H_2$ (pressure of 50 psi) at 40° in the presence of Pd/C (0.5 g) in EtOH (300 ml). After 10 h, the mixture was filtered, and the solvent was evaporated *in vacuo* to give *5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphthalen-2-amine* (**13**; 3.2 g, 56%; m.p. 148–150°). A mixture of **13** (0.5 g) and *ethyl 4-(chlorocarbonyl)benzoate* (**14**; 0.65 g) in pyridine (2 ml) was stirred at 60–80° for 6 h. The solvent was removed, the residue was extracted with AcOEt, and the extract was washed with $H_2O$, 1M HCl, brine, and was dried ($Na_2SO_4$). The evaporation of the solvent gave **15** (0.38 g, 80%; m.p. 196–200°). The mixture of **15** (0.5 g) and 4N aq. NaOH (4.0 ml) in EtOH (10 ml) was heated to reflux for 6 h. The solvent was removed, the residue was diluted with AcOEt, and the soln. was washed with $H_2O$, dil. HCl, and brine, and dried ($Na_2SO_4$). After evaporation of the solvent, the residue was recrystallized from hexane and AcOEt to give **5** (0.37 g, 70%). M.p. 128–130°. IR (KBr): 3275, 2965, 1696, 1649. $^1$H-NMR: 9.23 (*s*, 1 H); 8.13, 8.04 (*AA′BB′*, $J_{AB}$ = 8.0, 4 H); 7.57 (*s*, 1 H); 7.40 (*s*, 1 H); 2.27 (*s*, 3 H); 1.89–1.66 (*m*, 2 H); 1.39 (*d*, *J* = 11.6, 1 H); 1.32 (*s*, 3 H); 1.29 (*s*, 3 H); 1.26 (*s*, 3 H); 1.25 (*s*, 3 H); 0.99 (*d*, *J* = 6.8, 3 H). $^{13}$C-NMR: 168.5; 167.4; 152.4; 146.4; 143.8; 132.9; 130.6; 129.2; 127.6; 121.3; 120.2; 117.5; 43.7; 37.5; 34.6;

34.4; 32.4; 32.1; 28.7; 25.0; 17.7; 16.8. Anal. calc. for $C_{24}H_{29}NO_3$ (379.49): C 75.95, H 7.71, N 3.69; found: C 75.75, H 7.51, N 3.39.

*Synthesis of 4-{[(5,6,7,8-Tetrahydro-5,5,7,8,8-pentamethylnaphthalen-2-yl)methyl]amino}benzoic Acid* (**6**). To a soln. of **11** (20.0 g, 0.09 mol) in $CCl_4$ (200 ml), *N*-bromosuccinimide (NBS; 20.0 g, 0.1 mol) was added in small portions, and benzoyl peroxide (0.2 g) was added to initiate the reaction. The mixture was refluxed for 8 h on a water bath. The resulting soln. was filtered *in vacuo*, and the filtrate on evaporation gave *7-(bromomethyl)-1,2,3,4-tetrahydro-1,1,2,4,4-pentamethylnaphthalene* (**16**; 36.7 g, 80%) [17]. To the mixture of **16** (5.0 g, 0.01 mol) and *p*-aminobenzoic acid (PABA; 3.0 g, 0.02 mol) in acetone (100 ml) anh. $K_2CO_3$ (6.0 g, 0.04 mol) was added. The resulting mixture was refluxed for 14 h. The solvent was evaporated *in vacuo*, and the residue was acidified with 1N HCl, extracted with AcOEt, and the org. layer was washed with $H_2O$ and dried (anh. $Na_2SO_4$). After evaporation of the solvent *in vacuo*, the crude product was purified by CC. The product was recrystallized from hexane and AcOEt to give **6** (3.7 g, 80%). M.p. 174–176°. IR (KBr): 3417, 3369, 2964, 1725, 1667, 1602. $^1$H-NMR: 7.95, 6.65 (*AA′BB′*, $J_{AB}$ = 8.8, 4 H); 7.31 (*dd*, *J* = 6.0, 1.0, 1 H); 7.29 (*d*, *J* = 1, 1 H); 7.12 (*d*, *J* = 6.0, 1 H); 4.32 (*s*, 2 H); 1.80–1.90 (*m*, 2 H); 1.63 (*t*, *J* = 7.2, 1 H); 1.30 (*s*, 3 H); 1.29 (*s*, 3 H); 1.25 (*s*, 3 H); 1.05 (*s*, 3 H); 0.98 (*d*, *J* = 6.8, 3 H). $^{13}$C-NMR: 172.5; 152.6; 146.6; 144.3; 135.1; 132.4; 127.0; 126.4; 124.9; 117.5; 113.8; 111.6; 47.8; 43.6; 37.8; 34.5; 34.2; 32.4; 32.0; 29.7; 28.6; 25.0; 16.9. Anal. calc. for $C_{23}H_{29}NO_2$ (351.48): C 78.61, H 8.31, N 3.98; found: C 78.24, H 7.95, N 3.75.

*Synthesis of 4-{[(5,6,7,8-Tetrahydro-5,5,7,8,8-pentamethylnaphthalen-2-yl)carbonyl]amino}benzoic Acid* (**7**). To a soln. of hexamethylenetetramine (= 1,3,5,7-tetraazatricyclo[3.3.1.1$^{3,7}$]decane, HMT; 12.9 g, 0.08 mol) in $CHCl_3$ (100.0 ml) **16** (20.0 g, 0.04 mol) [17] was added. The resulting mixture was refluxed for 2 h, and filtered and acidified with 50% AcOH (10.0 ml) and conc. HCl (5.0 ml). The resulting soln. was again refluxed for 2 h. Two layers were separated, the aq. layer was extracted with $CHCl_3$, the org. layer was neutralized with $Na_2CO_3$, washed with $H_2O$, dried ($Na_2SO_4$), and filtered. The solvent was evaporated from the filtrate *in vacuo* to give *5,6,7,8-tetrahydro-5,5,7,8,8-pentamethylnaphthalene-2-carbaldehyde* (**17**; 10.0 g, 68%) [18]. The soln. of **17** (5.0 g, 0.01 mol) in acetone (20 ml) was stirred with an aq. soln. of $KMnO_4$ (13.4 g, 0.02 mol) in $H_2O$ (50.0 ml) for 3 h at r.t. to give *5,6,7,8-tetrahydro-5,5,7,8,8-pentamethylnaphthalene-2-carboxylic acid* (**18**; 2.2 g, 40%). Then, **18** (2.0 g, 0.01 mol) was converted to the acid chloride by using $SOCl_2$ (5.0 ml, excess). The excess $SOCl_2$ was recovered by distillation. A mixture of acid chloride of **18** (1.5 g, 0.01 mol), PABA (0.75 g, 0.015 mol), and pyridine (3.0 ml) was stirred at r.t. for 8 h. Pyridine was recovered *in vacuo*, and the residue was dissolved in AcOEt. The org. layer was washed with $H_2O$ and 1N HCl and finally with brine, and was dried ($Na_2SO_4$). After evaporation of the solvent *in vacuo*, the crude product was purified by CC to give **7** (1.2 g, 76%). M.p. 234–236° (hexane and AcOEt). IR (KBr): 3379, 3295, 2960, 1709, 1650, 1595. $^1$H-NMR: 8.07, 7.82 (*AA′BB′*, $J_{AB}$ = 8.4, 4 H); 7.96 (*d*, *J* = 2.4, 1 H); 7.64 (*dd*, *J* = 8.4, 2.4, 1 H); 7.40 (*d*, *J* = 8.4, 1 H); 1.80–1.90 (*m*, 2 H); 1.60 (*t*, *J* = 7, 1 H); 1.39 (*s*, 3 H); 1.28 (*s*, 3 H); 1.25 (*s*, 3 H); 1.11 (*s*, 3 H); 1.0 (*d*, *J* = 6.8, 3 H). $^{13}$C-NMR: 167.5; 166.0; 146.4; 143.8; 130.8; 128.5; 126.6; 124.0; 119.7; 119.3; 43.1; 40.5; 39.9; 37.8; 34.5; 32.2; 28.4; 24.9; 16.6; 14.1. Anal. calc. for $C_{23}H_{27}NO_3$ (365.46): C 75.58, H 7.44, N 3.83; found: C 75.88, H 7.74, N 3.54.

*Synthesis of 4-[(2,3-Dihydro-1,1,2,3,3-pentamethyl-1*H*-inden-5-yl)carbamoyl]benzoic Acid* (**8**). To a mixture of cold conc. $HNO_3$ and $H_2SO_4$ (1:1; 60 ml) *1,1,2,3,3-pentamethyl-2,3-dihydro-1*H*-indene* (**19**; 50.0 g, 0.24 mol) was added dropwise, and the resulting mixture was stirred at 0° for 15 min. The mixture was allowed to warm to r.t. and was heated at 60–70° for 30 min. The dark soln. was poured into ice-$H_2O$ and extracted with AcOEt. The org. layer was successively washed with $H_2O$, $NaHCO_3$, and brine, and dried (anh. $Na_2SO_4$). After removal of the solvent *in vacuo*, the residue was recrystallized from hexane to give *1,1,2,3,3-pentamethyl-5-nitroindane* (**20**; 7.2 g, 60%, m.p. 88–90°). Then, **20** (5.0 g, 0.02 mol) was subjected to hydrogenation under pressure of 50 psi at 40° in the presence of Pd/C (0.5 g) in EtOH (100 ml). After 10 h, the mixture was filtered, and the solvent was evaporated to give *1,1,2,3,3-pentamethylindan-5-amine* (**21**; 2.7 g, 59%; m.p. 179–180°). The condensation of **21** with *terephthalic acid ethyl ester monochloride* (**14**) and saponification were carried out as described above to give **8**. IR (KBr): 3373, 3290, 2960, 1691, 1650, 1595. $^1$H-NMR: 8.14, 8.0 (*AA′BB′*, $J_{AB}$ = 8.4, 4 H); 7.51 (*d*, *J* = 8.0, 1 H); 7.35 (*s*, 1 H); 7.13 (*d*, *J* = 8.0, 1 H); 1.88 (*q*, *J* = 7.2, 1 H); 1.29 (*s*, 3 H); 1.27 (*s*, 3 H); 1.09 (*s*, 3 H); 1.06 (*s*, 3 H); 0.99 (*d*, *J* = 7.2, 3 H). $^{13}$C-NMR: 168.7; 167.6; 152.4; 148.3; 136.3; 131.2; 130.5; 128.9; 127.5; 127.1; 123.1;

119.4; 115.1; 54.5; 44.9; 44.5; 29.7; 29.1; 29.0; 25.8; 8.5. Anal. calc. for $C_{22}H_{25}NO_3$ (351.43): C 75.18, H 7.16, N 3.98; found: C 75.35, H 7.33, N 3.68.

*Synthesis of 2-[(2,3-Dihydro-1,1,2,3,3-pentamethyl-1*H*-inden-5-yl)carbamoyl]benzoic Acid* (**9**). To a suspension of NaH (0.9 g) in THF (25 ml), a soln. of **21** (1.2 g, 0.02 mol) in THF (50.0 ml) was added, and the mixture was stirred for 15 min, then phthalic anhydride (3.6 g, 0.04 mol) was added. The resulting mixture was refluxed for 6 h. THF was recovered, and the residue was diluted with AcOEt, the org. layer was washed with 1N HCl and brine, and dried ($Na_2SO_4$). The evaporation of the solvent *in vacuo* and purification of the residue by CC gave **9** (1.2 g, 70%). M.p. 158–160° (hexane AcOEt, 1:1). IR (KBr): 3252, 2955, 1699, 1660, 1598. $^1$H-NMR: 8.06 (*d*, *J* = 6.8, 1 H); 7.80 (br. *s*, 1 H); 7.55–7.64 (*m*, 2 H); 7.50–7.58 (*m*, 1 H); 7.38–7.42 (*m*, 2 H); 7.13 (*d*, *J* = 8.4, 1 H); 1.88 (*q*, *J* = 6.8, 1 H); 1.26 (*s*, 6 H); 1.07 (*s*, 3 H); 1.06 (*s*, 3 H); 0.99 (*d*, *J* = 6.8, 3 H). $^{13}$C-NMR: 167.6; 152.4; 151.4; 134.3; 131.0; 131.9; 131.2; 130.0; 125.0; 123.6; 123.3; 121.1; 54.3; 44.8; 44.7; 29.0; 28.9; 25.9; 25.8; 8.5. Anal. calc. for $C_{22}H_{25}NO_3$ (351.43): C 75.18, H 7.16, N 3.98; found: C 75.45, H 7.41, N 3.59.

*Synthesis of Ethyl 2-Chloro-6-[(1,1,2,3,3-pentamethyl-2,3-dihydro-1*H*-inden-5-yl)amino]pyridine-3-carboxylate* (**10**). To a mixture of **21** (0.8 g, 0.02 mol) and *ethyl 2,6-dichloropyridine-3-carboxylate* (**22**; 0.20 g, 0.02 mol) in THF (20.0 ml) was added NaH (0.10 g). The resulting mixture was refluxed on a water bath for 6 h, THF was recovered, the residue was diluted with AcOEt, and the org. layer was washed with 1N HCl and brine, and dried ($Na_2SO_4$). Evaporation of the solvent *in vacuo* gave the crude product, which was purified by CC to give **10** (0.68 g, 50%). M.p. 118–120° (MeOH/AcOEt 1:3). IR (KBr): 2979, 1694, 1585. $^1$H-NMR: 8.40 (*d*, *J* = 8.4, 1 H); 7.2 (*s*, 1 H); 7.12 (*d*, *J* = 8.0, 2 H); 7.0 (*d*, *J* = 8.4, 1 H); 4.68 (*q*, *J* = 6.8, 2 H); 1.86 (*q*, *J* = 6.8, 1 H); 1.54 (*t*, *J* = 6.8, 3 H); 1.50 (*s*, 3 H); 1.25 (*s*, 3 H); 1.24 (*s*, 3 H); 1.05 (*s*, 3 H); 0.98 (*d*, *J* = 6.8, 3 H). $^{13}$C-NMR: 164.5; 160.7; 153.4; 145.0; 131.0; 121.3; 118.1; 111.0; 65.0; 54.4; 44.4; 44.3; 29.7; 29.1; 28.9; 25.8; 14.3; 8.5. Anal. calc. for $C_{22}H_{27}ClN_2O_2$ (398.92): C 68.29, H 7.03, N 7.24; found: C 67.95, H 7.41, N 7.49.

*Biology. Antitumor Activity.* In vitro *Anti-Tumor Activity Assay* (SRB Assay) [20]. The cell lines were grown in *RPMI 1640* medium containing 10% fetal bovine serum and 2 mM L-Gln (source of cell lines: NCI, USA). The cells were inoculated into 96-well microtiter plates in 90 µl of medium and incubated at 37° (5% $CO_2$, 95% air, and 100% relative humidity) for 24 h prior to addition of compounds. After 24 h, one 96-well plate containing $5 \times 10^3$ cells/well was fixed *in situ* with $Cl_3CCOOH$ (TCA) to represent a measurement of the cell population at the time of drug addition (time-zero (Tz)). Experimental drugs were initially solubilized in DMSO at 100 mg/ml and diluted to 1 mg/ml with $H_2O$ and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400, and 800 µg/ml with complete medium containing test sample. Aliquots of 10 µl of these different compound dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentration, *i.e.*, 10, 20, 40, and 80 µg/ml, resp.

*Endpoint Measurement.* After compound addition, plates were incubated at standard conditions for 48 h, and the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30% (*w*/*v*) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°. The supernatant was discarded, and the plates were washed five times with tap $H_2O$ and air-dried. Sulforhodamine B (SRB) soln. (50 µl) at 0.4% (*w*/*v*) in 1% AcOH was added to each of the wells, and the plates were incubated for 20 min at r.t. After staining, unbound dye was recovered, and the residual dye was removed by washing five times with 1% AcOH. The plates were dried in air. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690-nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of test wells to the average absorbance of the control wells $\times 100$.

Using the six absorbance measurements (Tz, control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)), the percent growth was calculated at each of the drug concentration levels. The dose–response parameters were calculated for each test sample. Growth inhibition of 50% ($IC_{50}$) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the incubation.

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