# Synthesis and Evaluation of Novel Pyrimido-Acridone, -Phenoxadine, and -Carbazole as Topoisomerase II Inhibitors

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**As part of a series of studies to discover new topoisomerase II inhibitors, novel pyrimidoacridones, pyrimidophenoxadines, and pyrimidocarbazoles were synthesized, and *in vitro* and *in vivo* antitumor activities and DNA-protein and/or DNA-topoisomerase II cross-linking activity as an indicator of topoisomerase II-DNA cleavable complex formation were evaluated. The pyrimidocarbazoles possessed high *in vitro* and *in vivo* potencies. Compound 26 (ER-37326), 8-acetyl-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione, showed *in vitro* growth inhibitory activity with respective $IC_{50}$ values of 0.049 $\mu$M and 0.35 $\mu$M against mouse leukemia P388 and human oral cancer KB. *In vivo*, this compound inhibited the tumor growth of mouse sarcoma M5076 implanted into mice with T/C values of 42% and 13% at 3.13 and 6.25 mg/kg/d respectively without significantly affecting the body weight. In addition, compound 26 (ER-37326) increased the formation of DNA-topoisomerase II cross-linking in P388 cells.**

**Key words** topoisomerase II inhibitor; cleavable complex; solid tumor; pyrimidocarbazole

Topoisomerase II (Topo II)-inhibiting antineoplastic agents, such as etoposide and doxorubicin are among the most effective antitumor drugs currently available for the treatment of human cancers.[2—6] These agents are shown to induce the accumlation of DNA-topo II cleavable complex (cleavable complex) which causes tumor cell death.[7]

Etoposide (**1**), an extensively used clinical topo II inhibitor elicits significant antitumor activity against a wide variety of neoplasms, including germ cell malignancies, small cell lung cancer (SCLC), non-Hodgkin's lymphomas, leukemias, Kaposi's sarcoma, neuroblastoma and soft-tissue sarcomas.[2,3] Use of etoposide and cisplatin (or carboplatin) is the standard therapy for patients with SCLC.[4] Doxorubicin is a topo II inhibitor with various activities such as nuclear helicase inhibitory activity[8] and free-radical formation activity.[9] Doxorubicin is a primary drug for the treatment of patients with lymphomas, breast cancer and sarcomas. ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) and CHOP (cyclophosphamide, doxorubicin and prednisone) are used as the standard therapy for advanced Hodgkin's lymphoma and intermediate-grade non-Hodgkin's lymphoma, respectively.[5,6]

However, these drugs are not used as therapeutics for commonly occurring solid tumors such as non-small-cell lung cancer (NSCLC), colon cancer, gastric cancer and pancreatic cancer.

Continuous effort is being made to apply topo II inhibitors for the treatment of various solid tumors. Amurubicin,[10,11] launched on the market in Japan in 2002, shows efficacy against NSCLC and SCLC. Several topo II inhibitors are currently under clinical studies against solid tumors.[12—14]

Amonafide (**3**),[15] which is the basis of our drug design in this program, is also a topo II inhibitor on which intensive clinical studies were conducted in the mid 1990s. In 2003, a phase study was launched in the United States to evaluate amonafide as a potential therapeutic for solid tumors.[16]

The objective of our study is to create a structurally novel topo II inhibitor effective against various solid tumors such as NSCLC, colon, gastric and pancreatic cancers.

This paper describes our medicinal chemistry program which leads to the discovery of a novel pyrimidocarbazole compound **26** (ER37326) with high *in vitro* and *in vivo* potencies.

## Chemistry

The structures of synthesized compounds for biological evaluation are presented in Table 1. The synthesis of pyrimidoacridones **6**—**10** is summarized in Chart 1. The amides **30a**—**c** were prepared from corresponding 9-oxoacridan-4-carboxylic acids **29a**—**c**.[17,18] Compounds **29a**—**c** were treated with *N*,*N*′-carbodiimidazole (CDI) in dimethylformamide (DMF) followed by *N*,*N*-dimethylethylenediamine to

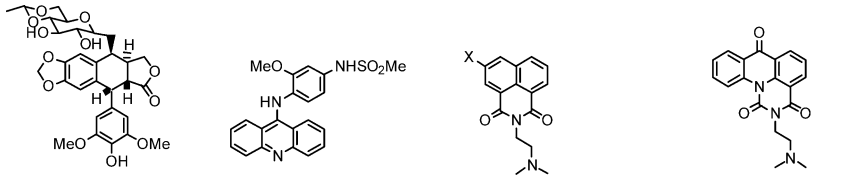


Fig. 1.

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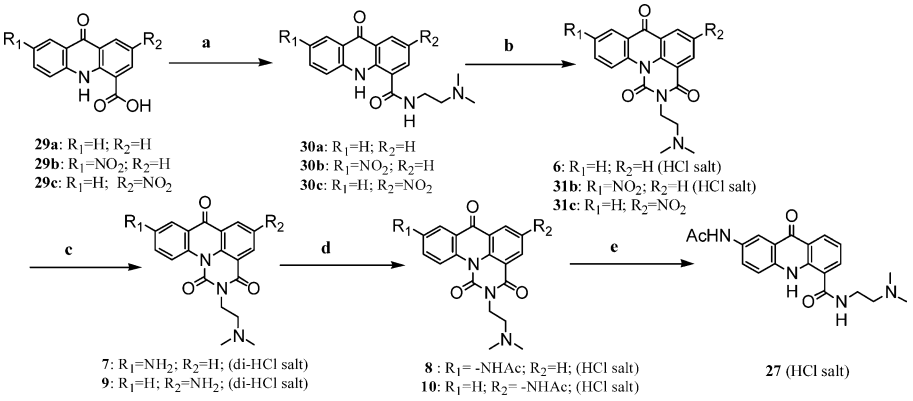



Chart 1. Synthesis of Pyrimidoacridones

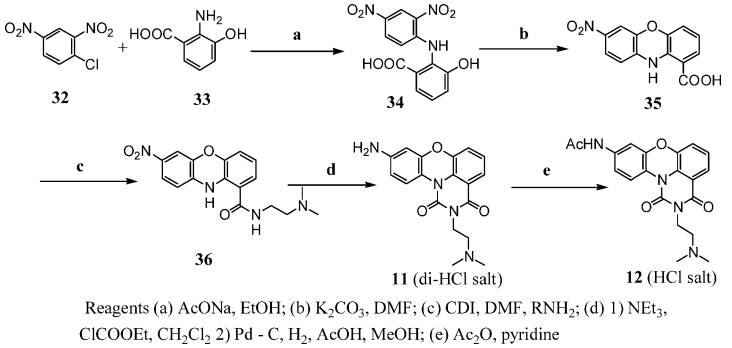



Chart 2. Synthesis of Pyrimidophenoxadines

produce amide **30a**—**c** respectively. Cyclization of **30a**—**c** provided **6**,[19] **31b** and **31c** respectively. Cyclization of **30a** and **30b** was carried out by treatment with sodium hydride followed by ethyl chloroformate, instead of the use of phosgene as previously reported in the synthesis of **6**.[19] Cyclization of **30c** was conducted by using Hunig's base and ethyl chloroformate.

The 5- or 9-nitro derivatives **31b** and **31c** were hydrogenated into the amino derivatives **7** and **9**, which were then acetylated with acetic anhydride to yield acetylamino derivatives **8** and **10** respectively. Ring opening reaction on **8** by potassium carbonate ($K_2CO_3$) was carried out to provide **27**.

The synthesis of pyrimidophenoxazines **11** and **12** (Chart 2) involved the coupling reaction of 1-chloro-2,4-dinitrobenzene and 3-hydroxyanthranilic acid in the presence of sodium acetate to yield 2-(2,4-dinitrophenylamino)-3-hydroxy benzoic acid **34**. Cyclization of **34** was conducted in a similar manner to the reported procedure for the synthesis of 7-nitro-10*H*-phenoxazine.[20] Compound **34** was heated in the presence of $K_2CO_3$ to give 7-nitro-10*H*-phenoxazine-1-carboxylic acid **35**. Then **35** was treated with CDI followed by *N*,*N*-dimethylethylenediamine to produce amide **36** which was cyclized in the presence of triethylamine and ethyl chloroformate and then hydrogenated into the amino derivative **11** before acetylation to acetylamino derivative **12**.

The syntheses of pyrimidocarbazoles **13**—**26** and **28** are shown in Charts 3—6. As shown in Chart 3, 9*H*-carbazole-1-carboxylic acid **39a** was synthesized by slight modification of the reported procedure.[21]

Fisher cyclization reaction of cyclohexanone and 2-hydrazino benzoic acid hydrochloride in acetic acid (AcOH) under reflux yielded 5,6,7,8-tetrahydro-9*H*-carbazole-1-carboxylic acid in one pot, which was aromatized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to 9*H*-carbazole-1-carboxylic acid **39a**. Treatment of the **39a** with potassium nitrate in sulfuric acid afforded the crude mixture of the mono-nitro regio isomers **39b** and **39c**, and then the mixture was amidated to afford the mixture of **40b** and **40c**. Compounds **40b** and **40c** were isolated by separation with silica gel ($SiO_2$) column chromatography. Each of them was cyclized into pyrimidocarbazoles **42b** and **42c** by treatment with sodium hydride followed by ethyl chloroformate. They were converted into amino and acetylamino derivatives **14**—**17** in a manner similar to the synthesis of pyrimidoacridones.

The 8-aminopyrimidocarbazole derivative **14** and its free base **44b** were used as key intermediates to produce various 8-substituted derivatives (Charts 3, 4). Compound **14** was mesylated with methanesulfonic anhydride to yield **22**. Compound **25** was prepared from **44b** by acylation with *n*-butyryl chloride. **44b** was diazotized, and then hydrolyzed in the presence of CuI and $Cu_2O$ to give hydroxy derivative **19**, which was converted into methoxy derivative **20** with sodium hydride and methyl iodide. Compound **23** was prepared by heating **44b** with 2,5-dimethoxy-tetrahydrofuran in AcOH.

As shown in Chart 5, 8-methyl derivative **18** was prepared by the Fisher cyclization reaction of 4-methylcyclohexanone and 2-hydrazino benzoic acid hydrochloride followed by the same procedure for the synthesis of **13**.

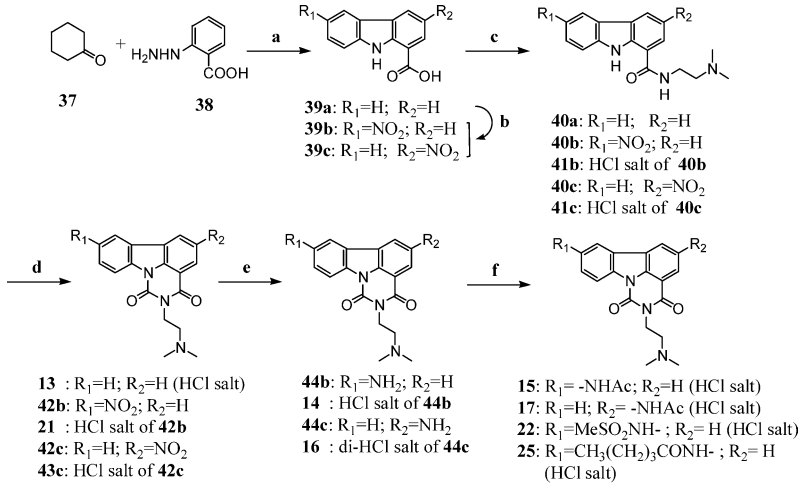



Chart 3. Synthesis of Pyrimidocarbazoles (I)

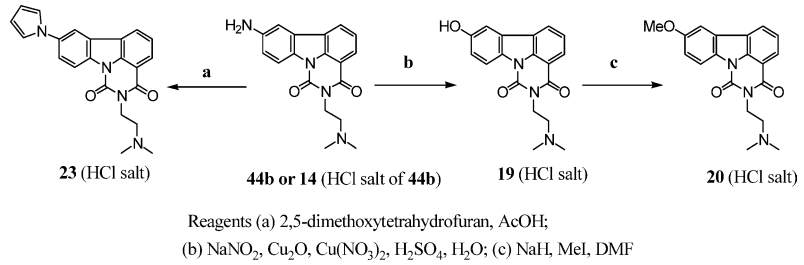



Chart 4. Synthesis of Pyrimidocarbazoles (II)

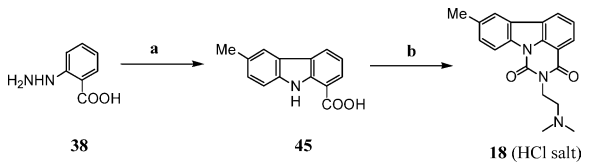



Chart 5. Synthesis of Pyrimidocarbazoles (III)

Friedel-Craft reaction of **39a** with acetic anhydride or *n*-butyryl chloride in the presence of $AlCl_3$ gave the desired 8-acylated products **46a** and **46b** respectively almost exclusively (Chart 6). Compounds **46a** and **46b** were converted into **26** and **24** in a similar manner described above.

## Results and Discussion

The results of *in vitro* growth inhibitory activity against murine leukemia P388 and human oral cancer KB and DNA–protein cross-linking formation activity of compounds **6**—**28** and reference compounds are presented in Table 1 and Fig. 2. DNA–protein cross-linking formation is considered to be an indicator for the ability of a compound to stabilize the cleavable complex of the compound, DNA, and topo II.

The results of the *in vivo* activity of selected compounds against P388 and M5076 are shown in Table 2 and Table 3 respectively.

At the commencement of our program, we evaluated *in vitro* growth inhibitory activities of amonafide (**3**), *N*-acetylamonafide (**4**) and unsubstituted 1,8-naphthalic imide (**5**) to examine the importance of the amino substituent of amonafide and the acetylamino substituent of *N*-acetylamonafide. Amonafide and *N*-acetylamonafide showed higher *in vitro* growth inhibitory activities than the unsubstituted 1,8-naphthalic imide (**5**) (Table 1).

Based on the above results of the amino and acetylamino substituent playing a key role in their activities, we designed and synthesized compounds (**7**—**10**) with pyrimidoacridone chromophore[19,22] bearing the amino or acetylamino substituent on 5- or 9-position. Compound **6**, previously reported by Antonini *et al.*[19] was also prepared and evaluated. In comparison to **6**, compounds **8** and **10** showed almost equal activities in the *in vitro* P388 model and higher activities *in vitro* KB model.

Compounds **6**, **8**, **10** were evaluated in the *in vivo* P388 model (Table 2). Compound **8** showed the most potent activity among them with a maximum increase in lifespan (ILS) of $>125\%$ at 25 mg/kg/d.

The superiority of **8** over **6** in their activities *in vivo* P388 could be attributed to the higher activity of **8** in the DNA–protein cross-linking formation activities than **6**.

As we found an active compound **8** in *in vivo* P388 model, this compound was evaluated in *in vivo* solid tumor model M5076 which is a more appropriate model for screening

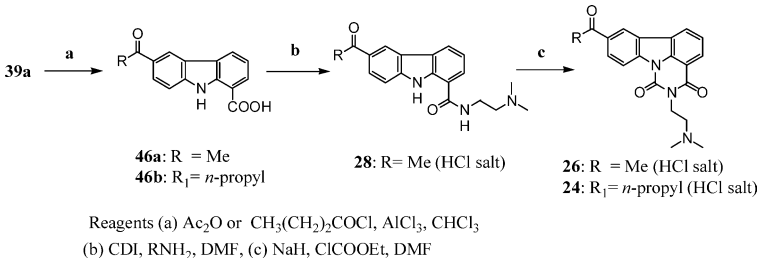
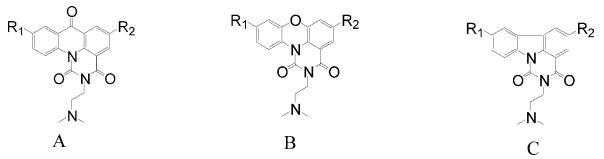



Chart 6. Synthesis of Pyrimidocarbazoles (IV)

Table 1. *In Vitro* Growth Inhibition and DNA–Protein Cross-Linking Formation




| No. | Form | $R_1$ | $R_2$ | *In vitro* growth inhibition $IC_{50}$ ($\mu$M)[a] | | DNA–protein cross linking[b] |
|---|---|---|---|---|---|---|
| | | | | P388 | KB | |
| **6** | A | H | H | 0.98 | 4.4 | + (2)[c] |
| **7** | A | $NH_2$ | H | 5.4 | 4.6 | + (2) |
| **8** | A | AcNH | H | 0.86 | 1.5 | ++ (10) |
| **9** | A | H | $NH_2$ | 63 | 22 | ++ (10) |
| **10** | A | H | AcNH | 0.85 | 0.70 | +++ (10) |
| **11** | B | $NH_2$ | H | 0.078 | 0.13 | ++ (10) |
| **12** | B | AcNH | H | 0.073 | 0.085 | +++ (10) |
| **13** | C | H | H | 0.11 | 0.12 | +++ (2) |
| **14** | C | $NH_2$ | H | 0.032 | 0.15 | ++ (2) |
| **15** | C | AcNH | H | 0.078 | 0.28 | +++ (10) |
| **16** | C | H | $NH_2$ | 0.011 | 0.075 | +++ (2) |
| **17** | C | H | AcNH | 0.015 | 0.068 | +++ (2) |
| **18** | C | Me | H | 0.13 | 0.54 | ++ (10) |
| **19** | C | OH | H | 0.020 | 0.13 | ++ (2) |
| **20** | C | OMe | H | 0.51 | 0.29 | ++ (2) |
| **21** | C | $NO_2$ | H | 0.29 | 1.1 | ++ (10) |
| **22** | C | $MeSO_2NH$ | H | 0.13 | 0.26 | ++ (2) |
| **23** | C | 1-Pyrrolyl | H | 0.43 | 0.43 | +++ (10) |
| **24** | C | *n*-Butyryl | H | 0.038 | 0.19 | +++ (10) |
| **25** | C | *n*-Pentanoylamino | H | 0.018 | 0.17 | +++ (10) |
| **26**[d] | C | Acetyl | H | 0.049 | 0.35 | ++ (10) |
| **27**[e] | | — | | 22 | 12 | + (10) |
| **28**[f] | | — | | 9.4 | 5.6 | n.d[g] |
| Etoposide (**1**) | | | | 0.16±0.01[h] | 0.25±0.01[h] | +++ (50) |
| mAMSA (**2**) | | | | 0.044±0.013[i] | 0.10±0.05[i] | —[j] |
| Amonafide (**3**) | | | | 0.28 | 1.4 | +++ (10) |
| Acetylamonafide (**4**) | | | | 0.75 | 2.4 | +++ (50) |
| **5**[k] | | | | 0.93 | 5.7 | ++ (50) |

*a*, *b*) See experimental. *c*) The dose ($\mu$M) at which maximum DNA–protein cross-linking was detected are indicated. As for the classification of +, ++, +++, see experimental. *d*) ER-37326. *e*) See Chart 1 for structure. *f*) See Chart 6 for structure. *g*) No data. *h*) SEM for 73 times of experiments. *i*) SEM for 2 times of experiments. *j*) mAMSA was used as the reference compound. See experimental. *k*) *N*,*N*-Dimethylaminoethyl 1,8-naphthalic imide. See Fig. 1 for structure.

compounds effective against solid tumors. As a result, compound **8** also showed good efficacy in *in vivo* M5076 model with 18% T/C (Table 3).

While **8** was attractive because of its efficacy, it indicated significant body weight loss in mice, (relative body weight (RBW) on day 7 was 0.78.), with chemical instability even under neutral condition,[23] which is an unwanted property as a drug.

Many anticancer topo II inhibitors, such as amonafide, amsacrine, doxorubicin, and ellipticine[24] contain a planer chromophore which can intercalate into the DNA helix. Since we speculated that these pyrimidoacridones are included in this class of intercalative topo II inhibitors, replacement of the acridone portion by other planer tricyclic chromophore was considered to be possible. Therefore we thought to explore other planer chromophores to improve the biological potency and chemical stability.

Thus the novel pyrimidophenoxadines **11**, **12** and pyrimidocarbazoles **13**—**26** were designed, synthesized, and evaluated.

Table 2. *In Vivo* Activity against P388 Leukemia in Mice[a)]

| No. | Dose (mg/kg/d) | ILS (%) | RBW-7[b)] | No. | Dose (mg/kg/d) | ILS (%) | RBW-7 |
|---|---|---|---|---|---|---|---|
| **6**[c)] | 6.25 | 0.0 | 1.18 | Amonafide (**3**) | 6.25[d)] | 20.0 | n.d |
| | 25 | 17.5 | 1.12 | | 25[d)] | 70.0 | n.d |
| | 100 | −30.0 | n.d.[e)] | | 100[d)] | −30.0 | n.d |
| **8** | 6.25 | 25.0 | 1.10 | *N*-Acetyl | 6.25 | 17.5 | 1.10 |
| | 25 | >125 | 0.81 | amonafide (**4**) | 25 | 82.5 | 1.01 |
| | 100 | −27.5 | 0.69 | | 100 | −27.5 | 0.72 |
| **10** | 1.56 | 14.8 | 1.09 | Etoposide (**1**) | 12.5 | >160 | 1.10 |
| | 6.25 | 24.2 | 1.05 | | | | |
| | 25 | −25.0 | 0.79 | | | | |

*a*) P388 leukemia cells were implanted i.p. and the compounds were administered i.p. with q1d×4 (days 1, 2, 3, 4). See experimental for details. *b*) Relative body weight on day 7. *c*) *In vivo* activity of this compound against P388 leukemia in mice was reported previously.[19,26)] The maximum effect of 60% ILS was shown at 25 mg/kg/d q1d×5 (days 1, 2, 3, 4, 5). *d*) This compound was administered ip with q1d×5 (days 1, 2, 3, 4, 5). *e*) All mice died before day 7.

Table 3. *In Vivo* Activity against M5076 Sarcoma in Mice[a)]

| No. | Dose[b)] | T/C (%) | RBW[c)] | Tox[d)] | No. | Dose | T/C (%) | RBW | Tox |
|---|---|---|---|---|---|---|---|---|---|
| **8** | 25 | 111 | 1.03 | 0/6 | **22** | 6.25 | 79 | 1.02 | 0/5 |
| | 50 | 51 | 0.88 | 0/6 | | 12.5 | 30 | 0.86 | 0/5 |
| | 100 | 18 | 0.78 | 0/6 | | 25 | 26[e)] | 0.78 | 2/5 |
| **12** | 6.25 | — | 0.84 | 6/6 | **23** | 12.5 | 31 | 1.02 | 0/5 |
| **14** | 1.56 | 52 | 1.07 | 0/5 | | 25 | 10 | 0.86 | 1/5 |
| | 3.13 | 26 | 1.02 | 0/5 | **26**[g)] | 3.13 | 42 | 1.01 | 0/5 |
| | 6.25 | 4[e)] | 0.82 | 3/5 | | 6.25 | 13 | 0.96 | 0/5 |
| **15** | 1.56 | 71 | 1.02 | 0/5 | | 12.5 | 9[e)] | 0.83 | 1/5 |
| | 3.13 | 41 | 1.01 | 0/5 | Amonafide (**3**) | 12.5 | 84 | 1.06 | 0/6 |
| | 6.25 | — | 0.78 | 5/5 | | 25 | 42 | 1.06 | 0/6 |
| **17** | 0.78 | 69 | 1.05 | 0/5 | | 50 | — | — | 6/6 |
| | 1.56 | — | 1.01 | 5/5 | Etoposide (**1**) | 6.25 | 59 | 1.07 | 0/6 |
| **19** | 12.5 | 53 | 1.06[f)] | 0/5 | | 12.5 | 50 | 1.10 | 0/6 |
| | 25 | — | — | 5/5 | | 25 | 18 | 0.85 | 1/6 |
| **20** | 12.5 | 69 | 1.09 | 0/5 | | | | | |
| | 25 | 34 | 1.01 | 0/5 | | | | | |
| | 50 | — | 0.75 | 5/5 | | | | | |

*a*) M5076 sarcoma cells were implanted sc and the compounds were administered i.p. with q1d×4 (days 1, 2, 3, 4). See experimental for details. *b*) mg/kg/d. *c*) Relative body weight on day 7 unless otherwise stated. *d*) Number of toxic death/total number of mice. *e*) Average T/C of survivors was indicated. *f*) Relative body weight on day 8. *g*) ER-37326.

In the subset of the amino and acetylamino substituent-containing analogues (Table 1), pyrimidophenoxadines and pyrimidocarbazoles were much more potent than their corresponding pyrimidoacridones in *in vitro* growth inhibitory activity and DNA–protein cross-linking formation assay (**7** *vs.* **11** *vs.* **14**, **8** *vs.* **12** *vs.* **15**, **9** *vs.* **16**, **10** *vs.* **17**). In addition, pyrimidocarbazoles were chemically more stable than pyrimidoacridones.[25)]

Although pyrimidophenoxadines were highly potent *in vitro*, *in vivo* efficacy of **12** was elusive because of its relatively severe toxicity. In most cases, the body weight of mice treated with our compounds recovered after the termination of drug administration. In the experiment, the body weight of mice treated by **12** continued to decrease even after day 7 with eventual death.

In the subset of pyrimidocarbazoles, the amino and acetylamino substituents contributed to their potency on the basis of *in vitro* growth inhibitory activities against P388 cells (**13** *vs.* **14**—**17**). 5-Substitution contributed more to the *in vitro* potency against both of P388 and KB cells than 8-substitution (**14** *vs.* **16**, **15** *vs.* **17**). *In vivo*, this trend reversed (Table 3). Compounds **14** and **15** were effective at a dose which did not cause the serious body weight decrease observed in case of **8**. However, **17** did not show potent *in vivo* activity.

This result indicated that 8-substitution in pyrimidocarbazoles is more favorable in terms of their potency in the M5076 model than 5-substitution. This trend is similar to the *in vivo* SAR of pyrimidoacridones in which 9-acetylamino **8** was more potent than 5-acetylamino **10**. Compound **27** was inactive *in vitro*, indicating the importance of pyrimidoacridone chromophore.

Based on the fact that 8-substituted pyrimidocarbazoles **14** and **15** are effective *in vivo*, other kinds of substitutent on position 8 of pyrimidocarbazole were further investigated (**18**—**26**).

In *in vitro* experiments, **19**, **24**, **25**, and **26** (ER37326) showed similar or higher activity than **14** and **15**.

As a result of screening using the M5076 model, we found ER-37326 with efficacy of 42% T/C at 3.13 mg/kg/d and 13% T/C at 6.25 mg/kg/d without significantly affecting the body weight, and its potency surpassed that of etoposide. DNA–protein cross-linking was detected in P388 cells treated with **26** (ER37326) as shown in Table 1 and Fig. 2. To determine if DNA–protein cross-linking by **26** (ER37326) was dependent on topo II, immunoblot assay was performed to detect DNA–topo II cross-linking. As shown in Fig. 3, **26** (ER37326) and etoposide indicated DNA–topo II cross-linking with 0.4, 2.0, 10 $\mu$M, but not with 50 $\mu$M of **26**

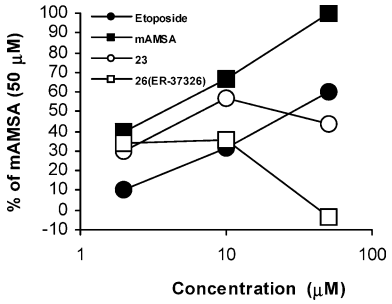



Fig. 2. Effects of Compounds on the Formation of DNA–Protein Cross-Linking in P388[a)]

*a*) See experimental for details.

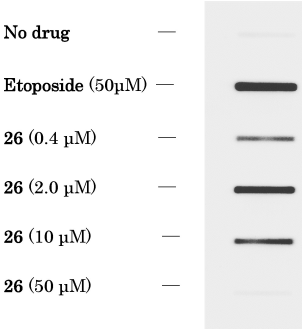



Fig. 3. Effects of **26** (ER-37326) and Etoposide (**1**) on the Formation of DNA–Topoisomerase II Cross-Linking in P388[a)]

*a*) See experimental for details.

(ER37326). This result is consistent with that derived from the DNA–protein cross-linking assay (Fig. 2), in which DNA–protein cross-linking was not observed at 50 $\mu$M of **26** (ER37326), indicating DNA–topo II cleavable complex in P388 cells was induced by **26** (ER37326).

## Conclusion

As part of a series of studies to discover potent topo II inhibitors, novel pyrimidoacridones, pyrimidophenoxadines, and pyrimidocarbazoles were synthesized and evaluated in the present study. The pyrimidocarbazoles possessed the most potent *in vitro* and *in vivo* antitumor activities.

Of these, **26** (ER-37326), in particular, showed more potent efficacy than etoposide without severe toxicity in the *in vivo* solid tumor model (M5076). In conclusion, we have found a novel lead compound **26** (ER-37326) worthy of further investigation as a potent anticancer agent against solid tumors.

### Experimental

**Syntheses of Compounds** Melting points (mp) were measured using a Yanako melting point apparatus and are uncorrected. The proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a varian Unity 400 (400 MHz) spectrometer or Mercury 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal standard unless otherwise stated. Mass spectra (MS) were obtained on a SSQ 7000 mass spectrometer. High resolution mass spectra (HR-MS) were obtained on a Q-Tof Ultima global mass spectrometer (micromass U.K.). Elemental analysis was performed with vario EL or vario ELIII or Yanaco MT-3 at Toray Research Center, or Yanaco MT-5 at the Analytical Chemistry Section of Eisai Research Laboratories. Samples for elemental analysis were dried under reduced pressure at 30 °C for 2 h, using a drying apparatus (MINI drier; Yazawa, 1-32 model) and an oil rotary vacuum pump (SATO vacuum machinery, SW-150).

Results obtained were within ±0.4% of the theoretical value. Materials used in the study were of commercial grades. $SiO_2$ (Kiesel 60, Merck) was used for column chromatography. Organic extracts were removed with a rotary evaporator under reduced pressure.

***N*-[2-(Dimethylamino)ethyl]-7-nitro-9,10-dihydro-9-oxo-4-acridinecarboxamide (30b)** CDI (6.25 g, 38.5 mmol) was added to a stirred solution of 7-nitro-9,10-dihydro-9-oxo-4-acridine carboxylic acid[17)] **29b** (5.47 g, 19.2 mmol) in DMF (100 ml) at room temperature (r.t.) and the mixture was stirred for 1 h. *N*,*N*-Dimethylethylenediamine (8.45 ml, 77.0 mmol) was added to the reaction mixture, which was stirred overnight and treated with water ($H_2O$). The precipitate obtained was collected by filtration to yield the title compound (4.54 g, 12.8 mmol, 67%) as a pale yellow solid. $^1$H-NMR (DMSO-$d_6$) $\delta$[27)] 2.30 (6H, s), 2.57—2.64 (2H, m), 3.51 (2H, q, $J$=6.0 Hz), 7.41 (1H, t, $J$=7.8 Hz), 7.90—7.97 (1H, m), 8.31 (1H, dd, $J$=1.6, 7.0 Hz), 8.39—8.44 (2H, m), 8.86 (1H, d, $J$=2.8 Hz).

**2-[2-(Dimethylamino)ethyl]-9-nitropyrimido[5,6,1-*de*]acridine-1,3,7-trione Hydrochloride (31b)** Sodium hydride (1.12 g, 25.7 mmol, 55% dispersion in oil) was added to a stirred solution of **30b** (4.54 g, 12.8 mmol) in DMF (50 ml) and the mixture was stirred for 30 min at r.t. The mixture was treated with ethyl chloroformate (2.46 ml, 25.7 mmol) at 0 °C, and then the mixture was stirred for 30 min at the same temperature. An excess amount of 1 N aqueous hydrogen chloride (1 N HCl (aq.) and AcOH (45 ml) were added to the reaction mixture and the precipitate obtained was collected by filtration to yield the title compound as a white solid (3.80 g, 9.12 mmol, 71%) $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.90 (6H, s), 3.45—3.54 (2H, m), 4.46 (2H, t, $J$=6.0 Hz), 7.84 (1H, t, $J$=7.6 Hz), 8.64 (1H, dd, $J$=1.6, 7.6 Hz), 8.69 (1H, dd, $J$=1.6, 7.6 Hz), 8.71 (1H, dd, $J$=2.8, 9.6 Hz), 9.00 (1H, d, $J$=2.8 Hz), 9.04 (1H, d, $J$=9.6 Hz), 9.97 (1H, br s).

**9-Amino-2-[2-(dimethylamino)ethyl]pyrimido[5,6,1-*de*]acridine-1,3,7-trione Dihydrochloride (7)** A mixture of **31b** (2.64 g, 6.33 mmol) and 10% palladium on carbon (Pd–C) (260 mg) in AcOH (300 ml) and 1 N HCl (aq.) (50 ml) was stirred under hydrogen at 1 atm for 5 h. The catalyst was removed by filtration and washed with methanol (MeOH), and the filtrate was concentrated *in vacuo*. The residue was neutralized with an aqueous sodium hydrogen carbonate solution ($NaHCO_3$ (aq.)) and the mixture was extracted with ethyl acetate (EtOAc)–tetrahydrofuran (THF) solution (1 : 1 in volume). The organic layer was washed successively with $H_2O$ and brine before being concentrated *in vacuo*. The solid obtained was washed with ethanol (EtOH) and isopropyl ether and suspended in EtOH with stirring and then acidified with an excess amount of HCl in MeOH solution. The precipitate obtained was collected by filtration, washed with EtOH and isopropyl ether and then dried *in vacuo* to yield the title compound as a orange solid (2.10 g, 4.96 mmol, 78%), mp 267—270 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.90 (6H, d, $J$=4.8 Hz), 3.51 (2H, q, $J$=5.6 Hz), 4.43 (2H, t, $J$=5.6 Hz), 7.29 (1H, d, $J$=9.2 Hz), 7.64 (1H, s), 7.72 (1H, t, $J$=7.6 Hz), 8.54 (1H, dd, $J$=1.6, 7.6 Hz), 8.62 (1H, dd, $J$=1.6, 7.6 Hz), 8.71 (1H, d, $J$=9.2 Hz), 9.92 (1H, br s). ESI-MS *m/z*: 351 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{18}N_4O_3 \cdot 2HCl \cdot H_2O$: C, 51.71; H, 5.02; N, 12.70. Found: C, 51.71; H, 5.12; N, 12.73.

**9-Acetylamino-2-[2-(dimethylamino)ethyl]pyrimido[5,6,1-*de*]acridine-1,3,7-trione Hydrochloride (8)** A mixture of **7** (450 mg, 1.06 mmol), acetic anhydride (15 ml) and pyridine (5 ml) was stirred under reflux for 1 h. The reaction mixture was concentrated *in vacuo* and $NaHCO_3$ (aq.) was added to the residue, which was extracted with EtOAc–THF solution (1 : 1 in volume). The organic layer was washed with $H_2O$ and dried over $MgSO_4$, and then concentrated *in vacuo*. The solid that formed during evaporation was collected by filtration, washed with EtOH and suspended in EtOH with stirring and followed by treatment with an excess amount of 1 N HCl (aq.). The solid obtained was collected by filtration to yield the title compound (200 mg, 0.47 mmol, 44%) as a pale yellow solid, mp 280—283 °C (dec.) (EtOH). ESI-MS *m/z*: 393 $(M+H)^+$, 807 $(2M+Na)^+$. $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.90 (6H, s), 3.45—3.51 (2H, m), 4.44 (2H, t, $J$=6.0 Hz), 7.76 (1H, t, $J$=7.6 Hz), 8.05 (1H, dd, $J$=2.4, 9.6 Hz), 8.57 (1H, dd, $J$=1.6, 7.6 Hz), 8.63—8.65 (2H, m), 8.85 (1H, d, $J$=9.6 Hz), 9.84 (1H, br s), 10.48 (1H, s). *Anal.* Calcd for $C_{21}H_{20}N_4O_4 \cdot HCl \cdot 2H_2O$: C, 54.25; H, 5.42; N, 12.05. Found: C, 54.55; H, 5.65; N, 12.21.

***N*-[2-(Dimethylamino)ethyl]-2-nitro-9,10-dihydro-9-oxo-4-acridinecarboxamide (30c)** This compound was obtained from 3-nitro-9,10-dihydro-9-oxo-4-acridine carboxylic acid[18)] **29c** (2.00 g, 7.04 mmol) in a similar manner to the preparation of **30b** as an orange solid (1.09 g, 3.08 mmol, 44%). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.31 (6H, s), 2.57 (2H, t, $J$=5.6 Hz), 3.55 (2H, q, $J$=5.6 Hz), 7.16—7.26 (1H, m), 7.64—7.72 (2H, m), 8.18 (1H, d, $J$=8.0 Hz), 9.02 (1H, d, $J$=1.6 Hz), 9.12 (1H, d, $J$=1.6 Hz).

**2-[2-(Dimethylamino)ethyl]-5-nitropyrimido[5,6,1-*de*]acridine-1,3,7-trione (31c)** To a stirred solution of **30c** (1.09 g, 3.08 mmol) in DMF

(50 ml) was added diisopropylethylamine (1.74 ml, 10 mmol) and ethyl chloroformate (0.96 ml, 10 mmol) at 0 °C and the mixture was stirred for 6 h at r.t. $H_2O$ was added to the reaction mixture, which was extracted with EtOAc–THF solution (1 : 1 in volume) and the organic layer was washed with brine and then concentrated *in vacuo*. The solid obtained was crystallized from EtOH–isopropyl ether to yield the title compound (530 mg, 1.39 mmol, 45%) as orange crystals. $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.27 (6H, s), 2.58—2.68 (2H, m), 4.22 (2H, t, $J$=6.8 Hz), 7.68 (1H, t, $J$=7.6 Hz), 7.96—8.01 (1H, m), 8.38 (1H, dd, $J$=2.0, 7.6 Hz), 8.77 (1H, d, $J$=8.4 Hz), 9.04 (1H, d, $J$=2.8 Hz), 9.13 (1H, d, $J$=2.8 Hz).

**5-Amino-2-[2-(dimethylamino)ethyl]pyrimido[5,6,1-*de*]acridine-1,3,7-trione Dihydrochloride (9)** The title compound was obtained from **31c** (530 mg, 1.39 mmol) in a similar manner to the preparation of **7** to yield an ocher solid (580 mg, 1.37 mmol, 99%), mp 294—297 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.91 (6H, d, $J$=5.2 Hz), 3.49 (2H, q, $J$=6.0 Hz), 4.42 (2H, t, $J$=6.0 Hz), 7.56—7.60 (1H, m), 7.83 (1H, d, $J$=2.8 Hz), 7.85 (1H, d, $J$=2.8 Hz), 7.85—7.92 (1H, m), 8.35 (1H, dd, $J$=1.6, 8.0 Hz), 8.95 (1H, d, $J$=8.0 Hz), 9.61 (1H, br s). ESI-MS *m/z*: 351 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{18}N_4O_3\cdot 2HCl\cdot 1.1H_2O$: C, 51.50; H, 5.05; N, 12.64. Found: C, 51.65; H, 5.30; N, 12.43.

**5-Acetylamino-2-[2-(dimethylamino)ethyl]pyrimido[5,6,1-*de*]acridine-1,3,7-trione Hydrochloride (10)** The title compound was obtained from **9** (300 mg, 0.71 mmol) in a similar manner to the preparation of **8** as a pale green solid (180 mg, 0.42 mmol, 59%), mp 289—291 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.91 (6H, s), 3.44—3.54 (2H, m), 4.44 (2H, t, $J$=5.6 Hz), 7.62 (1H, t, $J$=7.6 Hz), 7.91—7.96 (1H, m), 8.38 (1H, dd, $J$=2.0, 7.6 Hz), 8.80 (1H, d, $J$=2.4 Hz), 8.90 (1H, d, $J$=2.4 Hz), 8.92 (1H, d, $J$=9.2 Hz), 9.52 (1H, br s), 10.66 (1H, br s). ESI-MS *m/z*: 393 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{20}N_4O_4\cdot HCl\cdot 0.2H_2O$: C, 58.32; H, 4.99; N, 12.96. Found: C, 58.30; H, 5.06; N, 12.97.

**2-[2-(Dimethylamino)ethyl]pyrimido[5,6,1-*de*]acridine-1,3,7-trione Hydrochloride (6)** Synthesis of the title compound previously reported by Antonini *et al.*[19] was obtained in our laboratory from *N*-[2-(dimethylamino) ethyl]-9,10-dihydro-9-oxo-4-acridinecarboxamide[19] **30a** (457 mg, 1.48 mmol) using DMF (20 ml), triethylamine (0.56 ml, 4.0 mmol) and ethyl chloroformate (0.19 ml, 1.99 mmol) in a similar manner to the preparation of **31c**. Yield: 178 mg, 0.479 mmol, 32%. mp 278—279 °C (lit[19] 281—283 °C) ESI-MS *m/z*: 336 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{17}N_3O_3\cdot HCl\cdot H_2O$: C, 61.38; H, 4.88; N, 11.30. Found: C, 61.42; H, 4.86; N, 11.35.

**7-Acetylamino-*N*-[2-(dimethylamino)ethyl]-9,10-dihydro-9-oxo-4-acridinecarboxamide Hydrochloride (27)** A mixture of **8** (20 mg, 0.047 mmol) and $K_2CO_3$ (50 mg) in MeOH (2 ml) and $H_2O$ (2 ml) was stirred for 1 h. After additing ammonium chloride (200 mg) the mixture was concentrated *in vacuo* untill most of MeOH was evaporated. The mixture was extracted with EtOAc–THF solution (1 : 1 in volume). The organic layer was washed successively with $H_2O$ and dried over $MgSO_4$ before evaporation. EtOH (0.4 ml) and an excess amount of 1 N HCl (aq.) were added to the residue and then concentrated *in vacuo*. The residue was recrystallized from EtOH–isopropyl ether to yield 10 mg of the title compound as yellow crystals (10 mg, 0.025 mmol, 53%), mp 278—280 °C (dec.) (EtOH–isopropyl ether). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.09 (3H, s), 2.88 (6H, d, $J$=4.4 Hz), 3.32—3.39 (2H, m), 3.72 (2H, q, $J$=5.6 Hz), 7.36 (1H, t, $J$=8.0 Hz), 7.70 (1H, d, $J$=8.8 Hz), 7.99 (1H, dd, $J$=1.6, 8.8 Hz), 8.83 (1H, d, $J$=8.0 Hz), 8.45—8.48 (2H, m), 9.23 (1H, t, $J$=5.6 Hz), 9.78 (1H, br s) 10.19 (1H, s), 12.29 (1H, s). HR-MS *m/z*: 367.1776 (Calcd for $C_{20}H_{23}N_4O_3$ $([M+H]^+)$: 367.1770).

**2-(2,4-Dinitrophenylamino)-3-hydroxybenzoic Acid (34)** A mixture of 1-chloro-2,4-dinitrobenzene **32** (1.06 g, 5.23 mmol), 3-hydroxyanthranilic acid **33** (0.76 g, 4.96 mmol) and sodium acetate (1.72 g, 21.0 mmol) in EtOH (20 ml) and $H_2O$ (5 ml) was stirred under reflux for 24 h. The mixture was allowed to stand at r.t. and acidified with 1 N HCl (aq.). The precipitate obtained was collected by filtration and dissolved in EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $MgSO_4$ before concentration *in vacuo* to yield the title compound as a dark brown solid (1.40 g, 4.39 mmol, 89%), mp 270—273 °C (dec.) (ether–hexane). HR-MS *m/z*: 318.0372 (Calcd for $C_{13}H_8N_3O_7$ $([M-H]^-)$: 318.0362). $^1$H-NMR (DMSO-$d_6$) $\delta$ 6.66 (1H, d, $J$=9.6 Hz), 7.23 (1H, dd, $J$=1.6, 8.0 Hz), 7.32 (1H, t, $J$=8.0 Hz), 7.45 (1H, dd, $J$=1.6, 8.0 Hz), 8.23 (1H, dd, $J$=2.8, 9.6 Hz), 8.90 (1H, d, $J$=2.8 Hz), 0.31 (1H, s), 10.50 (1H, s).

**7-Nitro-10*H*-phenoxazine-1-carboxylic Acid (35)** A mixture of **34** (1.35 g, 4.23 mmol) and $K_2CO_3$ (350 mg) in DMF (10 ml) was stirred under reflux for 12 h. The mixture was allowed to stand at r.t. and acidified with 1 N HCl (aq.). The precipitate obtained was collected by filtration and washed with $H_2O$ and then, recrystallized from EtOH to yield the title compound as orange crystals (0.91 g, 3.3 mmol, 79%), mp >300 °C (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 6.75 (1H, t, $J$=8.0 Hz), 6.85 (1H, d, $J$=8.0 Hz), 7.03 (1H, d, $J$=8.8 Hz), 7.36—7.39 (2H, m), 7.70 (1H, dd, $J$=2.4, 8.8 Hz), 9.53 (1H, s). HR-MS *m/z*: 271.0401 (Calcd for $C_{13}H_7N_2O_5$ $([M-H]^-)$: 271.0355).

***N*-[2-(Dimethylamino)ethyl]-7-nitro-10*H*-phenoxazine-1-carboxamide (36)** DMF (0.5 ml) and phosphorus trichloride (2 ml) was added to a suspension of **35** (435 mg, 1.60 mmol) in chloroform ($CHCl_3$) (15 ml) and then, the mixture was stirred overnight at r.t. The mixture was then concentrated *in vacuo*. Toluene was added to the mixture, which was again concentrated *in vacuo*. The residue was dissolved in $CH_2Cl_2$ (10 ml) and treated with a solution of *N*,*N*-dimethylethylenediamine (1.0 ml, 9.1 mmol) in $CH_2Cl_2$ (20 ml) and stirred overnight at r.t. $NaHCO_3$ (aq.) was added to the mixture and the mixture was extracted with $CH_2Cl_2$. The organic layer was filterd through celite and the filtrate was washed with $H_2O$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CHCl_3$–MeOH) to yield the title compound as an amorphous solid (507 mg, 1.48 mmol, 93%). $^1$H-NMR ($CDCl_3$) $\delta$ 2.28 (6H, s), 2.52 (2H, t, $J$=6.2 Hz), 3.42 (2H, q, $J$=6.2 Hz), 6.39 (1H, d, $J$=8.6 Hz), 6.64—6.72 (2H, m), 6.94 (1H, dd, $J$=1.6, 7.5 Hz), 6.96—7.02 (1H, m), 7.41 (1H, d, $J$=2.5 Hz), 7.68 (1H, dd, $J$=2.5, 8.6 Hz), 9.88 (1H, br s). HR-MS *m/z*: 343.1384 (Calcd for $C_{13}H_8NO_2$ $([M+H]^+)$: 343.1406).

**9-Amino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimide[5,6,1-*kl*]phenoxadine-1,3(2*H*)-dione Dihydrochloride (11)** Compound **36** (49 mg, 0.143 mmol) was converted to the crude product of 9-Nitro-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*kl*]phenoxadine-1,3(2*H*)-dione (50 mg, 0.14 mmol) in a similar manner to the preparation of **31c** using triethylamine (0.30 ml, 2.2 mmol), dichloromethane ($CH_2Cl_2$) (5 ml) and ethyl chloroformate (0.03 ml, 0.3 mmol). The title compound was obtained from the crude product (50 mg, 0.14 mmol) in a similar manner to the preparation of **7** as a yellowish green solid (48 mg, 0.12 mmol, 86%), mp 286—289 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$[27] 2.84 (6H, d, $J$=4.4 Hz), 3.36—3.40 (2H, m), 4.29 (2H, t, $J$=5.6 Hz), 6.78—6.81 (2H, m), 7.30 (2H, t, $J$=8.0 Hz), 7.36 (1H, dd, $J$=1.2, 8.0 Hz), 7.62 (1H, dd, $J$=1.2, 8.0 Hz), 8.41 (1H, d, $J$=9.2 Hz), 10.16 (1H, br s). ESI-MS *m/z*: 339 $(M+H)^+$, *Anal.* Calcd for $C_{18}H_{18}N_4O_3\cdot 2HCl\cdot 1.1H_2O$: C, 50.15; H, 5.19; N, 13.00. Found: C, 49.94; H, 5.26; N, 13.09.

**9-Acetylamino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*kl*]phenoxadine-1,3(2*H*)-dione Hydrochloride (12)** The title compound was obtained from **11** (150 mg, 0.37 mmol) in a similar manner to the preparation of **8** as a white solid (114 mg, 0.27 mmol, 74%), mp 275—278 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.06 (3H, s), 2.88 (6H, s), 3.38—3.44 (2H, m), 4.32 (2H, t, $J$=5.6 Hz), 7.19 (1H, dd, $J$=1.2, 9.6 Hz), 7.31 (1H, td, $J$=1.6, 7.6 Hz), 7.39 (1H, dt, $J$=1.6, 7.6 Hz), 7.55 (1H, d, $J$=1.6 Hz), 7.64 (1H, dt, $J$=1.6, 7.6 Hz), 8.44 (1H, dd, $J$=1.6, 9.6 Hz), 9.80 (1H, br s), 10.30 (1H, s). ESI-MS *m/z*: 381 $(M+H)^+$, *Anal.* Calcd for $C_{20}H_{20}N_4O_4\cdot HCl\cdot 1.5H_2O$: C, 54.12; H, 5.45; N, 12.62. Found: C, 54.37; H, 5.53; N, 12.71.

**9*H*-Carbazole-1-carboxylic Acid (39a)**[21] A solution of cyclohexanone **37** (10.4 ml, 0.100 mol) in AcOH (20 ml) was dropped into a stirred suspension of 2-hydrazinobenzoic acid hydrochloride **38** (19.8 g, 0.105 mol) in AcOH (180 ml) at 80 °C and the mixture was stirred under reflux for 6 h, and allowed to stand at r.t. $H_2O$ was added to the mixture and the precipitate obtained was collected by filtration and washed with $H_2O$ to yield 5,6,7,8-tetrahydro-9*H*-carbazole-1-carboxylic acid[21,28] as a pale yellow solid (10.0 g, 0.465 mmol, 47% based on cyclohexanone), mp 198—200 °C (lit[21] 201—203 °C). A mixture of 5,6,7,8-tetrahydro-9*H*-carbazole-1-carboxylic acid (4.3 g, 20 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (10.0 g, 44 mmol) in toluene (100 ml) was stirred under reflux for 2 h before standing at r.t. The precipitate obtained was collected and purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–MeOH) to yield the title compound as a solid (2.46 g, 11.6 mmol, 58%) mp 271—272 °C (lit[21] mp 270—271 °C). $^1$H-NMR (DMSO-$d_6$) $\delta$ 7.19—7.23 (1H, m), 7.26 (1H, t, $J$=7.6 Hz), 7.40—7.45 (1H, m), 7.74 (1H, d, $J$=8.0 Hz), 8.00 (1H, dd, $J$=0.8, 7.6 Hz), 8.17 (1H, d, $J$=7.6 Hz), 8.41 (1H, dd, $J$=0.8, 7.6 Hz), 11.34 (1H, br s), 13.18 (1H, br s). HR-MS *m/z*: 210.0540 (Calcd for $C_{13}H_8NO_2$ $([M-H]^-)$: 210.0555).

***N*-[2-(Dimethylamino)ethyl]-9*H*-Carbazole-1-carboxamide (40a)** This compound was obtained from **39a** (1.06 g, 5.02 mmol) in a similar manner to the preparation of **30b** as an oil (1.40 g, 4.98 mmol, 99%). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.22 (6H, s), 2.46—2.54 (2H, m), 3.68 (2H, q, $J$=6.8 Hz), 7.15—7.24 (2H, m), 7.37—7.44 (1H, m), 7.70 (1H, d, $J$=7.6 Hz), 7.88 (1H, dd, $J$=0.8, 7.6 Hz), 8.14 (1H, d, $J$=7.6 Hz), 8.29 (1H, dd, $J$=0.8, 7.6 Hz), 8.56 (1H, t, $J$=7.6 Hz), 11.43 (1H, s). HR-MS *m/z*: 282.1604 (Calcd for $C_{17}H_{20}N_3O_1$ $([M+H]^+)$: 282.1606).

**2-[2-(Dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-]carbazole-1,3(2*H*)-**

**dione Hydrochloride (13)** This compound was obtained from **40a** (350 mg, 1.24 mmol) in a similar manner to the preparation of **31b** (200 mg, 0.58 mmol, 47%), mp 278—280 °C (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.90 (6H, br s), 3.44—3.49 (2H, m), 4.41 (2H, t, $J$=5.6 Hz), 7.55—7.60 (1H, m), 7.67—7.72 (2H, m), 8.10 (1H, dd, $J$=0.8, 7.6 Hz), 8.34—8.37 (1H, m), 8.38—8.41 (1H, m), 8.58 (1H, dd, $J$=0.8, 7.6 Hz), 9.62 (1H, br s). ESI-MS $m/z$: 308 $(M+H)^+$, *Anal.* Calcd for $C_{18}H_{17}N_3O_2\cdot$HCl: C, 62.88; H, 5.28; N, 12.22. Found: C, 62.64; H, 5.33; N, 12.12.

***N*-[2-(Dimethylamino)ethyl]-6-nitro-9*H*-carbazole-1-carboxamide (40b) and *N*-[2-(Dimethylamino)ethyl]-3-nitro-9*H*-carbazole-1-carboxamide (40c)** A solution of potassium nitrate (1.01 g, 10.0 mmol) in $H_2SO_4$ (2.5 ml) was added dropwise to a stirred solution of **39a** (2.0 g, 9.5 mmol) in AcOH (250 ml) at 0 °C. After stirring overnight at r.t., $H_2O$ was slowly added to the mixture. The precipitate was collected and washed with $H_2O$ and dried *in vacuo* to yield 2.3 g of crude product which was subsequently dissolved in DMF (100 ml). The solution was treated with CDI (2.9 g, 18 mmol), and stirred for 2 h. The reaction mixture was treated with *N*,*N*-dimethylethylenediamine (4.0 ml, 36 mmol), stirred overnight before addition of $H_2O$. The mixture was extracted with EtOAc–THF solution (1 : 1 in volume). The organic layer was washed successively with $H_2O$, $NaHCO_3$ (aq.), and brine and then concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–EtOH) to yield **40b** (1.46 g, 4.47 mmol, 47%) and **40c** (0.21 g, 0.64 mmol, 7%). Analytical samples were obtained by converting each free base into hydrochloride salts **41b** and **41c** in a usual manner as a yellow solid.

***N*-[2-(Dimethylamino)ethyl]-6-nitro-9*H*-carbazole-1-carboxamide Hydrochloride (41b)** $^1$H-NMR (DMSO-$d_6$) $\delta$[27] 2.85 (6H, br s), 3.26—3.38 (2H, m), 3.73 (2H, q, $J$=5.6 Hz), 7.38 (1H, t, $J$=7.6 Hz), 7.86 (1H, d, $J$=9.0 Hz), 8.09 (1H, d, $J$=7.6 Hz), 8.32 (1H, dd, $J$=2.2, 9.0 Hz), 8.60 (1H, d, $J$=7.6 Hz), 9.04 (1H, t, $J$=5.6 Hz), 9.22 (1H, d, $J$=2.2 Hz), 9.93 (1H, br s), 12.14 (1H, s).

***N*-[2-(Dimethylamino)ethyl]-3-nitro-9*H*-carbazole-1-carboxamide Hydrochloride (41c)** $^1$H-NMR (DMSO-$d_6$) $\delta$[27] 2.84 (6H, br s), 3.20—3.40 (2H, m), 3.65—3.80 (2H, m), 7.31 (1H, t, $J$=7.6 Hz), 7.52 (1H, t, $J$=7.6 Hz), 7.81 (1H, d, $J$=7.6 Hz), 8.41 (1H, d, $J$=7.6 Hz), 8.90 (1H, d, $J$=2.0 Hz), 9.20—9.28 (1H, m) 9.35 (1H, d, $J$=2.0 Hz), 9.64—9.86 (1H, m), 12.14 (1H, br s).

**2-[2-(Dimethylamino)ethyl]-8-nitro-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (21)** Sodium hydride (1.75 g, 40 mmol, 55% dispersion in oil) was added to a stirred solution of **40b** (5.82 g, 17.8 mmol) in DMF (200 ml) and the mixture was stirred for 1 h at r.t. under nitrogen atmosphere. Then the mixture was added a solution of ethyl chloroformate (3.8 ml, 40 mmol) in $CH_2Cl_2$ (10 ml) at 0 °C and stirred for 30 min at the same temperature. The reaction mixture was acidified with 1 N HCl (aq.) and the precipitate was collected before recrystallization from EtOH to yield the title compound as pale yellow crystals (5.54 g, 14.2 mmol, 80%), mp 288—290 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.89 (6H, br s), 3.46—3.50 (2H, m), 4.43 (2H, t, $J$=5.6 Hz), 7.76 (1H, t, $J$=7.6 Hz), 8.19 (1H, d, $J$=7.6 Hz), 8.53—8.60 (2H, m), 8.80 (1H, d, $J$=7.6 Hz), 9.38 (1H, d, $J$=2.0 Hz), 10.18 (1H, br s). ESI-MS $m/z$: 353 $(M+H)^+$, *Anal.* Calcd for $C_{18}H_{16}N_4O_4\cdot$HCl$\cdot$2.15$H_2O$: C, 50.57; H, 5.02; N, 13.10. Found: C, 50.49; H, 4.69; N, 13.09.

**8-Amino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione (44b) and 8-Amino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (14)** A mixture of **21** (5.54 g, 14 mmol) and 50% Pd–C (550 mg) in AcOH (200 ml) and 1 N HCl (aq.) (50 ml) was stirred under 1 atm of hydrogen overnight. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was treated with $H_2O$ and $NaHCO_3$ (aq.), and then the mixture was extracted with EtOAc. The organic layer was washed successively with $H_2O$ and brine and dried over $MgSO_4$ before being concentrated *in vacuo*. The residual solid was recrystallized from EtOH to yield **44b** (3.74 g, 12 mmol, 81%) as yellow crystals. Compound **44b** (430 mg, 1.3 mmol) was suspended in EtOH (20 ml) and 1 N HCl (aq.) was added and stirred overnight. The precipitate obtained was collected by filtration to yield the title compound **14** (480 mg, 100%) as pale yellow crystals, mp 281—283 °C (dec.) (EtOH).

**44b** (Free Base): $^1$H-NMR ($CDCl_3$) $\delta$ 2.36 (6H, s), 2.69 (2H, t, $J$=7.0 Hz), 4.32 (2H, t, $J$=7.0 Hz), 6.92 (1H, dd, $J$=2.4, 8.8 Hz), 7.29 (1H, d, $J$=2.4 Hz), 7.50 (1H, t, $J$=7.6 Hz), 8.09—8.12 (2H, m), 8.23 (1H, d, $J$=8.8 Hz).

**14** (HCl Salt): $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.91 (6H, br s), 3.44—3.50 (2H, m), 4.39 (2H, t, $J$=5.6 Hz), 7.04—7.08 (1H, m), 7.54—7.56 (1H, m), 7.63 (1H, t, $J$=5.6 Hz), 8.05 (1H, dd, $J$=0.8, 7.6 Hz), 8.12 (1H, d, $J$=8.8 Hz), 8.44 (1H, dd, $J$=0.8, 7.6 Hz), 9.60 (1H, br s). ESI-MS $m/z$: 323 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{18}N_4O_2\cdot$HCl$\cdot$1.3$H_2O$: C, 56.56; H, 5.70; N, 14.66. Found: C, 56.21; H, 5.31; N, 14.56.

**8-Acetylamino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (15)** A mixture of **44b** (1.61 g, 4.99 mmol) and acetic anhydride (15 ml) and pyridine (15 ml) was stirred at r.t. for 3 h. The reaction mixture was added ethyl acetate and the precipitate obtained was collected by filtration before being suspended in EtOH. The suspension was treated with an excess amount of 1 N HCl (aq.), and the precipitate obtained was collected to yield the title compound as a white solid (1.81 g, 4.51 mmol, 90%), mp 285—287 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.12 (3H, s), 2.92 (6H, br s), 3.45—3.52 (2H, m), 4.40 (2H, t, $J$=5.6 Hz), 7.67 (1H, t, $J$=7.6 Hz), 7.74 (1H, dd, $J$=2.0, 8.8 Hz), 8.10 (1H, dd, $J$=0.8, 7.6 Hz), 8.30 (1H, d, $J$=8.8 Hz), 8.53 (1H, dd, $J$=0.8, 7.6 Hz), 8.62 (1H, d, $J$=2.0 Hz), 9.29 (1H, br s), 10.32 (1H, s). ESI-MS $m/z$: 365 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{20}N_4O_3\cdot$HCl$\cdot H_2O$: C, 57.35; H, 5.53; N, 13.38. Found: C, 57.18; H, 5.42; N, 13.31.

**8-Pentanoylamino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (25)** A mixture of **44b** (64 mg, 0.20 mmol) and *n*-pentanoyl chloride (0.028 ml, 0.24 mmol) and triethylamine (0.084 ml, 0.60 mmol) in $CH_2Cl_2$ (10 ml) was stirred at r.t. for 3 h. The reaction mixture was added $H_2O$ and then extracted with EtOAc. The organic layer was washed successively with $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was dissolved in EtOH and treated with an excess amount of 1 N HCl (aq.) before evaporation. The residual solid was suspended in EtOH–hexane (1 : 1 in volume). The precipitate was collected to yield the title compound as a pale yellow solid (65 mg, 0.15 mmol, 75%), mp 234—236 °C (EtOH–hexane). $^1$H-NMR (DMSO-$d_6$) $\delta$ 0.93 (2H, t, $J$=7.6 Hz), 1.36 (2H, m), 1.63 (2H, m), 2.38 (2H, t, $J$=7.6 Hz), 3.46—3.49 (2H, m), 4.39 (2H, q, $J$=5.6 Hz), 7.66 (1H, t, $J$=7.6 Hz), 7.73 (1H, dd, $J$=2.0, 8.8 Hz), 8.09 (1H, d, $J$=7.6 Hz), 8.28 (1H, d, $J$=8.8 Hz), 8.51 (1H, d, $J$=7.6 Hz), 8.63 (1H, d, $J$=2.0 Hz), 9.25 (1H, br s), 10.22 (1H, s). ESI-MS $m/z$: 407 $(M+H)^+$, *Anal.* Calcd for $C_{23}H_{26}N_4O_3\cdot$HCl$\cdot$1.5$H_2O$: C, 58.78; H, 6.43; N, 11.92. Found: C, 58.71; H, 6.40; N, 12.22.

**2-[2-(Dimethylamino)ethyl]-8-methanesulfonylamino-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (22)** A mixture of **14** (200 mg, 0.56 mmol) and methanesulfonic anhydride (976 mg, 5.6 mmol) and pyridine (20 ml) was stirred under reflux for 1 h. The mixture was evaporated and $NaHCO_3$ (aq.) was added, which was extracted with EtOAc–THF solution (1 : 1 in volume). The organic layer was washed successively with $H_2O$ and brine, and dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–MeOH) to furnish the title compound as a free base, which was hydrochlorinated to yield the title compound as a brown solid (110 mg, 0.25 mmol, 45%), mp 285—288 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$[27] 2.90 (6H, br s), 3.05 (3H, s), 3.42—3.52 (2H, m), 4.35—4.42 (2H, m), 7.47—7.55 (1H, m), 7.67 (1H, t, $J$=7.6 Hz), 8.06—8.16 (2H, m), 8.32 (1H, d, $J$=8.8 Hz), 8.57 (1H, d, $J$=7.6 Hz), 9.35 (1H, br s), 10.01 (1H, s). ESI-MS $m/z$: 401 $(M+H)^+$, *Anal.* Calcd for $C_{19}H_{20}N_4O_4S\cdot$HCl$\cdot H_2O$: C, 50.16; H, 5.10; N, 12.32. Found: C, 50.17; H, 5.02; N, 12.30.

**2-[2-(Dimethylamino)ethyl]-5-nitro-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (43c)** The title compound's free base (**42c**) was obtained from **40c** (210 mg, 0.64 mmol) in a similar manner to the preparation of **21**. Yield of **42c**: 165 mg, 0.47 mmol, 73%. A part of **42c** (10 mg, 0.028 mmol) obtained above was converted into its HCl salt (**43c**) in a usual manner as a white solid (10 mg, 0.026 mmol, 93% based on the free base **42c**).

**42c** (Free Base of **43c**): $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.24 (6H, s), 2.56 (2H, t, $J$=6.8 Hz), 4.16 (2H, t, $J$=6.8 Hz), 7.61—7.65 (1H, m), 7.75—7.79 (1H, m), 8.42 (1H, d, $J$=8.4 Hz), 8.57 (1H, dd, $J$=0.8, 7.6 Hz), 8.75 (1H, d, $J$=2.0 Hz), 9.52 (1H, d, $J$=2.0 Hz).

**43c**: $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.83 (6H, br s), 3.28—3.46 (2H, m), 4.36—4.44 (2H, m), 7.63—7.68 (1H, m), 7.77—7.82 (1H, m), 8.41—8.44 (1H, m), 8.59—8.62 (1H, m), 8.78 (1H, d, $J$=2.0 Hz), 9.57 (1H, d, $J$=2.0 Hz), 9.60—9.88 (1H, m). *Anal.* Calcd for $C_{18}H_{16}N_4O_4\cdot$HCl$\cdot$0.4$H_2O$: C, 54.59; H, 4.53; N, 14.15. Found: C, 54.65; H, 4.36; N, 14.14.

**5-Amino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Dihydrochloride (16)** The title compound was obtained from **42c** (150 mg, 0.43 mmol) in a similar manner to the preparation of **7** as a white solid (170 mg, 0.43 mmol, 99%), mp 294—297 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.84—2.94 (6H, m), 3.42—3.52 (2H, m), 4.40 (2H, t, $J$=5.6 Hz), 7.54—7.59 (1H, m), 7.67—7.73 (1H, m), 7.86 (1H, d, $J$=1.6 Hz), 8.23 (1H, d, $J$=1.6 Hz), 8.32—8.38 (2H, m), 10.05 (1H, br s).

ESI-MS *m/z*: 323 $(M+H)^+$, *Anal.* Calcd for $C_{18}H_{18}N_4O_2 \cdot 2HCl \cdot 2H_2O$: C, 50.12; H, 5.61; N, 12.99. Found: C, 50.24; H, 5.26; N, 13.07.

**5-Acetylamino-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (17)** This compound was obtained from **16** (20 mg, 0.051 mmol) in a similar manner to the preparation of **15** (20 mg, 0.050 mmol, 98%) as a pale yellow solid, mp >300 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.15 (3H, s), 2.89—2.94 (6H, m), 3.45—3.51 (2H, m), 4.40 (2H, t, $J$=5.8 Hz), 7.54—7.58 (1H, m), 7.67—7.72 (1H, m), 8.30 (1H, d, $J$=7.6 Hz), 8.34—8.38 (2H, m), 8.66 (1H, d, $J$=1.6 Hz), 9.57 (1H, br s), 10.53 (1H, s). ESI-MS *m/z*: 365 $(M+H)^+$, *Anal.* Calcd for $C_{20}H_{20}N_4O_3 \cdot HCl \cdot 0.4H_2O$: C, 58.87; H, 5.38; N, 13.73. Found: C, 58.95; H, 5.40; N, 13.47.

**2-[2-(Dimethylamino)ethyl]-8-(1-pyrrolyl)-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (23)** To a stirred solution of **44b** (96 mg, 0.30 mmol) in AcOH (10 ml) was added 2,5-dimethoxytetrahydrofuran (0.039 ml, 0.30 mmol). The mixture was stirred under reflux for 30 min, and then allowed to stand at r.t. The reaction mixture was concentrated *in vacuo*. $NaHCO_3$ (aq.) was added to the residue and then the mixture was extracted with EtOAc. The organic layer was washed successively with $H_2O$ and brine and dried over $MgSO_4$, and then concentrated *in vacuo*. The residual solid was recrystallized from EtOH–hexane to yield the free base of the title compound (85 mg, 0.21 mmol) which was hydrochlorinated in a usual manner to yield the title compound as a pale yellow solid (80 mg, 0.196 mmol, 65%), mp 258—260 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.90 (6H, s), 3.47 (2H, m), 4.41 (2H, d, $J$=5.6 Hz), 6.34 (2H, t, $J$=2.0 Hz), 7.51 (2H, t, $J$=2.0 Hz), 7.71 (1H, t, $J$=7.6 Hz), 7.91 (1H, dd, $J$=2.0, 8.8 Hz), 8.12 (1H, dd, $J$=0.8, 7.6 Hz), 8.38 (1H, d, $J$=8.8 Hz), 8.59 (1H, dd, $J$=0.8, 7.6 Hz), 8.62 (1H, t, $J$=2.0 Hz), 9.62 (1H, br s). ESI-MS *m/z*: 373 $(M+H)^+$, *Anal.* Calcd for $C_{22}H_{20}N_4O_2 \cdot HCl \cdot 0.6H_2O$: C, 62.96; H, 5.33; N, 13.35. Found: C, 62.78; H, 5.42; N, 13.33.

**2-[2-(Dimethylamino)ethyl]-8-hydroxy-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (19)** To a stirred suspension of **44b** (160 mg, 0.50 mmol) in sulfuric acid (1.5 ml) and $H_2O$ (5 ml), a solution of sodium nitrite (42 mg, 0.61 mmol) in $H_2O$ (0.5 ml) was added dropwise with stirring at 0 °C. After stirring at the same temperature for 20 min, urea (60 mg, 1.0 mmol), a solution of copper nitrate trihydrate (2.4 g, 10 mmol) in $H_2O$ (30 ml), and copper (I) oxide (90 mg, 0.63 mmol) were added and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was added $NaHCO_3$ (aq.), before extraction with EtOAc. The organic layer was washed successively with $H_2O$ and brine, and then concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–EtOH) to furnish the title compound as a free base, which was converted into HCl salt in a usual manner to yield the title compound as a yellow solid (50 mg, 0.14 mmol, 28%), mp 286—287 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$[27] 2.87 (6H, br s), 3.36—3.48 (2H, m), 4.32—4.40 (2H, m), 7.09 (1H, dd, $J$=2.4, 8.8 Hz), 7.62 (1H, t, $J$=7.6 Hz), 7.63 (1H, d, $J$=2.4 Hz), 8.04 (1H, d, $J$=7.6 Hz), 8.15 (1H, d, $J$=8.8 Hz), 8.48 (1H, d, $J$=7.6 Hz), 9.30 (1H, br s), 9.84 (1H, s). ESI-MS *m/z*: 324 $(M+H)^+$, *Anal.* Calcd for $C_{18}H_{17}N_3O_3 \cdot HCl$: C, 60.09; H, 5.04; N, 11.68. Found: C, 59.91; H, 5.19; N, 11.53.

**2-[2-(Dimethylamino)ethyl]-8-methoxy-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (20)** Sodium hydride (5.0 mg, 0.11 mmol, 55% dispersion in oil) was added to **19** (18 mg, 0.050 mmol) in DMF (20 ml) and the mixture was stirred for 20 min at 0 °C. After addition of methyl iodide (0.003 ml, 0.05 mmol), the mixture was stirred for 1 h at 0 °C, and then acidified with 1 N HCl (aq.). The mixture was neutralized with $NaHCO_3$ (aq.), which was extracted with EtOAc–THF solution (1 : 1 in volume). The organic layer was washed successively with $H_2O$ and brine and then dried over $MgSO_4$ and the organic layer was concentrated *in vacuo*. The solid obtained was washed with isopropyl ether and EtOH to furnish the title compound as a free base, which was hydrochlorinated in a usual manner to yield the title compound as an orange solid (6.0 mg, 0.016 mmol, 32%), mp 280—281 °C (dec.) (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.92 (6H, br s), 3.46—3.51 (2H, m), 3.92 (3H, s), 4.40 (2H, t, $J$=5.6 Hz), 7.27 (1H, dd, $J$=2.6, 9.0 Hz), 7.68 (1H, t, $J$=7.6 Hz), 7.97 (1H, d, $J$=2.6 Hz), 8.09 (1H, dd, $J$=0.8, 7.6 Hz), 8.26 (1H, d, $J$=9.0 Hz), 8.57 (1H, dd, $J$=0.8, 7.6 Hz), 9.43 (1H, br s). ESI-MS *m/z*: 338 $(M+H)^+$, *Anal.* Calcd for $C_{19}H_{19}N_3O_3 \cdot HCl \cdot 0.2H_2O$: C, 60.46; H, 5.45; N, 11.13. Found: C, 60.43; H, 5.37; N, 11.08.

**6-Acetyl-9*H*-carbazole-1-carboxylic Acid (46a)** Acetic anhydride (0.208 ml, 2.20 mmol) was added to a stirred suspension of $AlCl_3$ (0.880 g, 6.60 mmol) in dry $CHCl_3$ (12 ml) with stirring and then a suspension of **39a** (422 mg, 2.00 mmol) in dry $CHCl_3$ (4 ml) was added to the mixture at 0 °C. After being allowed to stand at r.t. and stirred overnight. Then, the reaction mixture was poured into ice-water and an excess amount of 1 N HCl (aq.) was added and the mixture was extracted with EtOAc–THF solution (1 : 1 in volume). Organic layer was separated and $NaHCO_3$ (aq.) was added to the organic layer until the aqueous phase was neutralized to pH 7. The organic layer was separated and washed successively with $H_2O$ and brine, and dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–MeOH) to yield the title compound (370 mg, 1.46 mmol, 73%) as a solid. $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.68 (3H, s), 7.34 (1H, t, $J$=7.6 Hz), 7.80 (1H, d, $J$=8.8 Hz), 8.03—8.06 (1H, m), 8.06 (1H, dd, $J$=1.6, 8.8 Hz), 8.55 (1H, d, $J$=7.6 Hz), 8.91 (1H, d, $J$=1.6 Hz), 11.73 (1H, br s).

**6-Acetyl-*N*-[2-(dimethylamino)ethyl]-9*H*-carbazole-1-carboxamide Hydrochloride (28)** The free base of the title compound was obtained from **46a** (430 mg, 1.70 mmol) in a similar manner to the preparation of **30b**. Yield: 450 mg, 1.39 mmol, 82% based on **46a**. A portion of the free base (175 mg, 0.54 mmol) was hydrochlorinated in a usual manner to yield the title compound as a white solid (170 mg, 0.47 mmol, 87% based on the free base of the title compound), mp 188—190 °C (EtOH).

Free Base of **28**: $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.22 (6H, s), 2.47—2.52 (2H, m), 2.67 (3H, s), 3.48 (2H, q, $J$=6.8 Hz), 7.30 (1H, t, $J$=7.7 Hz), 7.76 (1H, d, $J$=7.7 Hz), 7.84 (1H, d, $J$=7.7 Hz), 8.04 (1H, dd, $J$=1.2, 7.7 Hz), 8.45 (1H, d, $J$=7.7 Hz), 8.65 (1H, br s), 8.87 (1H, d, $J$=1.2 Hz), 11.85 (1H, br s).

The Title Compound (**28**): $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.68 (3H, s), 2.87 (6H, s), 3.27—3.47 (2H, m), 3.74 (2H, q, $J$=5.6 Hz), 7.35 (1H, t, $J$=8.0 Hz), 7.78 (1H, d, $J$=8.4 Hz), 8.02—8.06 (2H, m), 8.50 (1H, d, $J$=8.0 Hz), 8.89 (1H, d, $J$=1.6 Hz), 8.96 (1H, t, $J$=5.6 Hz), 9.84 (1H, br s), 11.80 (1H, s). HR-MS *m/z*: 324.1711 (Calcd for $C_{19}H_{22}N_3O_2$ ($[M+H]^+$): 324.1712).

**8-Acetyl-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (26)** The free base of **26** (180 mg, 0.56 mmol) was obtained from **46a** (300 mg, 1.2 mmol) in a similar manner to the preparation of **30b** and **31b**. The free base of **26** (180 mg, 0.56 mmol) was hydrochlorinated into the title compound in a usual manner to yield a white solid, mp 282—284 °C (dec.) (EtOH). Yield: 125 mg, 0.32 mmol, 27% based on **46a**. $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.75 (3H, s), 2.90—2.94 (6H, m), 3.48—3.52 (2H, m), 4.42 (2H, t, $J$=5.6 Hz), 7.75 (1H, t, $J$=8.0 Hz), 8.16 (1H, d, $J$=8.0 Hz), 8.31 (1H, dd, $J$=1.6, 8.8 Hz), 8.49 (1H, d, $J$=8.8 Hz), 8.73 (1H, d, $J$=8.0 Hz), 9.05 (1H, d, $J$=1.6 Hz), 9.44 (1H, br s). ESI-MS *m/z*: 350 $(M+H)^+$, *Anal.* Calcd for $C_{20}H_{19}N_3O_3 \cdot HCl$: C, 62.26; H, 5.22; N, 10.89. Found: C, 61.97; H, 5.26; N, 10.80.

**6-Butyryl-9*H*-carbazole-1-carboxylic Acid (46b)** This compound was obtained from **39a** (422 mg, 2.00 mmol) using *n*-butyryl chloride (0.208 ml, 2.00 mmol) and $AlCl_3$ (0.800 g, 6.00 mmol) in a similar manner to the preparation of **46a** as a solid. (500 mg, 1.78 mmol, 89%). $^1$H-NMR (DMSO-$d_6$) $\delta$ 0.99 (3H, t, $J$=7.6 Hz), 1.66—1.76 (2H, m), 3.12 (2H, t, $J$=7.6 Hz), 7.34 (1H, t, $J$=8.0 Hz), 7.80 (1H, d, $J$=8.8 Hz), 8.03—8.06 (2H, m), 8.57 (1H, d, $J$=8.0 Hz), 8.92 (1H, d, $J$=1.6 Hz), 11.72 (1H, br s).

**8-Butyryl-2-[2-(dimethylamino)ethyl]-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (24)** CDI (580 mg, 3.6 mmol) was added to a stirred solution of **46b** (500 mg, 1.8 mmol) in DMF (30 ml) at r.t. and stirred for 1.5 h. Then, *N*,*N*-dimethylethylenediamine (0.79 ml, 7.2 mmol) was added and the mixture was stirred overnight. After being treated with $H_2O$, the mixture was extracted with EtOAc. The organic layer was washed successively with $H_2O$, $NaHCO_3$ (aq.) and brine, and dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was stirred in EtOAc–EtOH–hexane (1 : 1 : 1 in volume) and the precipitate obtained was collected to yield crude *N*-[2-(dimethylamino)ethyl]-6-butyryl-9*H*-carbazole-1-carboxamide (420 mg) which was dissolved in DMF (20 ml). Sodium hydride (100 mg, 2.3 mmol, 55% dispersion in oil) was added to the mixture at r.t. and stirred for 30 min and added ethyl chloroformate (0.23 ml, 2.4 mmol) at 0 °C and stirred for 30 min at the same temperature. The mixture was acidified with 1 N HCl (aq.) and then neutralized by $NaHCO_3$ (aq.) and then extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$ before being concentrated *in vacuo*. The residue was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$–EtOH) to furnish the title compound as a free base, which was hydrochlorinated to yield the title compound as a white solid (380 mg, 0.92 mmol, 92%), mp 219—220 °C (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.00 (3H, t, $J$=7.4 Hz), 1.67—1.78 (2H, m), 2.89 (6H, br s), 3.18 (2H, t, $J$=7.1 Hz), 3.38—3.55 (2H, m), 4.41 (2H, t, $J$=5.7 Hz), 7.75 (1H, t, $J$=7.7 Hz), 8.15 (1H, dd, $J$=0.5, 7.7 Hz), 8.31 (1H, dd, $J$=1.6, 8.6 Hz), 8.48 (1H, d, $J$=8.6 Hz), 8.73 (1H, dd, $J$=0.5, 7.7 Hz), 9.04 (1H, d, $J$=1.6 Hz), 9.54 (1H, br s). ESI-MS *m/z*: 378 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{23}N_3O_3 \cdot HCl \cdot 1.2H_2O$: C, 60.67; H, 6.11; N, 9.65. Found: C, 60.76; H, 5.92; N, 9.72.

**6-Methyl-9*H*-carbazole-1-carboxylic Acid (45)** A suspension of 2-hydrazinobenzoic acid hydrochloride **38** (1.0 g, 5.5 mmol) in AcOH (50 ml)

was refluxed under stirring and a solution of 4-methylcyclohexanone (0.61 ml, 5.0 mmol) in AcOH (10 ml) was dropped to the mixture, and AcOH (20 ml) was added. The mixture was stirred under reflux for 6 h and then allowed to cool. After adding $H_2O$, the precipitate that formed was collected by filtration, washed with $H_2O$ and dried *in vacuo* to yield crude 6-methyl-5,6,7,8,9*H*-carbazole-1-carboxylic acid (1.04 g) as a white solid. The crude product (1.04 g) was added mesitylene (50 ml) and 10% Pd–C (300 mg). The reaction mixture was stirred under reflux for 10 h and allowed to cool. The catalyst was removed by filtration and washed with THF. The filtrate was concentrated *in vacuo* and the crystals formed during evaporation was collected and washed with EtOH to yeild the title compound as a white solid (560 mg, 2.49 mmol, 50%). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.47 (3H, s), 7.20—7.26 (2H, m), 7.62 (1H, d, $J$=8.4 Hz), 7.95—7.98 (2H, m), 8.34 (1H, dd, $J$=0.8, 7.6 Hz).

**2-[2-(Dimethylamino)ethyl]-8-methyl-1*H*-pyrimido[5,6,1-*jk*]carbazole-1,3(2*H*)-dione Hydrochloride (18)** This compound was obtained from **45** (560 mg, 2.49 mmol) in a similar manner to the preparation of **24** as a white solid (400 mg, 1.12 mmol, 45%), mp 284—286 °C (EtOH). $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.51 (3H, s), 2.88 (6H, br s), 3.42—3.47 (2H, m), 4.38 (2H, t, $J$=5.8 Hz), 7.47—7.50 (1H, m), 7.65 (1H, t, $J$=7.6 Hz), 8.05 (1H, dd, $J$=0.8, 7.6 Hz), 8.12—8.14 (1H, m), 8.23 (1H, d, $J$=8.8 Hz), 8.49 (1H, dd, $J$=0.8, 7.6 Hz), 9.65 (1H, br s). ESI-MS $m/z$: 322 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{19}N_3O_2 \cdot HCl \cdot 0.2H_2O$: C, 63.14; H, 5.69; N, 11.63. Found: C, 63.00; H, 5.61; N, 11.58.

**Biological Experiment. Cells** Murine leukemia P388 was obtained from the Cancer Chemotherapy Center, Japan Foundation for Cancer Research (Tokyo, Japan). Human oral cancer KB was purchased from American Type Culture Collection (Rockville, MD, U.S.A.).

***In Vitro* Growth Inhibition Assay** Exponentially growing tumor cells ($1.25\times10^3$ cells) in 0.1 ml of medium were seeded in 96-well plates on day 0. On day 1, 0.1 ml aliquots of medium containing graded concentrations of test drugs were added to the cell plates. After incubation at 37 °C for 72 h, the cell number was determined by MTT assay.[29] 50% inhibitory concentration ($IC_{50}$) values were calculated as the drug concentrations that reduced the number of cells to 50% of the control number.

**DNA–Protein Complex Formation Assay Using the SDS-KCl** DNA–protein complex formation was detected by using the SDS-KCl method as described by Trask *et al.*[30,31] with some modifications. P388 cells ($1\times10^5$ cells in 1 ml of culture medium) were radiolabeled with 0.4 $\mu$Ci/ml of [$^3$H]thymidine at 37 °C for 24 h, washed with 1 ml of PBS and incubated in culture medium for 12 h. Cells were treated with each drug at the concentration of 2 $\mu$M, 10 $\mu$M and 50 $\mu$M at 37 °C for 1 h, collected using a microcentrifuge and then lysed at 65 °C in 1.25% SDS, 5 mM EDTA (pH 8.0), 0.4 mg/ml salmon testis DNA. The lysate was incubated at 65 °C for 10 min and brought to 65 mM KCl (using 325 mM KCl). This mixture was rapidly vortexed for 10 s and held on ice for 30 min. The precipitate that formed was collected using a microcentrifuge, suspended in 1 ml of 10 mM Tris–HCl, pH 7.5, 10 mM KCl, 2 mM EDTA and trapped on a GF/C filter (Whatman International Ltd., Maidstone, England). The filter was washed 5 times with 1 ml of 10 mM Tris–HCl, pH 7.5, 10 mM KCl, 2 mM EDTA and then transferred to a liquid scintillation vial for determination of the radioactivity in the pellet.

The ability of a compound to form DNA–protein cross-linking was graded according to the ratio of the maximum radio activity at any concentration (2.0, 10 or 50 $\mu$M) of a compound to the radioactivity of m-AMSA at 50 $\mu$M. For example, when the maximaum radioactivity of a compound at any concentration was 10—30% of the radioactivity of m-AMSA at 50 $\mu$M, the result was indicated as +. Other ratio of the maximum radio activity at any concentration of a compound to the radioactivity of m-AMSA at 50 $\mu$M were graded accordingly: 30—50% (++), 50—70% (+++).

***In Vivo* Antitumor Activity** The antitumor activity of the compounds was evaluated in two *in vivo* experimental murine models: P388 leukemia and M5076 ovarian sarcoma. P388 cells were innocuated ip ($10^6$ cells/mouse) into CDF1 mice on day 0. The drugs were dissolved or suspended in saline, and injected ip on day 1 to 4 once daily. The results are expressed as the percent increase in lifespan (ILS). (ILS (%)=100×(lifespan of treated animals−lifespan of controls)/(lifespan of controls)), using the mean values for groups of at least 5 mice each. M5076 cells were implanted sc into the flank of mice on day 0. The drugs were dissolved or suspended in saline, and injected ip on day 1 to 4 once daily. Mice were sacrificed on day 21, and respective tumor weights were measured. The results are expressed as percent T/C (mean tumor weight of treated animals divided by mean tumor weight of controls×100), using the mean values for the groups of at least 5 mice. In *in vivo* experiments, the body weight of mice was measured on day 7 unless as an indicator of toxicity. The relative body weight was expressed as the mean body weight of treated mice divided by the mean body weight of mice before the treatment on day 0.

**Detection of DNA–Topoisomerase II Cross-Linking Using Antibody to Topoisomerase II** DNA–topo II cross-linking was measured as described previously.[32,33] P388 cells ($2.5\times10^6$ cells in 2 ml of culture medium) in the exponential growth phase were treated with 0.4, 2, 10 and 50 $\mu$M 26 (ER-37326) or 50 $\mu$M etoposide and incubated for 1 h along with a negative control (no drug). Cells were centrifuged and lysed in 1 ml of TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA) containing 1% Sarkosyl. The lysate was layered onto a CsCl step gradient (1 ml each of 4 different CsCl concentrations) and centrifuged in a Beckman SW50.1 rotor (31000 rpm, 18 h, 25 °C). Fractions (200 $\mu$l each) were carefully collected from the top by using a Pipetman. DNA concentration was determined by reading the absorbance at 260 nm. The DNA peak fractions were collected and the DNA concentration of each solution was adjusted. One hundred microliters of the equal concentration of DNA solution was diluted with 200 $\mu$l of 25 mM sodium phosphate buffer (pH 6.5) and applied under vacuum to a nitrocellulose membrane, which had been presoaked in 28 mM sodium phosphate buffer (pH 6.5). The nonspecific binding sites were blocked by treatment with 5% Blotto (50 mM Tris–HCl, pH 7.4, 100 mM NaCl, 5% nonfat dry milk) in TBS-T (20 mM Tris–HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20). The membrane was incubated with a rabbit polyclonal antibody to topoisomerase II (TopoGEN, Columbus, OH, U.S.A.) as a primary antibody for 3 h. After three washings with TBS-T (10 min each), the membrane was incubated with goat anti-rabbit conjugated to horseradish peroxidase as a secondary antibody for 1 h. After washing with TBS-T, the final detection of the blot was carried out by using the ECL Western Blotting Detection system (Amersham, Arlington Heights, IL, U.S.A.).

## References and Notes


1) These authors contributed equally to this work.
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26) This compound was reported to have activity against P388 *in vivo*. (q1d×5, 60% of ILS at 25 mg/kg/d). See literature in ref. 19.
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