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Synthesis and Biological Evaluation of New Amino Acid and Dipeptide Derivatives of Neocryptolepine as Anticancer Agents

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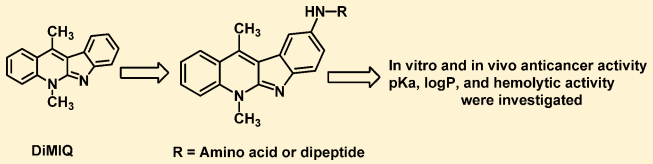
Supporting Information

ABSTRACT: The syntheses of neocryptolepine derivatives containing an amino acid or a dipeptide at the C-9 position and their evaluation for antitumor activity in vitro and in vivo are reported. To establish the influence of an amino acid or a peptide on the physicochemical properties of 5*H*-indolo[2,3-*b*]quinoline (DiMIQ), lipophilic and hemolytic properties were investigated. Most of the compounds displayed a high antiproliferative activity in vitro and strongly inhibited growth of tumor in mice compared to cyclophosphamide. The attachment of the hydrophilic amino acid or the peptide to the hydrophobic DiMIQ increased its hydrophilic properties and decreased its hemolytic activity. The glycylglycine conjugate (**7a**) was the most promising derivative. It strongly inhibited the growth of the tumor in mice (at dose 50 mg kg^{-1} day^{-1} it inhibited the tumor growth by 46−63% on days 11−16 and by 29−43% on days 18−23) and significantly decreased hemolytic activity and lowered the in vivo toxicity compared to DiMIQ.



INTRODUCTION

It is well-known that natural indoloquinoline alkaloids like neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline), cryptolepine (5-methyl-5*H*-indolo[3,2-*b*]quinoline), and isocryptolepine (5-methyl-5*H*-indolo[3,2-*c*]quinoline) display potent cytotoxic activity against several tumor cell lines, and it is believed that this is due to the interaction with the DNA. However, despite intensive investigations of indoloquinolines (hundreds of analogues in the past 30 years), the mechanism of their action is still controversial.[1]

It was found that 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline (**I**, DiMIQ), the analogue of neocryptolepine, is the most promising lead compound for potential anticancer agents among different derivatives of indoloquinolines.[2] Contrary to its 6,11-dimethyl-6*H*-analogue (**II**) (Figure 1), DiMIQ displays potent antiproliferative activity in vitro.[2] The activity of DIMIQ derivatives results from the ability to intercalate the DNA and create a drug−DNA−topoisomerase II complex.[3,4] Unfortunately, the solubility of DiMIQ in aqueous solutions, especially at neutral pH, is very low and seriously limits the practical application of this compound in the treatment of cancer.[5] In the hope to improve the bioavailability of the indoloquinoline system, the new derivatives of 5*H*- and 6*H*-indolo[2,3-*b*]quinolines substituted with methyl,[4] methoxy, 6-dimethylaminoalkyl,[6] glycosyl, aminoglycosyl,[7] and alkylaminoalkyl[8] were synthesized. Although a lot of work has been done, the potential of indoloquinolines as a drug is far from being completely explored because all of the new derivatives of indolo[2,3-*b*]quinoline, despite their high antitumor activity, exhibited high toxicity or poor solubility in water. The studies showed impaired bioavailability of 5*H*-indolo[2,3-*b*]quinoline derivatives which brought necessity for further work on the modification of the indoloquinoline ring structure.

Therefore, there is still a need to look for new derivatives of indolo[2,3-*b*]quinoline possessing a high anticancer activity

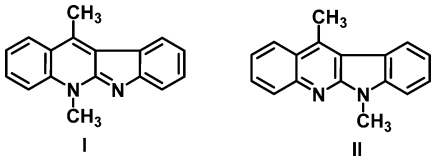


Figure 1. Structures of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline (**I**, DiMIQ, IC_{50} = 1.14 μM against KB cell line) and 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline (**II**) (IC_{10} = 12.3 mM against KB cell line).

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accompanied by a low general toxicity and a good solubility in aqueous medium, improving their bioavailability.

As it was presented in our previous paper, the transformation of inactive 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline (**II**, IC_{10} = 12.3 mM against oral carcinoma, KB cells) into its amino acid derivatives results in a pronounced cytotoxicity of the obtained conjugates.[9] The derivatives substituted with glycylamide (IC_{50} = 3.32 μM) and with L-prolylamide (IC_{50} = 4.39 μM) exhibit the highest antiproliferative activity, comparable to that of DiMIQ, (IC_{50} = 1.14 μM). Compounds bearing L-histidylamide and L-serinylamide also have a high antiproliferative activity, and the values of IC_{50} for these compounds range from 5.96 to 10.77 μM.

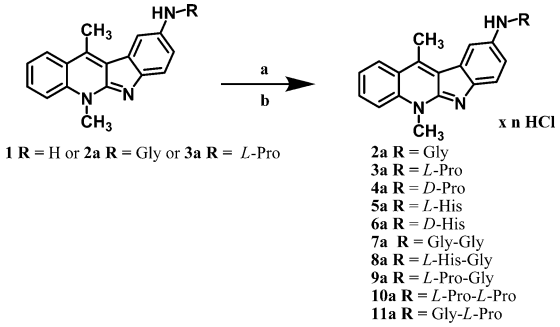
These results encourage us to continue the SAR study of amino acid or peptide derivatives of DiMIQ (**I**). It is well-known that attachment of an amino acid or a peptide to the drug can increase its selectivity.[10] Several L- and D- amino acids were introduced into the anthraquinone skeleton, and numerous derivatives were synthesized for the evaluation of the anticancer activity. For example, such conjugates as conjugates of doxorubicin, daunorubicin, and mitoxantrone with an amino acid or peptides show more selective antitumor activity than the drugs themselves.[11−20] The attachment of an amino acid or a peptide to the drug can also increase the solubility in water of the new compound.[21,22]

In light of these facts and in the hope to obtain some novel compounds with a significant anticancer activity, this work reports the synthesis of a novel series of amino acid and dipeptide derivatives of DiMIQ (**I**) and the testing of these compounds for their in vitro and in vivo activity. At first the antiproliferative activity in vitro against KB cells was screened for all the new obtained compounds. The most promising compounds, selected on the basis of this screening, were tested for their antiproliferative activity against non-small-cell lung cancer (A549), breast cancer (MCF-7), and colon cancer (LoVo). In addition to the above cancer cell lines, compounds were also screened for normal cell lines. The hemolysis of human erythrocytes was also investigated in order to obtain additional data on potential toxic effects of the tested compounds. On the basis of the above studies, four compounds were selected for the in vivo tests. Their physicochemical properties (pK_a and log *P*) were also examined.

RESULTS AND DISCUSSION

Chemistry. This paper presents the synthesis of the amino acid derivatives of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline (**I**) by the reaction of its 9-aminoderivative (**1**) with selected amino acids such as glycine, L-proline, D-proline, L-histidine, and D-histidine. The peptide derivatives of 5,11-dimethyl-5*H*-indolo-[2,3-*b*]quinoline were also synthesized. The derivatives of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline with such dipeptides as Gly-Gly, Gly-L-Pro, L-Pro-Gly, L-Pro-L-Pro, and L-His-Gly were obtained by a "step by step" method. The synthesis of the amino acid and peptide conjugates of **1** is outlined in Scheme 1. The structures of the new compounds and the yields are summarized in Tables 1 and 2. The starting **1** was not commercially available and was synthesized as described previously.[4,6,23] Compound **1** was then coupled with the commercially available *N*-Boc protected L- and D-amino acids. The coupling was achieved using the 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) method.[24] The activation method was selected for the coupling based on our recent results which showed that this method

Scheme 1. Synthesis of Hydrochloride Amino Acid and Dipeptide Derivatives of DiMIQ[a]



1 R = H or **2a R** = Gly or **3a R** = *L*-Pro

2a R = Gly
3a R = *L*-Pro
4a R = *D*-Pro
5a R = *L*-His
6a R = *D*-His
7a R = Gly-Gly
8a R = *L*-His-Gly
9a R = *L*-Pro-Gly
10a R = *L*-Pro-*L*-Pro
11a R = Gly-*L*-Pro

[a]Reagents and conditions: (a) Boc-AA, TBTU, HOBt, DIPEA, DMF, rt, 6−24 h; (b) 2.2 M HCl/methanol, rt, 2 h.

gave the best yields in a relatively short reaction time.[9] All the coupling reactions were performed in DMF (because of a very poor solubility of substrate **1** in the majority of organic solvents like DCM or THF) at room temperature for 2−24 h. In all cases Boc-N^{α}-L-amino acid or Boc-N^{α}-D-amino acids derivatives of **1**, compounds **2−6**, were separated by extraction and purified by column chromatography on silica gel, and the yield after the purification was between 55% and 67%. In the next step Boc groups were removed by 2.2 M HCl in methanol. The deprotection gave the corresponding hydrochlorides **2a−6a** as the final products with good yields ranging from 80% to 95%. The purity of the obtained final products was proved by elemental analysis and the C-18 RP-HPLC method using acetonitrile−water as the mobile phase.

The peptide derivatives of **1** were synthesized using the "step by step" method. The appropriate amino acid derivatives of **1** (*N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)glycylamide dihydrochloride (**2a**) and *N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-L-prolylamide dihydrochloride (**3a**)) were coupled with the Boc-N^{α}-L-amino acids by the TBTU method in DMF. The crude products, compounds **7−11**, were then separated by extraction and purified by column chromatography. The coupling yields of the amino acid derivatives of DiMIQ with the next Boc-amino acid were good and ranged from 80% to 88%. The synthetic yield for the second coupling was found to be better than the initial coupling. The superiority in the later coupling may be due to the improved solubility in DMF. The Boc-removal of compounds **7−11** with hydrogen chloride in methanol gave the hydrochloride peptide derivatives of DiMIQ, compounds **7a−11a**, with good yields ranging from 91% to 98%. The hydrochlorides of the deprotected derivatives of DiMIQ were purified by recrystallization. The structures and the yields of all the new peptide derivatives of **1** are displayed in Table 2.

The examination of the solubility of the resulting conjugates was important, since DiMIQ has a very limited solubility in water. We examined the solubility in water of the obtained hydrochloride amino acids and the peptide derivatives of **1** (**2a−11a**) in Tables 1 and 2, according to *European Pharmacopoeia, General Notices*.[25] It turned out that attaching of the amino acid or the peptide moiety to the sparingly soluble reference compound DiMIQ (hydrochloride of DiMIQ was tested and 10 mg needed 1 mL of water to completely dissolve, so it was sparingly soluble) substantially increased the solubility in water of the resulting conjugate, and compounds **2a−11a** were very soluble or freely soluble in water (for example, for **2a**,

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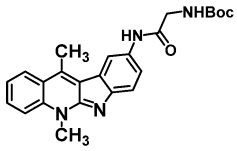
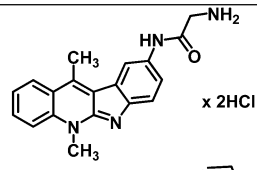
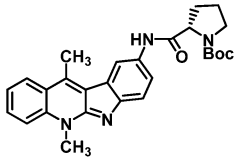
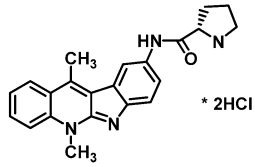
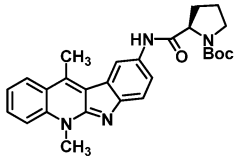
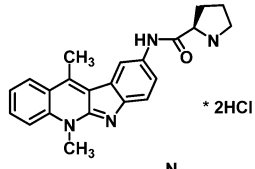
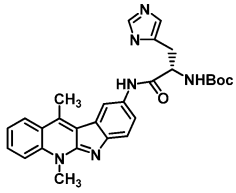
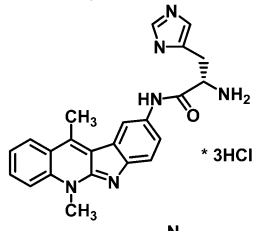
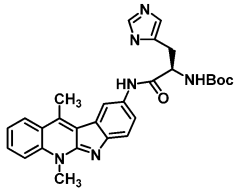
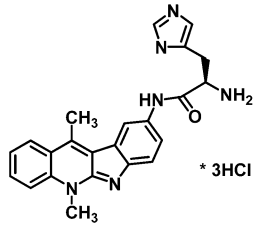
Table 1. Overall Coupling and Deprotection Yields (%) Obtained for All the Derivatives of DiMIQ and Solubility of the Obtained the Hydrochloride Amino Acid Derivatives of DiMIQ[a]

Compd	Structure	Coupl. yields (%)	Compd	Structure	Yields (%) Deprot.	Yields (%) Overall	Solub. in H_2O*/
2		65	2a		80	52	Very soluble
3		45	3a		87	39	Very soluble
4		67	4a		85	57	Very soluble
5		58	5a		87	50	Freely soluble
6		55	6a		88	48	Freely soluble

[a]The symbol "*/" indicates data according to European Pharmacopoeia.[25]

10 mg was completely dissolved in 0.01 mL of water and it was very soluble; for **9a**, 10 mg was completely dissolved in 0.1 mL of water and it was freely soluble). This better solubility results from the hydrophilic nature of the amino acid and the peptide part of the conjugates occurring in the hydrochloride form. When tested in vivo, these novel analogues with improved water solubility might play critical role in their bioavailability.

Antiproliferative Activity in Vitro. All the synthesized compounds **2a**–**11a** were evaluated for their antiproliferative activity in vitro against the KB cells (oral carcinoma cell). The results of the studies on the antiproliferative activity of the amino acid and peptide derivatives of DiMIQ are summarized in Table 3. The tested compounds showed diverse activity against the KB cells. The IC_{50} values of the amino acid derivatives **2a**, **3a**, and **4a** (values between 0.42 and 0.73 μM) and the dipeptide derivatives **10a** and **11a** (value 0.56 and 0.7, respectively) were lower than IC_{50} of the reference compounds DiMIQ (IC_{50} = 1.14 μM) and doxorubicin hydrochloride (DOX, IC_{50} = 0.84 μM). The maximal potency was achieved with the glycine (**2a**, IC_{50} = 0.67) and the L-proline (**3a**, IC_{50} = 0.42) side chain, but no significant differences in the cytotoxic activity between the L-amino acid and the D-amino acid derivatives (**3a**, IC_{50} = 0.42; **4a**, IC_{50} = 0.73) were observed. On the other hand, compounds **5a**, **6a**, **7a**, **8a**, **9a** appeared to be less active than DiMIQ (and also DOX). It is interesting that the antiproliferative activity of the dipeptide derivatives of DiMIQ was generally lower than the activity of the unsubstituted DiMIQ. The only exception was the dipeptide derivative, where the dipeptide moiety was attached to the indoloquinoline skeleton by the L-proline linker. For example, the IC_{50} value for compound **9a** is only 3.73 μM, but the IC_{50} for the compound **10a** is 0.56 μM. This different antiproliferative activity among the amino acid and the dipeptide derivatives to DiMIQ probably results from their different mechanisms of action and suggests that the kind of the amino acid linked with DiMIQ plays an important role in the antiproliferative activity of the tested compounds.

We selected three amino acid derivatives (**2a**, **3a**, **4a**) and four dipeptide derivatives (**7a**, **9a**, **10a**, and **11a**) for further study of the antiproliferative activity against the following cell lines: A549 (non-small-cell lung cancer), MCF-7 (breast cancer), LoVo (colon cancer), and normal mice fibroblasts (BALB/3T3). As seen in Table 4, the majority of the prepared compounds showed high cytotoxicity against all the cell lines. The most potent ones were derivatives **3a**, **4a**, **10a**, and **11a** (Table 4). The IC_{50} values for these derivatives (value between

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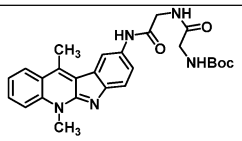
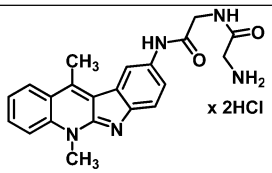
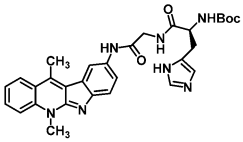
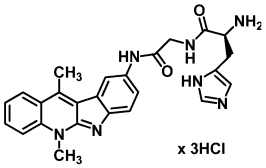
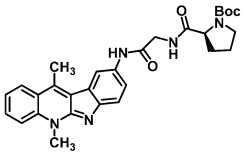
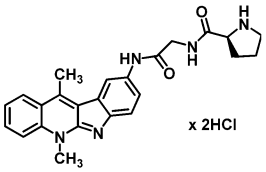
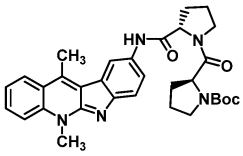
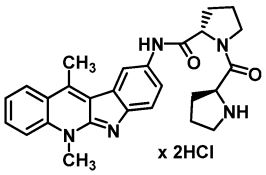
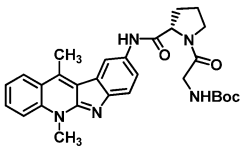
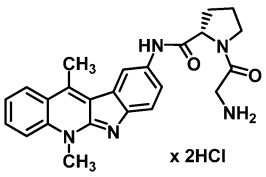
Table 2. Overall Coupling and Deprotection Yields (%) Obtained for All the Derivatives of DiMIQ and Solubility of the Obtained the Dihydrochloride Dipeptide Derivatives of DiMIQ[a]

Compd	Structure	Coupl yields (%)	Compd	Structure	Yields (%) Deprot.	Yields (%) Overall	Solub. in H_2O*/
7		86	7a		94	81	Very soluble
8		88	8a		94	83	Very soluble
9		84	9a		92	77	Freely soluble
10		88	10a		98	86	Freely soluble
11		80	11a		95	76	Freely soluble

[a]The symbol "*/" indicates data according to European Pharmacopoeia.[25]

0.6 and 0.96 μM) were greater than the IC_{50} of the reference compound DiMIQ (IC_{50} between 0.2 and 2.19 μM), and some of these differences were statistically significant. Good activity, comparable to that of DiMIQ, is shown by compounds **2a**, **7a**, and **9a** with IC_{50} ranging from 0.73 to 2.7 μM.

All the tested derivatives were cytotoxic against the normal mouse fibroblast (BALB/3T3). The antiproliferative activity of compounds **7a** and **9a** was comparable to that of the reference DiMIQ (IC_{50} of 1.71−3.96 μM vs 5.77 μM), but it was lower than against the A549 and LoVo tumors cell lines. The antiproliferative activity of the remaining compounds against BALB/3T3 (IC_{50} of 0.36−0.56 μM) was 5 times higher than the cytotoxicity of DiMIQ and comparable to the activity against cancer cells.

Antineoplastic Effect in Vivo. The collected study results allowed us to select derivatives for in vivo studies. We have chosen cyclophosphamide as a positive control because it is in clinical use for the treatment of malignant diseases for over 40 years and it is one of the most useful anticancer agents, used in the treatment of, for example, breast, lung, and ovary cancer. The evaluation of the antitumor activity in vivo was done for the following four conjugates of DiMIQ: with glycine (**2a**), L-proline (**3a**), and the peptides glycylglycine (**7a**), and glycyl-L-proline (**9a**). All the compounds were dissolved in water and investigated in the mouse Lewis lung cancer model (LLC) inoculated subcutaneously. We selected these compounds because **2a** and **3a** had the highest activity against the KB cell line and a very good activity against A549, MCF 7, and the LoVo cell line. Compound **3a** showed a higher activity in vitro against all the tested cell lines, in particular the cell lung cancer (A549). However, both compounds **2a** and **3a** showed a potent cytotoxic activity against the normal mouse fibroblast (BALB/3T). On the basis of the obtained data, it was quite clear that compounds **7a** and **9a**, a little less active against KB but highly active against the lung cancer cell line (A549) and against the colon cancer cell line (LoVo) and moderately cytotoxic against the normal mouse fibroblast (BALB/3T), were attractive candidates for a further assessment in vivo as antitumor agents.

The results are summarized in Figures 2−5 and Tables 5 and 6. Mice received tested compounds in doses 15 and 30 mg kg^{-1}

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Table 3. In Vitro Cytotoxic Activities of the Hydrochloride Amino Acid and Dipeptide Derivatives of DiMIQ against KB Cell Line[a]

compd	amino acid	IC_{50} [μM]
DiMIQ		1.14 ± 0.61
DOX		0.84 ± 0.03
2a	Gly	0.67 ± 0.93
3a	L-Pro	0.42 ± 0.16
4a	D-Pro	0.73 ± 0.24
5a	L-His	3.46 ± 0.68
6a	D-His	4.85 ± 0.93
7a	Gly-Gly	4.65 ± 0.75
8a	L-His-Gly	3.64 ± 1.4
9a	L-Pro-Gly	3.73 ± 1.0
10a	L-Pro-L-Pro	0.56 ± 0.24
11a	Gly-L-Pro	0.7 ± 0.21

[a]IC_{50}: compound concentration leading to 50% inhibition of cell proliferation. DiMIQ: reference compound. DOX: doxorubicin hydrochloride.

day^{-1} (**2a** and **3a**) or in doses 25 and 50 mg kg^{-1} day^{-1} (**7a** and **9a**) intraperitoneally (ip). One single dose (100 mg/kg) of cyclophosphamide (CY) was used as a positive control reference cytostatic. The doses of the **2a**, **3a**, **7a**, and **9a** agents used in the animal experiments were selected on the basis of the previous toxicity evaluation (Figures S1–S4 and Table S1 of Supporting Information) which was conducted in the range of doses from 5 to 50 mg/kg. The compounds **7a** and **9a** did not reveal any toxicity up to the highest dose; however, derivatives **2a** and **3a** were highly toxic at this dose.

Compound **3a** possessed the best antitumor properties (Figure 3b). At the dose 30 mg kg^{-1} day^{-1} it inhibited the tumor growth (TGI) by 60–74% on days 11–16 and by 38–50% on days 18–23. The antitumor properties were much higher than antitumor properties of a single dose of cyclophosphamide (100 mg/kg) whose TGI reached a maximum 30–49.8% on days 11–16. At the lower dose (15 mg kg^{-1} day^{-1}) of **3a** the maximal inhibition of the tumor growth reached 48% (Table 5). Compound **2a** had also antitumor properties (Figure 2b). At the highest dose used, it inhibited the tumor growth by 50–55% on days 11–16 and by 14–20% on days 18–23. The lower dose gave a similar effect (Table 5). The antitumor effects of **2a** were similar to the influence of cyclophosphamide on tumor growth. We also observed the antitumor properties of the dipeptide derivative **7a** (Figure 4b). At dose 50 mg kg^{-1} day^{-1} it inhibited the tumor growth by 46–63% on days 11–16 and 29–43% on days 18–23. The lower dose of this derivative inhibited the tumor growth by about 22–37% (Table 5). The derivative **7a** at the dose 50 mg kg^{-1} day^{-1} has antitumor activity similar to that of cyclophosphamide at the beginning of the experiment (days 11–16), but at the end of the experiment when the antitumor effect of CY was decreased (20–30% TGI), the activity of **7a** and also **3a** derivatives was still high (29–43% TGI). The dipeptide derivative **9a** did not reveal antitumor activity at dose 25 mg kg^{-1} day^{-1} (Figure 5b). At the highest dose used, it inhibited the tumor growth by 33–36% on days 14, 19, and 21 (Table 6), and the effect was similar to the anticancer activity of 100 mg/kg CY. In the groups of mice receiving the tested compounds some body weight loss, however not exceeding 15%, was observed (Figures 2a, 3a, 4a, and 5a).

Results demonstrated that the attachment of amino acid or peptide moiety to the indoloquinoline skeleton enhances its solubility in water, but there is no close dependency between solubility and anticancer activity of the "hybrid" compounds. It seems that the presence of the amino acid or peptide moiety may also have an influence on the mechanism of biological action of the "hybrid". The high anticancer activity of compounds **3a**, which has L-proline as a substituent, particularly proved that the kind of amino acid linked to DiMIQ plays a crucial role in the anticancer activity. We know that the mechanism of action of neocryptolepine depends on the topoisomerase II inhibition and on the DNA intercalation, but in this case we cannot exclude that the inhibitions of targets other than this system can contribute to the anticancer activity of the presented compounds. The exact mechanism of action of these compounds has not been established; however, it will be a subject of our further studies.

Determination of pK_a and Octanol–Water Partition Coefficients for Compounds 2a, 3a, 7a, and 9a. The pK_a values of **2a**, **3a**, **7a**, and **9a** were deduced from their UV spectra taken in buffers of various pH values in a range of 2–10 at 37 °C at a fixed wavelength. The final concentration of the tested compounds was 5 μM. Under such conditions, the linearity of the absorbance–compound concentration relationship was preserved. The pK_a values of **2a**, **3a**, **7a**, and **9a** were found to range from 7.2 to 7.5 with the formation of the isosbestic point at 280 nm (Table 7). This indicates that they are partially protonated under physiological conditions (pH 7.4).

Table 4. In Vitro Cytotoxic Activities of the Hydrochloride Amino Acid and Peptide Derivative of DiMIQ against Human Cancer Cell Lines and Normal Mice Fibroblasts[a]

		IC_{50} [μM]			
compd	amino acid	BALB/3T3	A549	MCF-7	LOVO
DiMIQ		5.77 ± 0.93	2.19 ± 0.48	1.54 ± 0.52	0.20 ± 0.40
DOX		1.08 ± 0.03	0.33 ± 0.10	0.44 ± 0.16	0.11 ± 0.03
2a	Gly	0.55 ± 0.01	1.31 ± 0.11	1.45 ± 0.39	0.88 ± 0.14
3a	L-Pro	0.56 ± 0.07	0.48 ± 0.32	0.82 ± 0.05	0.81 ± 0.12
4a	D-Pro	0.36 ± 0.04	0.32 ± 0.10	0.60 ± 0.07	0.62 ± 0.21
7a	Gly-Gly	1.71 ± 0.34	0.90 ± 0.03	2.70 ± 0.54	0.73 ± 0.13
9a	L-Pro-Gly	3.96 ± 0.51	1.15 ± 0.20	1.43 ± 0.65	0.98 ± 0.02
10a	L-Pro-L-Pro	0.48 ± 0.11	0.54 ± 0.13	0.96 ± 0.01	0,36 ± 0,05
11a	Gly-L-Pro	0.45 ± 0.12	0.86 ± 0.08	1.13 ± 0.13	0.44 ± 0.12

[a]IC_{50}: compound concentration leading to 50% inhibition of cell proliferation. DiMIQ: reference compound. DOX: doxorubicin hydrochloride. (*) $p < 0.05$ in comparison to DIMIQ, Mann–Whitney U test, Statistica 7.0.

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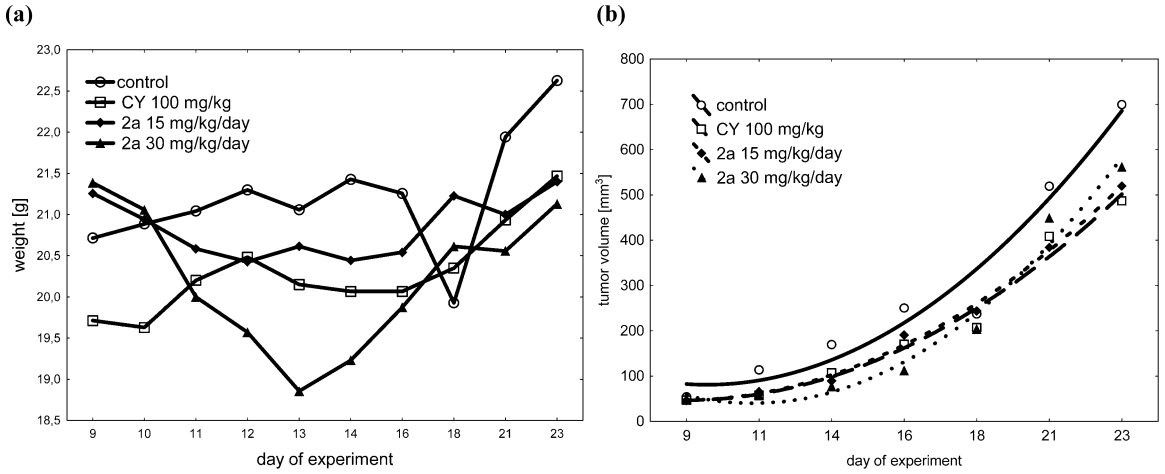

Figure 2. Effect of **2a** on the mice body weight (a) and the growth of the mouse Lewis lung cancer LLC inoculated sc (median) (b). Schedule was as follows: therapy with ip administration of **2a** in dose 15 mg kg^{-1} day^{-1} from day 9 to day 13 and in dose 30 mg kg^{-1} day^{-1} from day 9 to day 12. Cyclophosphamide (CY) in single dose 100 mg/kg was used as a cytostatic control. For **2a**, 30 mg kg^{-1} day^{-1}, the difference on day 16 was of statistical significance in comparison to control group: Kruskal–Wallis multiple comparison test, nonparametric, $p < 0.05$.

Table 5. Effect of Amino Acid (2a, 3a) and Dipeptide (7a) Derivatives of DiMIQ on Inhibition of Mouse Lewis Lung Cancer LLC Inoculated sc[a]

		TGI [%]					
treatment	dose [mg kg^{-1} day^{-1}]	day 11	day 14	day 16	day 18	day 21	day 23
CY	100	49.79	36.8	31.94	12.46	21.26	30.42
2a	15	42.4	47.43	23.85	0	26.1	25.78
	30	50.1	54.66	55.28	14.27	13.53	19.71
3a	15	41.98	42.61	47.73	8.38	0	24.65
	30	61.21	73.96	75.82	49.96	37.88	41.1
7a	25	37.41	31.76	27.25	22.41	28	35.82
	50	46.89	62.61	52.19	38.26	29.32	42.94

[a]TGI% was calculated for median. Schedule: therapy with ip administration of **2a** or **3a** at dose 15 mg kg^{-1} day^{-1} from day 9 to day 13 and at dose 30 mg kg^{-1} day^{-1} from day 9 to day 12; **7a** at doses 25 and 50 mg kg^{-1} day^{-1} from day 9 to day 13. Cyclophosphamide (CY) at single dose 100 mg/kg was used as a cytostatic control.

Table 6. Effect of Dipeptide (9a) Derivative of DiMIQ on Inhibition of the Mouse Lewis Lung Cancer LLC Inoculated sc[a]

		TGI [%]					
treatment	dose [mg kg^{-1} day^{-1}]	day 12	day 14	day 17	day 19	day 21	day 24
CY	100	24.13	48.01	37.02	40.01	26.99	0
9a	25	12.93	11.6	0	3.1	0	0
	50	16.29	36.52	18.53	33.11	32.46	8.19

[a]TGI%: calculated for median. Schedule: therapy with ip administration of **9a** at doses 25 and 50 mg kg^{-1} day^{-1} from day 10 to day 14. Cyclophosphamide (CY) at single dose 100 mg/kg was used as a cytostatic control.

The hydrophobic parameter (log *P*) of **2a**, **3a**, **7a**, and **9a** was estimated under physiological as well as in acidic and basic conditions. The determined partition coefficients covered a range of over 4.0 log units (Figure 6). The log *P* values for all the compounds were negative at pH 2.5 and positive at pH 9.0. At low pH values, the tested compounds occurred in the form of salts, and because of a better water solubility their log *P* values became negative (values ranged between −1.3 and −1.9), similar to that of DiMIQ whose log *P* is −1.0 at pH 2.2.[4] Nevertheless, we observed distinct differences in more basic conditions, at pH 7.4, because the log *P* values of compounds **2a** and **3a** were positive (0.6 and 1.4 respectively), but at the same condition the log *P* values of compounds **7a** and **9a** were negative with values −0.2 and −0.1, respectively. These compounds appeared to be much more hydrophilic in contrast to the unsubstituted DiMIQ, which is more hydrophobic (log *P* of 2.0 in pH 5.0). The presence of the hydrophilic amino acid chain or even more hydrophilic peptide chain like Gly-Gly or Gly-L-Pro in conjugates with DiMIQ increased their hydrophilic properties, and it corresponded with the good biological profile of these compounds, for example, the anticancer activity in vivo. It is possible that the hydrophilic compounds react mainly with the cytosol soluble targets like glutathione, leading to its depletion, causing oxidative stress and triggering cell death.[26]

DiMIQ, 2a, 3a, 7a, and 9a Induced Isotonic Hemolysis of Human Erythrocytes. The effect of the tested compounds on human erythrocytes to determine whether they are able to interact with the natural membranes and lead to the disruption of red blood cells was also estimated. The effects of the studied

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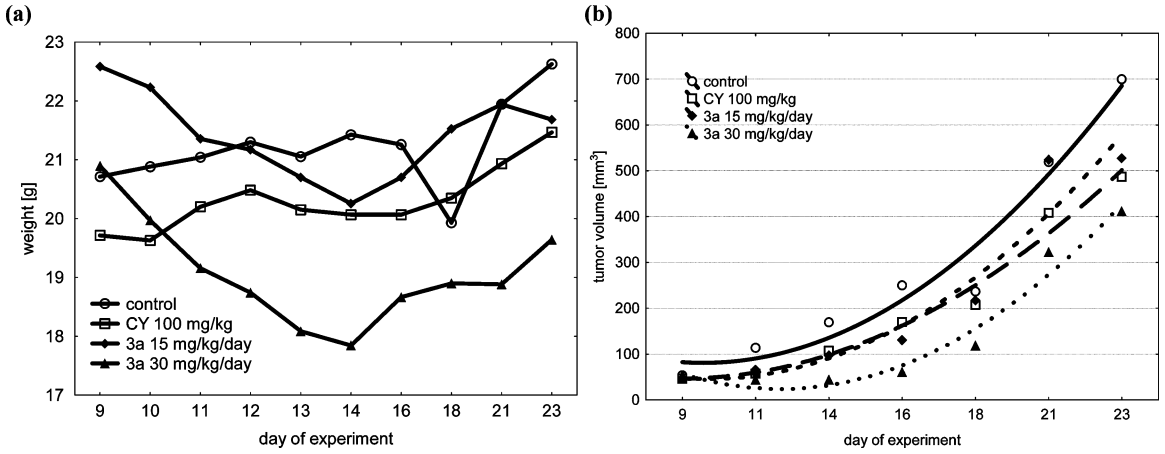

Figure 3. Effect of **3a** on the mice body weight (a) and the growth of the mouse Lewis lung cancer LLC inoculated sc (median) (b). Schedule was as follows: therapy with sc administration of **3a** in dose 15 mg kg^{-1} day^{-1} from day 9 to day 13 and in dose 30 mg kg^{-1} day^{-1} from day 9 to day 12. Cyclophosphamide (CY) in single dose 100 mg/kg was used as a cytostatic control. For **3a**, 30 mg kg^{-1} day^{-1}, differences in days 14, 16, and 23 were of statistical significance in comparison to control group: Kruskal–Wallis multiple comparison test, nonparametric, $p < 0.05$.

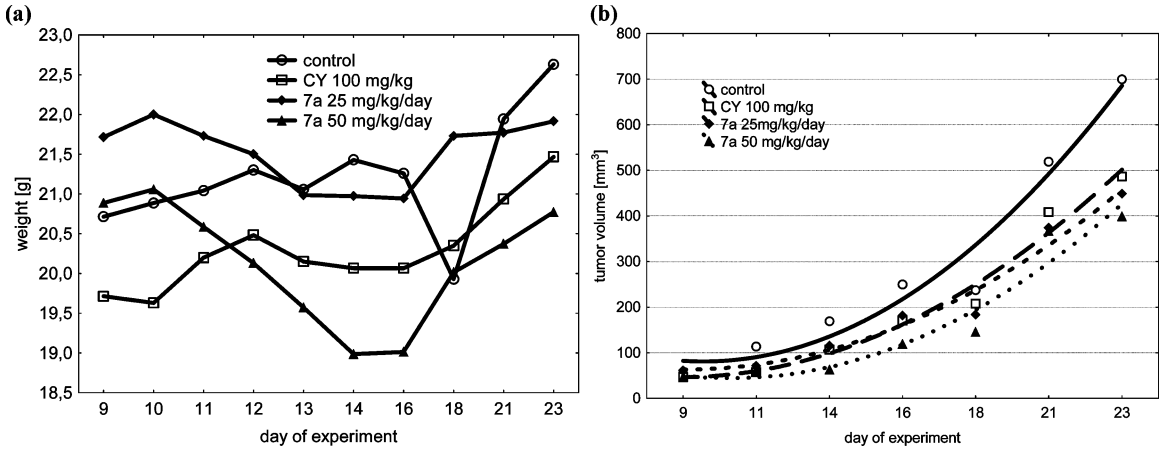

Figure 4. Effect of **7a** on the mice body weight (a) and the growth of the mouse Lewis lung cancer LLC inoculated sc (median) (b). Schedule was as follows: therapy with sc administration of **7a** in doses 25 and 50 mg kg^{-1} day^{-1} from day 9 to day 13. Cyclophosphamide (CY) in single dose 100 mg/kg was used as a cytostatic control. For **7a**, 50 mg kg^{-1} day^{-1}, differences in days 16, 18, and 23 were of statistical significance in comparison to control group: Kruskal–Wallis multiple comparison test, nonparametric, $p < 0.05$.

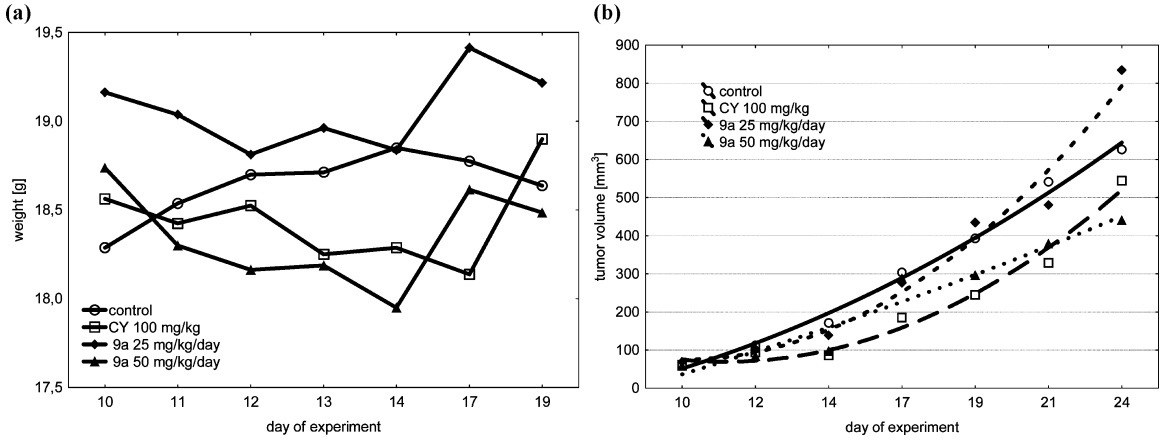

Figure 5. Effect of **9a** on the mice body weight (a) and the growth of the mouse Lewis lung cancer LLC inoculated sc (median) (b). Schedule was a follows: therapy with sc administration of **9a** at doses 25 and 50 mg kg^{-1} day^{-1} from day 9 to day 13. Cyclophosphamide (CY) at single dose 100 mg/kg was used as a cytostatic control.

compounds were assayed as a function of the concentration. As shown in Figure 7, all the compounds present in the erythrocyte suspension caused the disruption of red cells in isotonic conditions (pH 7.4, hematocrit of 50%). Among the

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Table 7. Isosbestic Points and pK_a of 2a, 3a, 7a, and 9a

compd	isosbestic point [nm]	pK_a [a]	λ [nm][a]
2a	280	7.2	290
3a	280	7.2	290
7a	280	7.5	290
9a	278	7.3	290

[a]Determination of pK_a was performed at indicated wavelength.

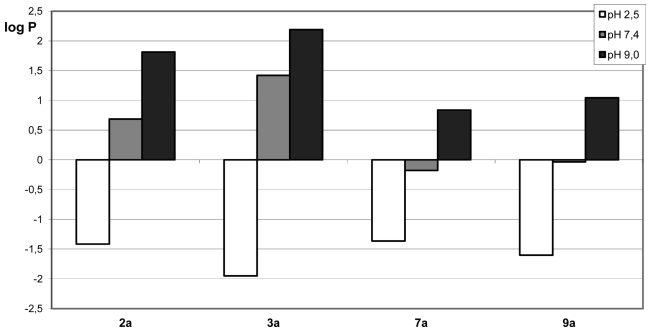

Figure 6. Octanol–water partition coefficients (log P) of compounds **2a**, **3a**, **7a**, **9a**. The log P was defined as $\log(C_{octanol}/C_{water})$. The amount of the compound in the water phase was determined spectrophotometrically, and the amount in the octanol phase was obtained by subtracting the supernatant concentration from the total concentration. Presented values are the mean of two independent experiments done in triplicate.

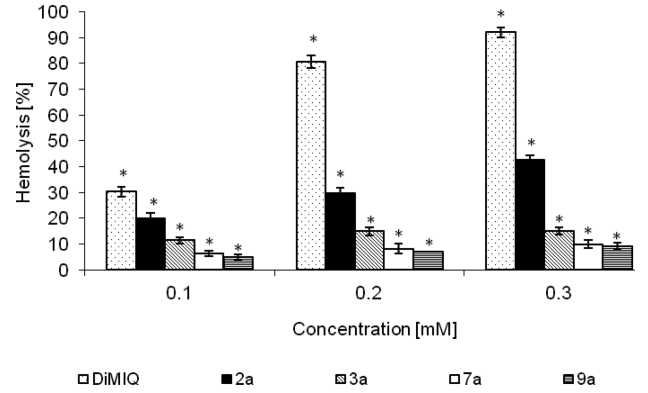

Figure 7. DiMIQ, **2a**, **3a**, **7a**, **9a** induced isotonic hemolysis of human erythrocytes (pH 7.4, hematocrit of 50%, incubation time of 30 min at 37 °C). Data are given as the mean ± SD of three individual determinations: (*) $p < 0.05$ in comparison to DIMIQ, Mann–Whitney U test, Statistica 7.0.

newly synthesized agents, **2a** exhibits the strongest hemolytic behavior (42.5% at 0.3 mM). On the other hand the hemolytic activity of all the tested compounds is lower than the hemolytic activity of DiMIQ. Moreover, as shown earlier, compounds **7a** and **9a** seemed to be the most promising ones because of the lowest hemolytic activity (only 9.97% at 0.3 mM) which is correlated with the low in vivo toxicity of these derivatives.

CONCLUSION

On the basis of the previous reports indicating that the amino acids Gly, L-Pro, and L-His are a significant requirement for the antiproliferative activity of conjugates with 6*H*-indolo[2,3-*b*]quinoline (**II**), a series of DiMIQ analogues containing the amino acid and the dipeptide chains was synthesized with the initial objective to increase the selectivity of the action and the solubility in water of these conjugates. These novel neocryptolepine derivatives were obtained by the reaction of the key intermediate 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-amine (**1**) with the appropriate amino acid to give products with a moderate to good yield. Attaching the following amino acid to the amino acid derivatives of DiMIQ gave the dipeptide derivatives of DiMIQ. All the new hydrochloride amino acid and peptide derivatives of DiMIQ were tested for their solubility in water, and all of the new compounds showed very good or free solubility in water. During this study the new amino acid and dipeptide derivatives of DiMIQ were tested for their antiproliferative activity against tumor cells. As a result, four of the new compounds were selected for the investigation of their anticancer activity in vivo, and their physicochemical properties (pK_a and log P) were also examined. The hemolysis of human erythrocytes was also investigated in order to obtain additional data on potential toxic effects of the tested compounds.

Summing up, we arrived at the following conclusions: (1) All the amino acid and peptide derivatives of 5*H*-indolo[2,3-*b*]quinoline displayed good antiproliferative activity against the KB cells. (2) The majority of the new derivatives displayed good antiproliferative activity against A549, MCF-7, LoVo. (3) The cytotoxic activity of the new compounds against the normal mice fibroblasts was mixed. (4) The amino acids and peptide derivatives of 5*H*-indolo[2,3-*b*]quinoline showed a high antitumor activity in vivo. (5) The conjugate 5*H*-indolo[2,3-*b*]quinoline with peptide glycylglycine (**7a**) was the most promising derivative because it strongly inhibited the growth of the tumor in mice and it did not show any disadvantageous effects. (6) The attachment of the hydrophilic amino acid or the peptide chain to the hydrophobic 5*H*-indolo[2,3-*b*]-quinoline increased its hydrophilic properties. (7) The attachment of the amino acid or the peptide chain to 5*H*-indolo[2,3-*b*]quinoline significantly decreased its hemolytic activity compared to DiMIQ, which is correlated with the low in vivo toxicity (especially for compounds **7a**, and **9a**).

Our results clearly show that the attachment of an amino acid or a peptide chain to DiMIQ significantly improves its physicochemical properties, although the antiproliferative activity in vitro remains similar to that of DiMIQ. On the basis of our studies, concerning the encouraging anticancer activity in vivo and low hemolytic activity, we choose compound **7a** as our new lead compound. These results are very promising and the detailed studies of the mechanisms of action, the pharmacokinetics, and the tissue distribution of these new amino acid and dipeptide derivatives of neocryptolepine will be the subject of the next investigations. Nevertheless, the problem of low solubility, high toxicity, and maybe the drug selectivity has been at least partially solved by synthesizing the salts of the amino acid and peptide derivatives of DiMIQ. Overall, the neocryptolepine derivatives containing the amino acid or the peptide side chains have been confirmed as promising lead compounds for the development of new, highly potent, and selective agents against cancer.

EXPERIMENTAL SECTION

General. Melting points were determined using a Kofler type apparatus and were uncorrected. The IR spectra were recorded with a Perkin-Elmer 1640 FTIR BX spectrophotometer in KBr pellets. The ^{1}H and ^{13}C NMR spectra were measured using Varian-NMR-vnmrs500, Varian-NMR-vnmrs600, and Varian Gemini 200 spectrometers. The ^{1}H and ^{13}C NMR spectral data are given in relation to

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the TMS and DSS signal at 0.0 ppm for DMSO and D_2O solutions, respectively. The ESI mass spectra were recorded on a PE Biosystems Mariner mass spectrometer. Progress of the reaction was monitored by thin layer chromatography (TLC) with Merck DC-Alufolien Kieselgel 60 F_{254}. The chemicals and solvent were purchased from Fluka Company. Column chromatography was performed on Merck silica gel 60 (230−400 mesh). Measurement of optical rotation is performed using a Jasco digital polarimeter P-2000, and measurements of optical rotation are made at 589 nm at 20 °C. HPLC experiments were carried out on a Waters HPLC system (Waters Associates, Milford, MA, U.S.) consisting of a multisolvent delivery system 600E, a photodiode array detector 2996, a Rheodyne model 7725i injector, chromatography manager Empower 2 software for PC coputations, and Luna C-18 column (Phenomenex, U.S.) with 5 μm particles, 250 mm × 4.6 mm. The detection was performed by using UV at λ = 275 nm. The compound concentration was about 2.0 mg/mL, and the injection volume was 20 μL. Method 1 was as follows: mobile phase consisting of A (H_2O + 0.1% TFA, v/v) and B (acetonitrile + 0.1% TFA, v/v) was used with a linear gradient from 20% B to 100% B for 20 min (**2a**) and from 30% B to 100% B for 20 min (**3a**, **7a**). Method 2 was as follows: mobile phase consisting of A (H_2O + 0.05% TFA, v/v) and B (acetonitrile) was used with a linear gradient from 10% B to 80% B for 20 min (**9a**). All key compounds were proven by HPLC method to have ≥95% purity.

General Procedure for the Synthesis Compounds 2 and 3. To a solution of N^{α}-protected L-amino acid (0.50 mM), TBTU (1 mM), and HOBt (1 mM) in DMF (3 mL) was added DIPEA (1.5 mM). The mixture was stirred for 15 min at room temperature. Then a solution of 5,11-dimethyl-5*H*-indolo[2.3-*b*]quinolin-9-ylamine (**1**) (0.76 mM) in 2 mL of DMF was added, and the reaction mixture was stirred at room temperature for 6−24 h (TLC monitoring). After the reaction was completed, the solvent was evaporated under reduced pressure at ∼40 °C. The resulting oil was treated with water and $CHCl_3$, and the organic layer was separated and washed successively with aqueous $NaHCO_3$ solution and aqueous NaCl solution. The extract was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)glycylamide (2).** Compound **2** was obtained as a red solid from Boc-Gly-OH and **1**. The crude product was recrystallized from ethyl acetate. Yield 206 mg (65%). Mp (°C): 212−215. IR: 3278, 2976, 1688, 1651, 1569, 1523, 1461, 1249, 1167 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) [ppm]: 9.89 (1H, s), 8.56 (1H, s), 8.29 (1H, d, J = 7.5), 7.92 (1H, d, J = 9.0 Hz), 7.83 (1H, m), 7.59 (1H, d, J = 9.0 Hz), 7.50 (1H, m), 7.49 (1H, d, J = 8.0 Hz), 4.23 (3H, s), 3.76 (2H, d, J = 6.0 Hz), 3.07 (3H, s), 1.40 (9H, s). ESI-MS: calcd for $(M + H)^+$, 419.2; found, 419.2. Anal. Calcd for $C_{24}H_{26}N_4O_3$: C 68.88, H 6.26, N 13.39. Found: C 68.34, H 6.42, N 13.07.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-L-prolylamide (3).** Compound **3** was obtained as an orange solid from Boc-L-Pro and **1**. The crude product **3** was purified by chromatography on silica gel column with $CHCl_3$/CH_3OH, 9:1 (v/v), and crystallized from diethyl ether. Yield 187 mg (45%). Mp (°C): 210−212. IR: 3311, 2975, 1673, 1635, 1525, 1460, 1166, 748 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) (two rotamers) [ppm]: 9.98 (1H, s), 8.67 and 8.64 (1H, s and s), 8.33 (1H, d, J = 8.0 Hz), 7.96 (1H, d, J = 8.5 Hz), 7.86 (1H, m), 7.61 (1H, dd, J = 1.0 and 8.5 Hz), 7.53 (2H, m), 4.29 (1H, m), 4.27 (3H, s), 3.47 (1H, m), 3.38 (1H, m), 3.11 (3H, s), 2.25 (1H, m), 1.96 (2H, m), 1.83 (1H, m), 1.43 and 1.33 (9H, s and s). ESI-MS: calcd for $(M + H)^+$, 459.3; found, 459.3. Anal. Calcd for $C_{27}H_{30}N_4O_3 \cdot 2H_2O$: C 65.57, H 6.93, N 11.33. Found: C 65.44, H 6.76, N 11.43.

***N*-(5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)glycylamide Dihydrochloride (2a).** Compound **2** (206 mg, 0.49 mM) was treated with 2.2 M HCl/CH_3OH (3 mL) with stirring for 2 h (TLC monitoring). When TLC showed that the Boc group was completely removed, the precipitated salt **2a** was collected by filtration and recrystallized from ethyl acetate. Yield 153 mg (80%). Mp (°C): 270. IR: 3418, 2957, 1682, 1642, 1573, 1476, 1240 cm^{-1}. ^1H NMR (600 MHz, D_2O) [ppm]: 8.00 (1H, d, J = 8.1 Hz), 7.91 (1H, m), 7.70 (1H, d, J = 8.1 Hz), 7.63 (1H, m), 7.49 (1H, d, J = 1.8 Hz), 6.74 (1H, d, J = 8.3 Hz), 6.47 (1H, dd, J = 1.8 and 8.3 Hz), 3.78 (2H, m), 3.74 (3H, s), 2.51 (3H, s). ^{13}C NMR (150.83 MHz, D_2O) [ppm]: 166.9, 151.4, 147.0, 136.9, 136.72, 136.70, 135.1, 129.1, 128.8, 124.6, 122.8, 121.2, 119.6, 118.6, 114.7, 114.3, 43.6, 38.2, 18.2. ESI-MS: calcd for $(M + H)^+$, 319.3; found, 319.3. Anal. Calcd for $C_{19}H_{18}N_4O \cdot 2H_2O \cdot 2HCl$: C 53.40, H 5.66, N 13.11, Cl 16.59. Found: C 53.54, H 5.67, N 13.07, Cl 16.58. Purity (HPLC): 99.3%.

***N*-(5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-L-prolylamide Dihydrochloride (3a).** Compound **3** (115 mg, 0.25 mM) was treated with 2.2 M HCl/CH_3OH (2 mL) with stirring for 3 h (TLC monitoring). When TLC showed that the Boc group was completely removed, the precipitated salt **3a** was collected by filtration and recrystallized from ethyl acetate/diethyl ether. Yield 97 mg (87%). Mp (°C): 255. IR: 3411, 2943, 1675, 1640, 1575, 1477, 1269, 764 cm^{-1}. ^1H NMR (500 MHz, D_2O) [ppm]: 8.34 (1H, d, J = 8.5 Hz), 8.08 (1H, s), 8.07 (1H, m), 8.00 (1H, d, J = 8.5 Hz), 7.79 (1H, m), 7.29 (1H, d, J = 8.0 Hz), 7.17 (1H, d, J = 8.0 Hz), 4.50 (1H, dd, J = 6.5 and 8.5 Hz), 4.12 (3H, s), 3.58 (2H, m), 2.95 (3H, s), 2.66 (1H, m), 2.23 (3H, m). ^{13}C NMR (125.68 MHz, D_2O) [ppm]: 169.8, 151.8, 148.3, 138.4, 137.3, 136.4, 134.9, 129.1, 128.5, 125.1, 124.2, 122.4, 120.7, 118.7, 117.0, 114.9, 62.7, 49.2, 38.1, 32.6, 26.4, 18.2. ESI-MS: calcd for $(M + H)^+$, 359.3; found, 359.3. Anal. Calcd for $C_{22}H_{22}N_4O \cdot 2H_2O \cdot 2HCl$: C 56.53, H 6.04, N 11.99 Cl 15.17. Found: C 56.97, H 6.27, N 12.05, Cl 15.20. Purity (HPLC): 98.4%. $[\alpha]_D^{20}$ −12.3 (c 0.1, H_2O)

General Procedure for the Synthesis 7 and 9. To a solution of N^{α}-protected amino acid (0.8 mM) in DMF (6 mL) were added TBTU (0.8 mM), HOBt (0.8 mM), and DIPEA (0.4 mL, 2.2 mM) at room temperature, and the solution was stirred for 15 min. Next **2a** (0.5 mM) was added to the solution, and the reaction mixture was stirred for 24 h. After the reaction was completed the solvent was evaporated under reduced pressure at ∼40 °C. The resulting oil was treated with water and $CHCl_3$, and the organic layer was separated and washed successively with aquoeus $NaHCO_3$ solution and aqueous NaCl solution. The extract was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)glycylglycylamide (7).** Compound **7** was obtained from Boc-Gly and **2a**. The crude products were purified by crystallization from ethyl acetate to afford compound **7** as an orange crystal. Yield 231 mg (86%). Mp (°C): 240. IR: 3383, 2976, 2930, 1684, 1639, 1534, 1482, 1367, 1228, 1169, 760 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) [ppm]: 8.83 (1H, br s), 8.63 (1H, d, J = 9.5 Hz), 8.32 (1H, d, J = 8.8 Hz), 8.13 (1H, m), 7.86 (1H, m), 7.76 (1H, d, J = 8.8 Hz), 7.62 (1H, d, J = 8.8 Hz), 4.45 (3H, s), 4.09 (2H, s), 3.82 (2H, s), 3.33 (3H, s), 1.47 (9H, s). ESI-MS: calcd for $(M + H)^+$, 476.3; found, 476.3. Anal. Calcd for $C_{26}H_{29}N_5O_4 \cdot H_2O$: C 63.27, H 6.33, N 14.19. Found: C 63.31, H 6.31, N 14.12.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*-(5,11-dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-L-prolylglycylamide (9).** Compound **9** was obtained from Boc-L-Pro and **2a**. The crude products were purified by crystallization from ethyl acetate to afford compound **9** as an orange crystal. Yield 186 mg (84%). Mp (°C): 225−227. IR: 3436, 3302, 2973, 1703, 1675, 1565, 1168, 747 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) (two rotamers) [ppm]: 10.28 and 9.85 (1H, s and s), 8.77 and 8.73 (1H, s and s), 8.53 (1H, d, J = 8.4 Hz), 8.23 (1H, d, J = 8.4 Hz), 8.05 (1H, m), 7.98 (1H, d, J = 8.4 Hz), 7.74 (1H, m), 7.67 (1H, d, J = 9.0 Hz), 4.39 (3H, s), 4.19 (1H, m), 3.94 (2H, m), 3.60 (2H, m), 3.23 (3H, s), 2.16 (1H, m), 1.86 (3H, m), 1.41 and 1.37 (9H, s and s). ESI-MS: calcd for $(2M + H)^+$ 1031.6, $(M + H)^+$ 516.3; found, 1031.6, 516.3. Anal. Calcd for $C_{29}H_{33}N_5O_4 \cdot 3H_2O$: C 61.14, H 6.90, N 12.29. Found: C 59.84, H 6.87, N 12.29.

***N*-(5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-glycylglycylamide Dihydrochloride (7a).** Compound **7** (124.1 mg, 0.26 mM) was treated with 2.2 M HCl/CH_3OH (3 mL) with stirring for 1 h (TLC monitoring). When TLC showed that Boc group was completely removed, the precipitated salt **7a** was collected by filtration and recrystallized from ethyl acetate. Yield 110 mg (94%). Mp (°C): 252−254. IR: 3330, 3100, 1788, 1682, 1645, 1430, 1200, 1135, 795 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6 with small addition of H_2O)

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[ppm]: 10.56 (1H, s), 8.92 (1H, t, J = 5.4 Hz), 8.79 (1H, s), 8.58 (1H, d, J = 8.2 Hz), 8.32 (1H, d, J = 8.8 Hz), 8.27 (3H, br s), 8.12 (1H, m), 7.83 (1H, m), 7.76 (1H, d, J = 8.6 Hz), 7.63 (1H, d, J = 8.6 Hz), 4.48 (3H, s), 4.08 (2H, d, J = 6.0 Hz), 3.71 (2H, s), 3.24 (3H, s). ^{13}C NMR (125.68 MHz, DMSO-d_6 with small addition of H_2O) [ppm]: 167.4, 166.7, 148.3, 146.9, 136.4, 135.4, 134.4, 133.4, 126.9, 125.6, 122.7, 120.9, 120.6, 119.6, 116.9, 114.1, 112.9, 42.9, 40.4, 36.6, 16.0. ESI-MS: calcd for $(M + H)^+$ 376.3, $(M + 2H)^{2+}$ 188.8; found, 376.3, 188.8. Anal. Calcd for $C_{21}H_{21}N_5O_2 \cdot 2HCl \cdot 6H_2O$: C 45.33, H 6.34, N 12.59, Cl 12.74. Found: C 45.30, H 6.24, N 12.45, Cl 12.98. Purity (HPLC): 98.6%.

***N*-(5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinolin-9-yl)-L-prolylglycylamide Dihydrochloride (9a).** The compound **9** (159 mg, 0.31 mM) was treated with 2.2 M HCl/CH_3OH (2 mL) with stirring for 2 h (TLC monitoring). When TLC showed that Boc group was completely removed, the precipitated salt **9a** was collected by filtration and recrystallized from ethyl acetate. Yield 139 mg (92%). Mp (°C): 267. IR: 3410, 3300, 2927, 1686, 1649, 1482, 1225, 766 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6 with small addition of H_2O) [ppm]: 10.47 (1H, s), 8.91 (1H, t, J = 5.8 Hz), 8.74 (1H, d, J = 1.7 Hz), 8.60 (1H, d, J = 8.6 Hz), 8.32 (1H, d, J = 8.9 Hz), 8.12 (1H, m), 7.85 (1H, m), 7.76 (1H, dd, J = 1.8 and 8.8 Hz), 7.67 (1H, d, J = 8.8 Hz), 4.39 (3H, s), 4.26 (1H, m), 4.05 (2H, d, J = 5.6 Hz), 3.26 (3H, s), 3.22 (2H, m), 2.36 (1H, m), 1.92 (3H, m). ^{13}C NMR (125.68 MHz, DMSO-d_6 with small addition of H_2O) [ppm]: 169.1, 167.6, 149.2, 147.3, 136.3, 135.8, 134.8, 133.8, 127.3, 126.1, 123.3, 121.6, 121.1, 120.0, 117.3, 114.9, 113.3, 59.5, 46.1, 43.1, 36.7, 29.9, 23.9, 16.3. ESI-MS: calcd for $(M + H)^+$ 416.2, $(M + H)^{++}$ 208.6; found, 416.2, 208.6. Anal. Calcd for $C_{24}H_{25}N_5O_2 \cdot 2HCl \cdot 2H_2O$: C 54.96, H 5.96, N 13.35, Cl 13.52. Found: C 54.15, H 5.63, N 13.39, Cl 14.01. Purity (HPLC): 98.2%. $[\alpha]_D^{20}$ −16.96 (c 0.1, H_2O).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for compounds **4**, **5**, **6**, **8**, **10**, **11**, **4a**, **5a**, **6a**, **8a**, **10a**, **11a**; ^{1}H NMR, ^{13}C NMR, IR, MS, elemental analysis, purity (HPLC), and toxicity evaluation of compounds **2a**, **3a**, **7a**, and **9a**; biological procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

Boc, *tert*-butyloxycarbonyl; DIPEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid; HCl, hydrochloride acid; HOBt, *N*-hydroxybenzotriazole monohydrate; HPLC, high performance liquid chromatography; IR, infrared; MS, mass spectrometry; NMR, nuclear magnetic resonance; SAR, structure−activity relationship; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl

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