Synthesis and Antitumor Activity of Conjugates of Muramyldipeptide or Normuramyldipeptide with Hydroxyacridine/Acridone Derivatives

Krystyna Dzierzbicka* and Aleksander M. Kołodziejczyk

Department of Organic Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-952 Gdansk, Poland

Received July 10, 2002

A series of MDP (muramyldipeptide) or nor-MDP (normuramyldipeptide) analogues modified at the C-terminus post of the molecule by a formation of an ester bond between the carboxylic group of isoglutamine and the hydroxyl function of the respective derivatives of 4-carboxamide-acridine/9-acridone or 1-nitro-9-hydroxyalkylaminoacridines were synthesized as potential anticancer agents. The compounds *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-(ethylamino)-1-nitroacridine ester **3j** and *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-propylamino-1-nitroacridine ester **3k** exhibited high in vitro cytotoxic activity against a panel of human cell lines, prostate cancer and AIDS-related lymphoma (ARL). Analogue **3j** was also active in vivo in the hollow fiber assay. Antitumor activity of both compounds were tested in vivo against difference human tumor xenograft, but only analogue **3k** showed in vivo activity against sc UACC-62 melanoma in mice.

Introduction

Synthesis and antitumor activity of conjugates of MDP (muramyldipeptide) or nor-MDP (normuramyldipeptide) obtained in the acylation of the C6 hydroxyl group in the sugar moiety MDP by *N*-substituted acridine/acridone ω-aminoalkano-carboxylic acids and in the amide bond formation between the carboxylic group of isoglutamine and the amine function of the respective acridine/acridone derivatives have been already presented.[1] MDP and nor-MDP analogues modified with acridine/acridone derivatives in the peptide part demonstrated high cytotoxic activity, and two among them appeared to be active in vivo in the hollow fiber assay.[1]

The carboxyl group of MDP is a convenient site for chemical modifications that lead to derivatives that are interesting from the biological point of view. Among them are Murabutide (administered in clinical trials as adjuvants for vaccines), MDP-Lys(L18) (Muroctasin), and MTP-PE.[2] Due to Muroctasin activation of peripheral blood leukocytes, this particular compound is expected to be a useful drug for the treatment of leukopenia induced either by cancer chemotherapy or radiation therapy.[3] Muramyl tripeptide phosphatidylethanolamine (MTP-PE) stimulates in vitro and in vivo monocytes/macrophages to kill a variety of tumor cells. Encapsulation of MTP-PE into multilamellar liposomes (L-MTP-PE) is presently undergoing clinical trials in patients with recurrent osteosarcoma and melanoma. There is an expectation that L-MTP-PE combined with other anticancer agents may improve long-term cure rates of patients with these diseases.[4]

Continuing our program of syntheses of conjugates of MDP or nor-MDP with anticancer active compounds[1,5−8] we present syntheses of several new MDP or nor-MDP analogues which are modified at the C-terminus of the peptide residue by the formation of ester bond between the isoglutamine carboxylic group and the hydroxyl group of 4-carboxamide-hydroxyalkylacridine/9-acridone and 1-nitro-9-hydroxyalkylaminoacridines. 4-Carboxamide-acridine/9-acridone derivatives are known as effective anticancer agents. Among them *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), a lipophilic DNA intercalating reagent synthesized in the laboratory of Auckland in New Zealand,[9] is a dual topoisomerase I/II poison[10] showing high in vivo activity against two experimental murine solid tumors, Lewis lung and Colon 38.[9,11] DACA is able to overcome transport multidrug resistance (MDR) mediated by both P-glycoprotein and multidrug resistance protein (MRP).[12−14] On the basis of both these properties, DACA has undergone clinical trials.[15,16] Unfortunately, clinically accepted 4-carboxamide-acridine/9-acridone derivatives with the strongest antitumor activity, such as DACA, could not be coupled to MDP, as they are devoided of functional groups capable of forming a covalent bond with the MDP molecule. Acridine/acridone moieties in the conjugates presented herein correspond to variously modified structures of acridine derivatives whose biological activities have not been evaluated. On the other hand, the 1-nitro-9-hydroxyalkylaminoacridines have high antineoplastic activity, which was confirmed by many tests in vitro and in vivo.[17] Pharmacological examination showed that they are less toxic than the other known derivatives of acridines. These compounds have been patented by B. Wysocka-Skrzela at al.[17] who adapted their use for the synthesis of some conjugates with MDP and nor-MDP. One of the derivatives of 1-nitro-9-hydroxyethylaminoacridine containing a methyl group at C4 was selected for preclinical studies for prostate cancer.[18]

Chemistry

The synthesis of 4-carboxamide-hydroxyalkylacridine/9-acridone and 1-nitro-9-hydroxyalkylaminoacridine con-

* Address for correspondence: Department of Organic Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza St, 80−952 Gdansk, Poland. Telephone: (48 58) 347−27−36. Fax number: (48 58) 347−26−94. E-mail: kd@chem.pg.gda.pl.

10.1021/jm020991m CCC: $25.00
Published on Web 11/28/2002

Scheme 1

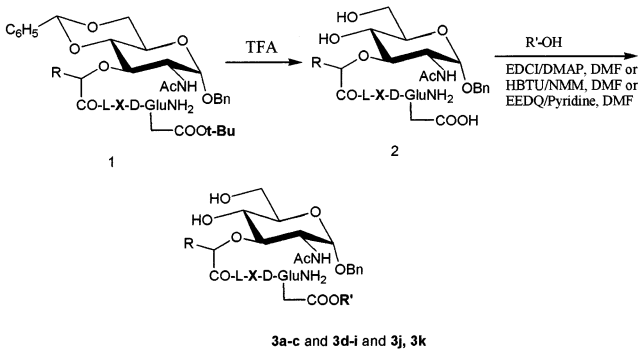
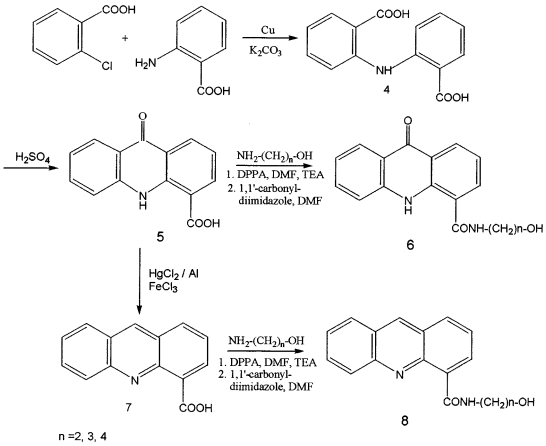


Table 1. 4-Carboxamidoalkyl-acridine/9-acridone and 1-Nitro-9-hydroxyalkylaminoacridines Derivatives of MDP and nor-MDP

compd	R	*n*	X	empirical formula[a]	yield (%)	mp (°C)
3a	CH_3	2	Val	$C_{44}H_{54}N_6O_{13}$	38	169−174
3b	H	2	Ala	$C_{41}H_{48}N_6O_{13}$	39	198−203
3c	H	3	Ala	$C_{42}H_{50}N_6O_{13}$	38	163−168
3d	H	3	Val	$C_{44}H_{54}N_6O_{13}$	36	154−157
3e	CH_3	2	Ala	$C_{42}H_{50}N_6O_{12}$	38	172−176
3f	CH_3	3	Val	$C_{45}H_{56}N_6O_{12}$	36	183−187
3g	CH_3	3	Ala	$C_{43}H_{52}N_6O_{12}$	40	170−173
3h	CH_3	4	Ala	$C_{44}H_{54}N_6O_{12}$	37	179−183
3i	H	2	Ala	$C_{41}H_{48}N_6O_{12}$	37	200−205
3j	CH_3	2	Ala	$C_{41}H_{49}N_7O_{13}$	47	157−159
3k	CH_3	3	Ala	$C_{42}H_{51}N_7O_{13}$	50	143−147

jugates of muramyl- or normuramyldipeptide was carried out according to Scheme 1. The protected MDP or nor-MDP **1**, synthesized as described previously,[19−21] were subjected to a cautious hydrolysis at the carboxyl group with 90% TFA at room temperature to give compounds **2**, followed by a formation of an ester bond at the C-terminal with the 4-carboxamide-hydroxyalkyl-acridine/9-acridone derivatives **6**, **8** or with 1-nitro-9-hydroxy-alkylaminoacridines **9**. Treating compounds **1** with 90% TFA for 20 min caused removal of the protecting *tert*-butyl and 4,6-benzylidene groups. Unexpectedly the formation of an ester bond between the carboxyl group of isoglutamine belonging to MDP molecule and the hydroxyl group of 4-carboxamide-hydroxyalkylacridine/9-acridone turned out to be complicated. When popular coupling reagents such us DCC, EEDQ, CCBT, and CCMT have been used, the reaction yields usually not exceed 5%. Fortunately synthesis of conjugates **3j** and **3k** by use of EEDQ in pyridine gave good yields. In other cases, after many attempts, we have found reaction conditions enabling preparation of these products with yields nearly 35−40%. HBTU or EDCI used as coupling reagent and applied as a 2-fold surplus of the hydroxy component in the presence of NMM or DMAP at 0 °C for 2 h and then at room temperature for next 72 h afforded satisfactory yields of the ester. The final products **3a**−**i** were purified with radial chromatography and preparative TLC. The composition of the conjugates was confirmed by ^{1}H NMR (500 MHz) spectroscopy, elemental analysis, and TLC qualitative amino acid analysis. Yield and melting points of the products are collected in Table 1. The 4-carboxamide-acridine/9-acridone derivatives (**6a**, **6b**,

Scheme 2



and **8a**−**c)** were obtained according to Scheme 2. Starting with 2-chlorobenzoic acid and 2-aminobenzoic acid in the Ullmann condensation followed by cyclization in the presence of sulfuric acid, 9-acridone-4-carboxylic acid **5** was obtained.[22] Acridine-4-carboxylic acid **7** was prepared by reduction of the corresponding 9-acridone-4-carboxylic acid **5** with aluminum/mercury amalgam, followed by $FeCl_3$ reoxidation of the resulting acridans.[23,24] Both acids **5** and **7** were condensed with amino alcohols hydrochloride in DMF by means of either DPPA in the presence of TEA or 1,1′-carbonyldiimidazole. Structures of all products (**6a**, **6b**, and **8a**−**c**) were established on the basis of NMR spectra and microanalyses. Synthesis of *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-ethylamino-1-nitroacridine ester **3j** and *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-propylamino-1-nitroacridine ester **3k** have been described previously.[5] Their anticancer activities are presented now.

Biological Results and Discussion

In this paper we describe the synthesis as well as cytotoxic and anticancer activities of conjugates of MDP or nor-MDP with 4-carboxamide-hydroxyalkylacridine/9-acridone derivatives **3a**−**i** and 1-nitro-9-hydroxyalkylaminoacridines **3j** and **3k**. All results of the biological assay obtained so far confirmed high cytotoxicity of the conjugates **3j** and **3k** containing the 9-aminoacridine skeleton and a nitro group at position 1, whereas conjugates **3a**−**i** containing 4-carboxamide-acridine/9-acridone are devoided this activity. It was next found that the presence of a nitro group is of great importance in anticancer activity of this family of compounds and that the acridine derivatives are more active than acridone ones. The effect of a nitro group can result from a markedly changed lipophilicity of the conjugates **3j** and **3k**, bearing also in mind that the nitro group is per se a redox active moiety. The role of the nitro group in acridines has been discussed in detail in many publications.[25−28] Coupling of acridine anticancer agents to MDP or nor-MDP molecules can increase their activity. The comparison of cytotoxic activity of 1-nitro-9-hydroxyethylaminoacridine dihydrochloride **9** and its conjugate with MDP (**3j**) (see Table 2) showed that conjugate **3j** was more active than the acridine derivative alone. The influence of muramyldipeptides on the properties of acridine derivatives has been, up to now, difficult to establish.

Table 2. Cytotoxicity of Selected Conjugates in the NCI Cell Line Panel[a]

compd	MID log LC_{50} (M)	Δ	sensitive cell lines[b]
3c	−4.04	0.20	LOX IMVI, MALME-3M, SK-MEL-5, CAKI-1, DU-145
3j	−6.85	1.40	HOP-92, NCI-H23, NCI-H460, NCI-H522, DMS 273, DMS 114, COLO 205, HCC-2998, HCT-116, CNS XF498, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-62, OVCAR-3, OVCAR-8, RXF 393
3k	−5.78	2.20	COLO 205, HCC-2998, MALME-3M
9[c]	−5.90	2.10	LXFL 529, COLO 205, HCC-2998, SF-295, SF-539, MALME-3M, M14 M19-MEL, SK-MEL-2, UACC-257, SK-MEL-5, OVCAR-8, U 251, XF 498, A 498, RXF-393, UO-31

[a] MID = the calculated mean panel LC_{50}concentrations (M). LC_{50} = half-lethal concentration. Δ is the number of log units by which the Δ of the most sensitive lines of the panel differs from the corresponding MID. The individual Δ values are calculated by subtracting each log LC_{50} from the panel mean. [b] Sensitive cell lines correspond to Δ values reported in the table. [c] **9** =1-nitro-9-hydroxyethylaminoacridine dihydrochloride.

Table 3. Results of in Vivo Hollow Fiber Assay[a]

compd	ip score	sc score	ip + sc	cell kill activity
3j	12	8	20	Y
3k	2	2	4	N[b]

[a] ip = intraperitoneal, sc = subcutaneous; full information on meaning of ip and sc scores can be described elsewhere;[33] Y = yes, and N = no. [b] Although the compound did not show activity in the hollow fiber assay, it was directed to investigations on xenograft assays.

a. Evaluation of Cytotoxicity.[29−31] All the final compounds were tested for cytotoxicity in the National Cancer Institute (NCI, Bethesda, MD) screening system based on 60 human tumor lines. This primary antitumor screen is designed to discover selective, disease-specific drugs. For the past 10 years, the Developmental Therapeutics Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), NCI, has used an in vitro panel consisting of 60 human tumor cell lines as the primary anticancer screen. An analysis of the data indicated that approximately 95% of the active compounds, based on the 60 cell-line screen, can be identified using only three of these cell lines: MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). Compounds which reduce the growth of any one of the cell lines to 32% or less are passed on for an evaluation on the full panel of 60 cell lines over a 5-log dose range. Only compound **3c** was active on all three cell lines: MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). All the other compounds **3a**, **3b**, and **3d**−**i** were inactive. The results were contrary to what we had expected. The analogues of MDP or nor-MDP esterified with 4-carboxamide-acridine/9-acridone **3a**−**i** were inactive whereas similar compounds **3j** and **3k** appeared to be very active in vitro and have qualified for further tests in vivo. The results obtained from the National Cancer Institute (NCI) screen showed differentiation of activity of these compounds on the GI_{50} and LC_{50} levels. The selected compounds **3j** and **3k** were of high activity on the LC_{50} level but of low activity on the TGI and GI_{50} levels (the latter data not shown in Table 2). These data suggest that the selected compounds **3j** and **3k** are cytotoxic but not cytostatic. The screen revealed a consistent fingerprint of highly sensitive colon, melanoma, and lung cancer cell lines. They also showed a high activity against prostate cancer. Furthermore, compound **3j** exhibited high activity in the AIDS-related lymphoma (ARL) test. Analogues **3j** and **3k**, by virtue of their activity and the subpanel disease selectivity, were selected by the NCI Biological Evaluation Committee for further testing in vivo.

b. Hollow Fiber Assay for Preliminary in Vivo Testing. Two of our compounds **3j** and **3k** have been evaluated in vivo in the hollow fiber assay (Table 3). That test have been described in detail previously.[1] Although only one compound (**3j**) met the criteria of activity, both of them have been selected by the NCI Biological Evaluation Committee for evaluations in human tumor xenograft assays.

c. In Vivo Biological Evaluation. Compound **3k** was extensively examined in mice in the Department of Immunology, CSK WAM of Warsaw, Poland. After

Table 4. Antitumor Effects of Compounds **3j** and **3k** Administered to Nude Mice Bearing sc Human Tumor Xenografts

		treatment[a]			growth delay	
compd	tumor	schedule days	dose/units (mg/kg)	opt % *T*/*C* (day)	% *T* − *C*/*C*	toxic deaths[b]
3j	NCI-H460 non-small cell lung	IP 7	1.34	54 (19)	21	1(6)
	UACC-62 melanoma	IP 10	1.50	89 (23)	2	0(6)
	HCT-15 colon	IP 5	0.90	79 (26)	5	2(6)
	RXF-393 renal	IP 7	0.90	81 (13)	32	0(6)
	COLO 205 colon	IP 12	0.67	75 (22)	11	0(6)
	AS283 lymphoma	IP 7	1.00	63 (21)	9	0(10)
	MDA-MB-435 breast	IV 24(38)	1.80	60 (52)	17	2(10)
3k	NCI−H522 non-small cell lung	IP 10	3.35	89 (24)	1	1(6)
	UACC-62 melanoma	**IP 8**	**8.40**	**1 (38)**	**107**	**0(6)**
	UACC-257 melanoma	IP 15	5.60	60 (33)	1	2(6)
	SK-MEL-28 melanoma	IP 7	3.35	105 (15)	−3	0(6)
	COLO 205 colon	IP 8	5.60	65 (29)	−7	1(6)
	HCC-2998 colon	IP 10	5.60	60 (20)	16	1(6)
	A498 renal	IP 11	5.60	68 (30)	3	1(6)

[a] Treatment = This section defines the test compound and dose, route, and schedule at which the compound was administered. The routes include intravenous [IV] or intraperitoneal [IP]. Opt. % *T*/*C* (day) = Percent treated/control is calculated by dividing the median treated tumor weight by the median control tumor weight on each observation day and multiplying by 100. The day on which this optimum *T*/*C* occurs is shown in parentheses. A *T*/*C* % of greather than 40 is considered inactive. Growth delay % *T* − *C*/*C* = expressed as the percentage by which the treated group median tumor weight is delayed in achieving the specified tumor size compared to the controls. A positive number indicates that the treated tumor reached *X* mg more slowly than did the control tumor. The greather this positive value, the longer the delay in the treated tumor reaching *X* mg. [b] Number of toxic deaths/total number of mice.

intraperitoneal administration of **3k**, peripheral blood and peritoneal lavage cell counts were determined, beginning day +1 until +7. It was observed that **3k** caused an increase in the number of peritoneal macrophages reaching the levels of severalfold the baseline and an increase in the peritoneal lymphocyte number beginning on day +2. In the same time these changes were accompanied by the significant increase in peripheral blood granulocyte number and slight lymphopenia. Moreover, moderate myelosuppression was observed indicated by the decrease in cellularity of femoral bone marrow cavity and decrease in reticulocyte count. Subsequently, the effect of **3k** on intraperitoneal growth of transplantable Lewis lung carcinoma (LL2) was studied. Tumor-bearing mice were treated ip with either **3k** (0.5 mL of 10^{-4} M solution in saline) or saline (control) and were observed for survival and ascites development. Treatment was initiated 1 day after tumor inoculation and administered daily. Control mice died about three weeks after inoculation, while at the same time **3k**-treated mice were in good condition, without evident ascities.[31]

Further compounds **3j** and **3k** were tested in growing human carcinoma xenografts[32] (Table 4) by NCI in Bethesda. The properties of these compounds were determined at three concentrations, but Table 4 presents just the best ones. Compound **3k** exhibited significant antitumor activity against UACC-62 melanoma. However, results obtained were not sufficient for further investigation of **3k**.

Experimental Section

Melting points were determined with a Kofler-block apparatus and are uncorrected.

^{1}H NMR spectra were measured in DMSO solutions with a Varian 500 and 200 NMR spectrometer. Preparative column chromatography and radial chromatography were performed on silica gel (Kieselgel 60, 100−200 mesh) in solvent systems specified in the text. All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Merck F_{254} silica gel precoated plates. The following solvent systems (by vol) were used for TLC development: n-BuOH−H_2O−AcOH (4:1:1) (A), $CHCl_3$−MeOH (4:1) (B), $CHCl_3$−MeOH (9:1) (C), and the organic layer of a n-BuOH−acetone−H_2O (3:2:1) mixture diluted with MeOH 1:10 (D). Elemental analyses were performed by Laboratory of Elemental Analysis, Faculty of Chemistry, University of Gdansk. Qualitative amino acid analyses of the hydrolyzates of these compounds were accomplished on TLC plates. Benzyl 1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetyl-(muramyl or normuramyl)-L-(alanyl or valyl)-D-isoglutaminate **1** and 1-*O*-benzyl-*N*-acetyl-(muramyl- or normuramyl)-L-(alanyl or valyl)-D-isoglutamine **2** were prepared as described elsewhere.[19−21] The 9-acridone-4-carboxylic acid **5** was obtained according to the known procedure.[22] The acridine-4-carboxylic acid **7** was manufactured as described.[23,24] The general procedures for the synthesis of compounds *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-ethylamino-1-nitroacridine ester **3j** and *O*-(1-*O*-benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-9-propylamino-1-nitroacridine ester **3k** were published previously.[5]

The following abbreviations also apply: Bn (benzyl), CCBT [6-chloro-1-(*p*-chlorophenylsulfonyloxy)benzotriazole], CCMT [1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate], DCC (*N*,*N*″-dicyclohexylcarbodiimide), DMAP [4-(dimethylamino)pyridine], DPPA (diphenyl azidophosphate), DMF (dimethylformamide), EDCI [1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride], HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), NMM (*N*-methylmorpholine), TEA (triethylamine), TFA (trifluoroacetic acid).

***N*-(ω-Hydroxyalkyl)-9-acridone-4-carboxamide (6a, 6b) and *N*-(ω-Hydroxyalkyl)acridine-4-carboxamide (8a−c). General Method: Procedure A.** To a stirred solution of acridine-4-carboxylic acid **7** (0.24 g, 1 mmol) or 9-acridone-4-carboxylic acid **5** (0.24 g, 1 mmol) and hydroxy ω-aminoalkyl hydrochloride (1.1 mmol) in anhydrous DMF (4 mL) was added a solution of DPPA (0.24 mL, 1.1 mmol) in DMF (1 mL) at 0 °C followed by addition of TEA (2.2 mmol). Initially the mixture was stirred at 0 °C for 3 h and after that at room temperature for 48 h. After evaporation, of the solvent the reaction mixture was purified using radial chromatography and preparative TLC in solvent B or C to obtain compounds **6a**, **6b**, and **8a**−**c** (yield 60−70%).

Procedure B. Acridine-4-carboxylic acid **7** (1.18 g, 5 mmol)- or 9-acridone-4-carboxylic acid **5** (1.19 g, 5 mmol) was suspended in anhydrous DMF (10 mL), and 1,1′-carbonyldiimidazole (1.24 g, 7.4 mmol) was added into the reaction medium. The mixture was warmed to 50 °C until all solids dissolved and then cooled to room temperature, followed by addition of hydroxy ω-aminoalkyl (14.8 mmol) and stirring. After 50 min, water was added, and the solvents were removed under reduced pressure. The residue was dissolved in ice-cold solution of 1 N Na_2CO_3 and extracted with EtOAc. The organic layer was washed with water and dried, and after evaporation of the solvent, the product was purified using preparative TLC in solvent C to obtain compounds **6a**, **6b**, and **8a**−**c** (yield 70−80%).

***N*-(2-Hydroxyethyl)-9-acridone-4-carboxamide (6a):** yield 0.18 g (63%, Procedure A), 0.22 g (78%, Procedure B); mp 218−220 °C; ^{1}H NMR (DMSO) δ 3.44 (q, J = 5.8 Hz, 2H, CH_2CH_2OH), 3.60 (q, J = 5.8 Hz, 2H, CH_2CH_2OH), 4.84 (t, J = 5.8 Hz, 1H, CH_2CH_2OH), 7.32 (t, J = 7.8 Hz, 1H, C7-H), 7.34 (d, J = 7.8 Hz, 1H, C2-H), 7.72 (d, J = 7.3 Hz, 1H, C5-H), 7.74 (d, J = 8.3 Hz, 1H, C6-H), 8.22 (d, J = 7.8 Hz, 1H, C8-H), 8.28 (d, J = 7.3 Hz, 1H, C3-H), 8.43 (d, J = 7.8 Hz, 1H, C1-H), 8.96 (t, J = 5.4 Hz, 1H, CONH), 12.4 (s, 1H, N10-H). Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

***N*-(3-Hydroxypropyl)-9-acridone-4-carboxamide (6b):** yield 0.18 g (64%, Procedure A), 0.23 g (80%, Procedure B); mp 176−179 °C; ^{1}H NMR (DMSO) δ 1.75 (t, J = 6.2 Hz, 2H, $CH_2CH_2CH_2$OH), 3.40 (q, J = 5.8 Hz, 2H, $CH_2CH_2CH_2$-OH), 3.50 (q, J = 5.8 Hz, 2H, $CH_2CH_2CH_2$OH), 4.55 (t, J = 5.2 Hz, 1H, $CH_2CH_2CH_2OH$), 7.28 (t, J = 7.3 Hz, 1H, C7-H), 7.36 (d, J = 7.3 Hz, 1H, C2-H), 7.72 (d, J = 7.3 Hz, 1H, C5-H), 7.74 (d, J = 8.3 Hz, 1H, C6-H), 8.22 (d, J = 7.8 Hz, 1H, C3-H), 8.24 (d, J = 7.8 Hz, 1H, C8-H), 8.42 (d, J = 7.8 Hz, 1H, C1-H), 8.98 (t, J = 5.4 Hz, 1H, CONH), 12.46 (s, 1H, N10-H). Anal. ($C_{17}H_{16}N_2O_3$) C, H, N.

***N*-(2-Hydroxyethyl)acridine-4-carboxamide (8a):** yield 0.17 g (62%, Procedure A), 0.21 g (78%, Procedure B); mp 145−148 °C; ^{1}H NMR (DMSO) δ 3.63 (q, J = 5.8 Hz, 2H, CH_2CH_2-OH), 3.73 (q, J = 5.8 Hz, 2H, CH_2CH_2OH), 5.10 (t, J = 5.4 Hz, 1H, CH_2CH_2OH), 7.63−8.42 (m, 6H, acridine protons), 8.75 (d, J = 6.2 Hz, 1H, ArH-3), 9.35 (s, 1H, ArH-9), 11.85 (t, J = 5.4 Hz, 1H, CONH). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

***N*-(3-Hydroxypropyl)acridine-4-carboxamide (8b):** yield 0.16 g (60%, Procedure A), 0.20 g (75%, Procedure B); mp 118−120 °C (lit.[24] mp 120−122 °C); ^{1}H NMR (DMSO) δ 1.90 (quinted, J = 5.8 Hz, 2H, $CH_2CH_2CH_2$OH), 3.60 (q, J = 5.8 Hz, 2H, $CH_2CH_2CH_2$OH), 3.70 (q, J = 5.8 Hz, 2H, $CH_2CH_2CH_2$-OH), 4.70 (t, J = 5.4 Hz, 1H, $CH_2CH_2CH_2OH$), 7.57−8.40 (m, 6H, acridine protons), 8.75 (d, J = 6.2 Hz, 1H, ArH-3), 9.30 (s, 1H, ArH-9), 11.45 (t, J = 5.4 Hz, 1H, CONH). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

***N*-(3-Hydroxybutyl)acridine-4-carboxamide (8c):** yield 0.19 g (64%, Procedure A), 0.22 g (74%, Procedure B); mp 132−134 °C; ^{1}H NMR (DMSO) δ 1.60−1.90 (m, 4H, $CH_2(CH_2)_2CH_2$-OH), 3.60 (q, J = 5.8 Hz, 2H, $CH_2(CH_2)_2CH_2$OH), 3.70 (q, J = 5.8 Hz, 2H, $CH_2(CH_2)_2CH_2$OH), 4.70 (t, J = 5.4 Hz, 1H, $CH_2(CH_2)_2CH_2OH$), 7.57−8.40 (m, 6H, acridine protons), 8.75

(d, J = 6.2 Hz, 1H, ArH-3), 9.30 (s, 1H, ArH-9), 11.45 (t, J = 5.4 Hz, 1H, CONH). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

General Procedures for the Syntheses of Compounds 3a−i. Method A. A solution of the compound **2** (2.2 mmol), 4-(dimethylamino)pyridine (1.1 mmol), and the ω-(hydroxyalkyl)-9-acridone/acridine-4-carboxamides (**6a**, **6b** or **8a−c**) (4 mmol) in anhydrous DMF (4 mL) was cooled and stirred in an ice bath to achieve temperature 0 °C, followed by addition of EDCI (2.4 mmol). The reaction mixture was stirred at 0 °C for 2 h and after that at room temperature for 72 h. After evaporation of the solvent, the reaction mixture was purified using radial chromatography and preparative TLC in solvent B or C to obtain compounds **3a−i**.

Method B. A solution of the compound **2** (1 mmol), 4-(dimethylamino)pyridine (1.1 mmol), and the ω-(hydroxyalkyl)-9-acridone/acridine-4-carboxamide (**6a**, **6b**, and **8a−c**) (2 mmol) in anhydrous DMF (3 mL) was cooled with stirring in an ice bath to achieve temperature 0 °C, and HBTU (1 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h and after that at room temperature for 72 h. After evaporation of the solvent the reaction mixture was purified using radial chromatography and preparative TLC in solvent B or C to obtain compounds **3a−i**.

***O*-[(1-*O*-Benzyl-*N*-acetyl-muramyl-L-valyl-D-γ-isoglutaminyl)-*N*-2-ethyl]-9-acridone-4-carboxamide ester (3a):** yield 0.72 g (38%); mp 169−174 °C; ^{1}H NMR (DMSO) δ 0.78 and 0.80 (2d, J = 6.8 Hz, J = 6.8 Hz, 6H, CHCH(CH_3)$_2$-CO), 1.20 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.74 (m, 1H, βCH$_2$-isoGln), 1.80 (s, 3H, AcMur), 1.92 (m, 1H, βCH$_2$-isoGln), 2.06 (m, 1H, CHCH(CH$_3$)$_2$CO), 2.14 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.32−3.55 (m, 3H, H-C3,4,5-Mur), 3.58−3.68 (m, 3H, CH$_2CH_2$NHCO, OH-C6-Mur), 3.88−3.96 (m, 1H, H-C2-Mur), 4.06−4.40 (m, 6H, αCH-Mur, αCH-Val, αCH-isoGln, CH_2CH$_2$-NHCO, CHCH(CH$_3$)$_2$CO), 4.50 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.68 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.90 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.70 (brs, 1H, OH-C4-Mur), 7.14 and 7.34 (2s, 2H, NH$_2$-isoGln), 7.28−7.40 (m, 7H, Ph, AcrH-2, AcrH-7), 7.75 (brs, 1H, AcrH-5), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, AcrH-8), 8.24 (d, J = 8.3 Hz, 1H, AcrH-3), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.44 (d, J = 7.8 Hz, 1H, AcrH-1), 9.12 (brs, 1H, Acr-CONH), 12.3 (s, 1H, N10-H). Anal. ($C_{44}H_{54}N_6O_{13}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-normuramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-2-ethyl]-9-acridone-4-carboxamide ester (3b):** yield 0.72 g (39%); mp 198−203 °C; ^{1}H NMR (DMSO) δ 1.20 (d, J = 7.3 Hz, 3H, CH_3-Ala), 1.74 (m, 1H, βCH$_2$-isoGln), 1.82 (s, 3H, AcMur), 2.2 (m, 1H, βCH$_2$-isoGln), 2.36 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.46−3.56 (m, 3H, H-C3,4,5-Mur), 3.58−3.68 (m, 3H, CH$_2CH_2$NHCO, OH-C6-Mur), 3.80−3.88 (m, 1H, H-C2-Mur), 4.14−4.32 (m, 5H, αCH-Mur, αCH-Ala, αCH-isoGln, CH_2CH$_2$NHCO), 4.44 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.66 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.72 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.8 (d, J = 5.9 Hz, 1H, OH-C4-Mur), 7.14 and 7.34 (2s, 2H, NH$_2$-isoGln), 7.28−7.40 (m, 7H, Ph, AcrH-2, AcrH-7), 7.75 (brs, 1H, AcrH-5), 8.06 (d, J = 6.8 Hz, 1H, NH-Ala), 8.24 (d, J = 8.3 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, AcrH-8), 8.24 (d, J = 8.3 Hz, 1H, AcrH-3), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.44 (d, J = 7.8 Hz, 1H, AcrH-1), 9.12 (brs, 1H, Acr-CONH), 12.3 (s, 1H, N10-H). Anal. ($C_{41}H_{48}N_6O_{13}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-normuramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-3-propyl]-9-acridone-4-carboxamide ester (3c):** yield 0.70 g (38%); mp 163−168 °C; ^{1}H NMR (DMSO) δ 1.22 (d, J = 7.3 Hz, 3H, CH_3-Ala), 1.74 (m, 1H, βCH$_2$-isoGln), 1.76 (m, 2H, CH$_2CH_2$ CH$_2$NHCO), 1.82 (s, 3H, AcMur), 2.2 (m, 1H, βCH$_2$-isoGln), 2.36 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.46−3.56 (m, 3H, H-C3,4,5-Mur), 3.58−3.68 (m, 3H, CH$_2$CH$_2CH_2$-NHCO, OH-C6-Mur), 3.80−3.88 (m, 1H, H-C2-Mur), 4.14−4.32 (m, 5H, αCH-Mur, αCH-Ala, αCH-isoGln, CH_2CH$_2$CH$_2$NHCO), 4.44 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.66 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.72 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.8 (d, J = 5.9 Hz, 1H, OH-C4-Mur), 7.14 and 7.34 (2s, 2H, NH$_2$-isoGln), 7.28−7.40 (m, 7H, Ph, AcrH-2, AcrH-7), 7.75 (brs, 1H, AcrH-5), 8.06 (d, J = 6.8 Hz, 1H, NH-Ala), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, AcrH-8), 8.24 (d, J = 8.3 Hz, 1H, AcrH-3), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.44 (d, J = 7.8 Hz, 1H, AcrH-1), 9.12 (brs, 1H, Acr-CONH-), 12.3 (s, 1H, N10-H). Anal. ($C_{42}H_{50}N_6O_{13}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-normuramyl-L-valyl-D-γ-isoglutaminyl)-*N*-3-propyl]-9-acridone-4-carboxamide ester (3d):** yield 0.67 g (36%); mp 154−157 °C; ^{1}H NMR (DMSO) δ 0.78 and 0.80 (2d, J = 6.8 Hz, J = 6.8 Hz, 6H, -CHCH(CH_3)$_2$-CO), 1.74 (m, 1H, βCH$_2$-isoGln), 1.80 (s, 3H, AcMur), 1.92 (m, 1H, βCH$_2$-isoGln), 2.06 (m, 1H, CHCH(CH$_3$)$_2$CO), 2.14 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.32−3.55 (m, 3H, H-C3,4,5-Mur), 3.58−3.68 (m, 3H, CH$_2$CH$_2CH_2$NHCO, OH-C6-Mur), 3.80−3.88 (m, 1H, H-C2-Mur), 4.14−4.32 (m, 6H, αCH-Mur, αCH-isoGln, αCH-Val, CH_2CH$_2$CH$_2$NHCO), 4.44 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.66 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.72 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.8 (d, J = 5.9 Hz, 1H, OH-C4-Mur), 7.14 and 7.34 (2s, 2H, NH$_2$-isoGln), 7.28−7.40 (m, 7H, Ph, AcrH-2, AcrH-7), 7.75 (brs, 1H, AcrH-5), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, AcrH-8), 8.24 (d, J = 8.3 Hz, 1H, AcrH-3), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.44 (d, J = 7.8 Hz, 1H, AcrH-1), 9.12 (brs, 1H, Acr-CONH), 12.3 (s, 1H, N10-H). Anal. ($C_{44}H_{54}N_6O_{13}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-2-ethyl]-acridine-4-carboxamide ester (3e):** yield 0.70 g (38%); mp 172−176 °C; ^{1}H NMR (DMSO) δ 1.18 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.23 (d, J = 6.4 Hz, 3H, CH_3−Ala), 1.74 (m, 1H, βCH$_2$-isoGln), 1.76 (s, 3H, AcMur), 1.74 (m, 1H, βCH$_2$-isoGln), 1.94−2.02 (m, 1H, βCH$_2$-isoGln), 2.30 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.42−3.52 (m, 3H, H-C3,4,5-Mur), 3.52−3.68 (m, 3H, CH$_2CH_2$NHCO, OH-C6-Mur), 3.76−3.82 (m, 1H, H-C2-Mur), 4.10−4.30 (m, 5H, CH_2CH$_2$NHCO, αCH-isoGln, αCH-Mur, αCH-Ala), 4.42 (d, J = 12.4 Hz, 1H, PhCH$_2$), 4.65 (d, J = 12.4 Hz, 1H, PhCH$_2$), 4.73 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.29 (d, J = 6.8 Hz, 1H, OH-C4-Mur), 7.08 and 7.32 (2s, 2H, NH$_2$-isoGln), 7.24−7.38 (m, 5H, Ph), 7.72−8.46 (m, 6H, acridine protons), 7.70 (d, J = 7.3 Hz, 1H, NH-Ala), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.38 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.72 (d, J = 5.4 Hz, 1H, ArH-3), 9.32 (s, 1H, ArH-9), 11.40 (t, J = 5.6 Hz, 1H, CONH). Anal. ($C_{42}H_{50}N_6O_{12}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-muramyl-L-valyl-D-γ-isoglutaminyl)-*N*-3-propyl]-acridine-4-carboxamide ester (3f):** yield 0.69 g (36%); mp183−187 °C; ^{1}H NMR (DMSO) δ 0.78 and 0.80 (2d, J = 6.8 Hz, J = 6.8 Hz, 6H, -CHCH(CH_3)$_2$-CO), 1.20 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.74 (m, 1H, βCH$_2$-isoGln), 1.80 (s, 3H, AcMur), 1.86−1.96 (m, 3H, βCH$_2$-isoGln, CH$_2CH_2$ CH$_2$NHCO), 2.06 (m, 1H, CHCH(CH$_3$)$_2$CO), 2.14 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.32−3.55 (m, 3H, H-C3,4,5-Mur), 3.58−3.68 (m, 3H, CH$_2$CH$_2$CH$_2$NHCO, OH-C6-Mur), 3.88−3.96 (m, 1H, H-C2-Mur), 4.06−4.40 (m, 6H, αCH-Mur, αCH-Val, αCH-isoGln, CH_2CH$_2$ CH$_2$NHCO, CHCH(CH$_3$)$_2$CO), 4.50 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.68 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.90 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.70 (brs, 1H, OH-C4-Mur), 7.05 and 7.35 (2s, 2H, NH$_2$-isoGln), 7.4 (m, 5H, Ph), 7.57−8.40 (m, 6H, acridine protons), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.70 (d, J = 6.2 Hz, 1H, ArH-3), 9.30 (s, 1H, ArH-9), 11.40 (t, J = 5.4 Hz, 1H, CONH). Anal. ($C_{45}H_{56}N_6O_{12}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-3-propyl]-acridine-4-carboxamide ester (3g):** yield 0.74 g (40%); mp 170−173 °C; ^{1}H NMR (DMSO) δ 1.18 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.23 (d, J = 6.8 Hz, 3H, CH_3−Ala), 1.74 (m, 1H, βCH$_2$-isoGln), 1.76 (s, 3H, AcMur), 1.74 (m, 1H, βCH$_2$-isoGln), 1.94−2.02 (m, 3H, βCH$_2$-isoGln, CH$_2CH_2$ CH$_2$NHCO), 2.30 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.42−3.52 (m, 3H, H-C3,4,5-Mur), 3.52−3.68 (m, 3H, CH$_2$CH$_2CH_2$NHCO, OH-C6-Mur), 3.76−3.82 (m, 1H, H-C2-Mur), 4.10−4.30 (m, 5H, CH_2CH$_2$CH$_2$NHCO, αCH-isoGln, αCH-Mur, αCH-Ala), 4.42 (d, J = 12.4 Hz, 1H, PhCH$_2$), 4.65 (d, J = 12.4 Hz, 1H, PhCH$_2$), 4.73 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.29 (d, J = 6.8 Hz, 1H, OH-C4-Mur), 7.08 and 7.32 (2s, 2H, NH$_2$-isoGln), 7.24−7.38 (m, 5H, Ph), 7.72−8.44 (m, 6H, acridine protons), 7.70 (d, J = 7.3 Hz, 1H, NH-Ala), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.38 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.72 (d, J = 5.4 Hz, 1H, ArH-

3), 9.32 (s, 1H, ArH-9), 11.40 (t, J = 5.6 Hz, 1H, CONH). Anal. ($C_{43}H_{52}N_6O_{12}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-muramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-4-butyl]-acridine-4-carboxamide ester (3h):** yield 0.74 g (37%); mp 179−183 °C; ^{1}H NMR (DMSO) δ 1.18 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.23 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.74 (m, 1H, βCH_2-isoGln), 1.76 (s, 3H, AcMur), 1.74 (m, 1H, βCH_2-isoGln), 1.94−2.02 (m, 3H, βCH_2-isoGln, CH_2-(CH_2)$_2$ CH_2NHCO), 2.30 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.42−3.52 (m, 3H, H-C3,4,5-Mur), 3.52−3.68 (m, 3H, CH_2-$(CH_2)_2CH_2$NHCO, OH-C6-Mur), 3.76−3.82 (m, 1H, H-C2-Mur), 4.10−4.30 (m, 5H, $CH_2(CH_2)_2CH_2$ NHCO, αCH-isoGln, αCH-Mur, αCH-Ala), 4.42 (d, J = 12.4 Hz, 1H, $PhCH_2$), 4.65 (d, J = 12.4 Hz, 1H, $PhCH_2$), 4.73 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.29 (d, J = 6.8 Hz, 1H, OH-C4-Mur), 7.08 and 7.32 (2s, 2H, NH_2-isoGln), 7.24−7.38 (m, 5H, Ph), 7.72−8.44 (m, 6H, acridine protons), 7.70 (d, J = 7.3 Hz, 1H, NH-Ala), 8.14 (d, J = 8.3 Hz, 1H, NH-Mur), 8.38 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.72 (d, J = 5.4 Hz, 1H, ArH-3), 9.32 (s, 1H, ArH-9), 11.40 (t, J = 5.6 Hz, 1H, CONH). Anal. ($C_{44}H_{54}N_6O_{12}$) C, H, N.

***O*-[(1-*O*-Benzyl-*N*-acetyl-normuramyl-L-alanyl-D-γ-isoglutaminyl)-*N*-2-ethyl]-acridine-4-carboxamide ester (3i):** yield 0.65 g (37%); mp 200−205 °C; ^{1}H NMR (DMSO) δ 1.22 (d, J = 6.8 Hz, 3H, CH_3−Ala), 1.74 (m, 1H, βCH_2-isoGln), 1.83 (s, 3H, AcMur), 2.02 (m, 1H, βCH_2-isoGln), 2.12 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.42 (m, 3H, H-C3,4,5-Mur), 3.44−3.68 (m, 3H, CH_2CH_2NHCO, OH-C6-Mur), 3.80−3.88 (m, 1H, H-C2-Mur), 4.04−4.32 (m, 5H, CH_2CH_2NHCO, αCH-isoGln, αCH-Mur, αCH-Ala), 4.42 (d, J = 12.2 Hz, 1H, $PhCH_2$), 4.66 (d, J = 12.2 Hz, 1H, $PhCH_2$), 4.70 (d, J = 3.4 Hz, 1H, H-C1-Mur), 5.83 (br, 1H, OH-C4-Mur), 7.09 and 7.34 (2s, 2H, NH_2-isoGln), 7.24−7.42 (m, 5H, Ph), 7.72−8.44 (m, 6H, acridine protons), 7.68 (d, J = 7.3 Hz, 1H, NH-Ala), 8.08 (d, J = 7.8 Hz, 1H, NH-Mur), 8.14 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.72 (d, J = 5.9 Hz, 1H, ArH-3), 9.33 (s, 1H, ArH-9), 11.44 (t, J = 5.6 Hz, 1H, CONH). Anal. ($C_{41}H_{48}N_6O_{12}$) C, H, N.

Acknowledgment. This work was supported by the Polish State Committee for Scientific Research (Grant No. 4 P05F 017 16). We express our thanks to Dr. V. L. Narayanan from NCI (Bethesda, MD) for the in vitro screening and O. C. Yoder, Ph.D. from NCI (Brussels, Belgium) for the in vivo Hollow Fiber Assay. We are grateful to Danuta Laskowska for her skillful technical assistance and to Dr. Barbara Wysocka-Skrzela for her cooperation.

References

(1) Dzierzbicka, K.; Kołodziejczyk, A. M.; Wysocka-Skrzela, B.; Myśliwski, A.; Sosnowska, D. Synthesis and antitumor activity of conjugates of muramyldipeptides, normuramyldipeptide, and desmuramyldipeptides with acridine/acridone derivatives. *J. Med. Chem.* **2001**, *44*, 3606−3614.

(2) Baschang, G. Muramylpeptides and lipopeptides: studies towards immunostimulants. *Tetrahedron* **1989**, *45*, 6331−6360.

(3) Sosnowska, D.; Dzierzbicka, K.; Myśliwski, A.; Kołodziejczyk, A. M. Muroctasin, a muramyl dipeptide derivative. *Postepy Hig. Med. Dośw.* **1992**, *46*, 73−82.

(4) Dzierzbicka, K.; Gozdowska, M.; Kołodziejczyk, A. M. L-MTP-PE − A potential drug for cancer therapy. *Postepy Hig. Med. Dośw.* **1997**, *51* (2), 227−236.

(5) Kołodziejczyk, A. M.; Dzierzbicka, K.; Kołodziejczyk, A. S. A new class of anticancer agents: conjugates of MDP and acridine/acridone derivatives. *Polish J. Chem.* **1994**, *68*, 1023−1029.

(6) Gozdowska, M.; Dzierzbicka, K.; Wysocka-Skrzela, B.; Kołodziejczyk, A. M. Synthesis and in vitro anticancer activity of conjugates of MDP with amino-acridine/acridone derivatives. *Polish J. Chem.* **1997**, *71*, 767−773.

(7) Dzierzbicka, K.; Gozdowska, M.; Wysocka-Skrzela, B.; Kołodziejczyk, A. M. Synthesis and anticancer activites of new MDP conjugates with amino-acridine/acridone derivatives. *Acta Pol. Pharm. Drug Res.* **1999**, *56*, 20−24.

(8) Dzierzbicka, K.; Kołodziejczyk, A. M.; Wysocka-Skrzela, B.; Kołodziejczyk, A. S. Synthesis of muramyl dipeptide conjugated with acridine derivatives, showing anti-HIV-1 and anticancer activity. *Pol. J. Chem.* **1994**, *68*, 37−45.

(9) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. Potential antitumor agents. 50. In vivo solid tumor activity of derivatives of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* **1987**, *30*, 664−669.

(10) Finlay, G. J.; Riou, J. F.; Baguley, B. C. From amsacrine to DACA (N-[2-(dimethylamino) ethyl]acridine-4-carboxamide): selectivity for topoisomerases I and II among acridine derivatives. *Eur. J. Cancer* **1996**, *32A*, 708−714.

(11) Baguley, B. C.; Zhuang, L.; Marshall, E. Experimental solid tumor activity of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *Cancer Chemother. Pharmacol.* **1995**, *36*, 244−248.

(12) Schneider, E.; Darkin, S. J.; Lawson, P. A.; Ching, L. M.; Ralph, R. K.; Baguley, B. C. Cell line selectivity and DNA breakage properties of the antitumour agent N-[2-(dimethylamino) ethyl]-acridine-4-carboxamide: role of DNA topoisomerase II. *Eur. J. Cancer and Clin. Oncol.* **1988**, *24*, 1783−1790.

(13) Finlay, G. J.; Marshall, E. S.; Matthews, J. H. L.; Paull, K. D.; Baguley, B. C. In vitro assessment of N-[2-(dimethylamino)ethyl]-acridine-4-carboxamide, a DNA-intercalating antitumour drug with reduced sensitivity to multidrug resistance. *Cancer Chemother. Pharmacol.* **1993**, *31*, 401−406.

(14) Davey, R. A.; Su, G. M.; Hargrave, R. M.; Harvie, R. M.; Baguley, B. C.; Davey, M. W. The potential of N-[2-(dimethylamino)ethyl]-acridine-4-carboxamide to circumvent three multidrug-resistance phenotypes in vitro. *Cancer Chemother. Pharmacol.* **1997**, *39*, 424−.

(15) de Bono, J. S.; Propper, D.; Ellard, S.; Steiner, J.; Bevan, P.; Dobbs, N.; Flannigan, E.; Ganesan, T. S.; Talbot, D. C.; Campbell, S.; Twelves, C.; Harris, A. A phase I study of XR5000 (DACA) by 120 h intravenous infusion. In *Proceedings of the 10th NCI-EORTC Symposium on New Drugs in Cancer Therapy*; 1998; p 449.

(16) Haldane, A.; Finlay, G. J.; Hay, M. P.; Denny, W. A.; Baguley, B. C. Cellular uptake of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA). *Anti-Cancer Drug Design* **1999**, *14*, 275−280.

(17) Wysocka-Skrzela, B.; Ledóchowski, A.; Radzikowski, C. 1-Nitro-9-hydroxyalkylamino-acridines or their salts. European Patent Application, 0038572, 1981; *Chem. Abstr.* **1982**, *96*, 68847u.

(18) Chen, Y.; Badithe, A. T.; Garikapaty, V. P. S.; Liu, X.; Szostek, A.; Wysocka-Skrzela, B.; Banerjee, D.; Konopa, J.; Miller, D. G.; Tiwari, K. R. Pre-clinical studies of 1-nitroacridine derivatives: Effective antitumor agents for prostate cancer. *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, 77.

(19) Lefrancier, P.; Choay, J.; Derrieu, M.; Lederman, I. Synthesis of *N*-acetylmuramyl-L-alanyl-D-isoglutamine, an adjuvant of the immune response and some *N*-acetylmuramyl peptide analogues. *Int. J. Pept. Protein Res.* **1977**, *9*, 249−257.

(20) Gozdowska, M. Ph.D. Thesis, Gdansk University of Technology, 1998.

(21) Dzierzbicka, K.; Kołodziejczyk, A. M. A convenient synthesis of isoglutamine and glutamine derivatives. *Pol. J. Chem.* **1991**, *65*, 1437−1439.

(22) Rewcastle, G. W.; Atwell, G. J.; Chambers, D.; Baguley, C.; Denny, W. A. Potential antitumor agents. 46. Structure−activity relationships for acridine monosubstituted derivatives of the antitumor agent N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* **1986**, *29*, 471−477.

(23) Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. Potential antitumor agents. Part 43. Synthesis and biological activity of dibasic 4-carboxamido-9-amino-acridines, a new class of antitumor agent. *J. Med. Chem.* **1984**, *27*, 1481−1485.

(24) Lee, H. H.; Palmer, B. D.; Baguley, B. C.; Chin, M.; McFadyen, W. D.; Wickham, G.; Thorsbourne-Palmer, D.; Wakelin, L. P. G.; Denny, W. A. DNA-Directed alkylating agents. 5. Acridinecarboxamide derivatives of (1,2-diaminoethane)dichloroplatinum (II). *J. Med. Chem.* **1992**, *35*, 2983−2987.

(25) Pawlak, J. W.; Pawlak, K.; Konopa, J. Cytotoxic and antitumor activity of 1-nitroacridines as an aftereffect of their interstrand DNA cross-linking. *Cancer Res.* **1984**, *44*, 4289−4296.

(26) Gorlewska, K.; Mazerska, Z.; Sowiński, P.; Konopa, J. Products of metabolic activation of the antitumor drug Ledakrin (Nitracrine) in vitro. *Chem. Res. Toxicol.* **2001**, *14* (1), 1−10.

(27) Wilson, W. R.; Denny, W. A.; Twigden, S.; Baguley, B. C.; Probert, J. C. Selective toxicity of nitracrine to hypoxic mammalian cells. *Br. J. Cancer* **1984**, *49*, 215−223.

(28) Wilson, W. R.; Denny, W. A.; Steward, G. M.; Fenn, A.; Probert, J. C. Reductive metabolism and hypoxia-selective metabolism of nitracrine. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1235−1238.

(29) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(30) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. The National Cancer Institute: Cancer Drug Discovery and Development Program. *Semin. Oncol.* **1992**, *19*, 622−638.
(31) Kruszewski, A. A.; Dzierzbicka, K.; Urbanowska, E.; Kot, M.; Jędrzejczak, W. W.; Kołodziejczyk, A. The effects of intraperitoneal administration of MDP-C867 on cell kinetics and transplantable tumour growth in peritoneal cavity, preliminary results. Presented at the 2nd Israel-Poland Symposium on the Chemistry and Biology of Peptides and Proteins, Jerusalem, 1995, s.41.
(32) Personal communication from NCI, Bethesda, MD.
(33) Plowman, J.; Dykes, D. J.; Hollingshead, M.; Simpson-Herren, L.; Alley, M. C. Human Tumor Xenograft Models in NCI Drug Development. In *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Teicher, B., Ed.; Humana Press: Totowa, NJ, 1997; pp 101−125.

JM020991M