Synthesis and Cytotoxic Activity of Conjugates of Muramyl and Normuramyl Dipeptides with Batracylin Derivatives

Krystyna Dzierzbicka,*,† Piotr Trzonkowski,‡ Przemysław Sewerynek,§ and Andrzej Myśliwski‡

Department of Organic Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-952 Gdansk, Poland, and Department of Histology and Immunology and Intercollegiate Faculty of Biotechnology, Medical University of Gdansk, Debinki 1 Street, 80-210 Gdansk, Poland

Received October 10, 2002

The synthesis of MDP (muramyl dipeptide) or nor-MDP (normuramyl dipeptide) conjugates modified at the peptide part with batracylin (BAT) or batracylin derivatives is described. Batracylin was synthesized by our modified method (Scheme 3). The synthesis of BAT via this modified route now appears to be feasible on a multigram scale. Preliminary screening data obtained at the National Cancer Institute (NCI, Bethesda, MD) have revealed that the conjugates did not expose any cytotoxic activity even at $10^{-4}-10^{-8}$ M or μg/mL. During tests performed at Medical University of Gdansk, Poland, two analogues **11c** and **11e** reduced the proliferation of Ab melanoma cells in vitro compared with batracylin alone (Table 2, Figure 1).

Introduction

MDP (*N*-acetylmuramyl-L-alanyl-D-isoglutamine), the minimal structure of the *Mycobacterium* cell wall, retains most of its biological activity, in particular the adjuvant activity stimulates nonspecific resistance to microbial infections, exhibits some residual anticancer and antiviral potency, and also acts synergistically with many drugs including antibiotics and anticancer agents.[1,2] Immunoactive properties of muramyl peptides depend on the stimulation or inhibition of the biosynthesis of many cytokines.[3] As we have already shown, MDP and some of its analogues are able to increase the anticancer activity of compounds possessing acridine rings.[4] On the other hand, batracylin, 8-aminoisoindolo-[1,2-*b*]quinazolin-1,2-(10*H*)-one (BAT), displays antitumor activity in vivo against murine leukemia P-388 and colon adenocarcinoma 38 in mice sublines with acquired resistance to adriamycin, cisplatin, and methotrexate.[5,6] Oral administration of batracylin is effective against other murine solid tumors including pancreatic ductal adenocarcinoma, colon adenocarcinoma no. 51, and hepatoma 129.[7] BAT is inactive against B16 melanoma, CD8F1 mammary carcinoma, L1210 leukemia, Lewis lung carcinoma, and human MX-1 mammary xenograft.[5] Recently, the chemotherapeutic mechanism of BAT was shown to differ from that leading to genotoxicity.[8,9] BAT acts as a topoisomerase II inhibitor and induces unscheduled DNA synthesis (UDS) of nonproliferating cells. Susceptibility to BAT toxicity was species-dependent.[10,11] The greater sensitivity of the rat has been associated with a high plasma concentration of the *N*-acetyl metabolite of BAT. The authors suggested that the presence of the metabolite may be associated with its acute toxicity and initiation of the genotoxic response in nonproliferating cells. The role of acetylation in the genotoxicity of the BAT was evaluated in *Salmonella typhimurium* strains expressing various levels of *N*- and *O*-acetyltransferase activity. The results demonstrate that the mutagenicity of BAT is directly related to *N*-acetyltransferase activity.[12] These data suggest that the genotoxic effects of BAT require the free amine while the antitumor effects are independent of the amino group. The limitations associated with the chemotherapeutic potential of BAT, along with the large dose levels required for anticancer activity and high toxicity, especially in rats, therefore turn our attention to the synthesis of its analogues.[13−15] Up to now, several BAT analogues have been synthesized with modifications in positions C8 (Cl, Br, NO_2, CH_3, NH_2) and C7 (Cl).[16] Recently, three patents on different types of BAT analogues were published.[17−19] BAT and its analogues are poorly soluble in water. To increase their solubility in water, they were acylated with amino acids, dipeptide, and tripeptide[17] or coupled to sugar moieties.[18]

Continuing our program of syntheses of MDP (muramyl dipeptide) and nor-MDP (normuramyl dipeptide) conjugates with anticancer active compounds,[20−23] we present syntheses of MDP and nor-MDP analogues that are modified at the C-terminus of the peptide residue by the formation of an amide bond between the isoglutamine carboxylic group and the amine group of the respective BAT or *N*-(*N*^{n}-amino acid)-BAT derivatives. In this paper we describe a modified method of batracylin (BAT) synthesis (Scheme 3). The synthesized conjugates have been submitted to the National Cancer Institute (NCI, Bethesda, MD) and the Medical University of Gdansk, Poland, for testing of cytotoxic activity.

Chemistry

Conjugates of MDP and nor-MDP with BAT or BAT derivatives [*N*-(*N*^{n}-amino acid)-BAT] were synthesized according to Scheme 1. The protected MDP or nor-MDP **1** were synthesized as described previously.[24,25] These substrates were subjected to a cautious hydrolysis at

* To whom correspondence should be addressed. Telephone: (48 58) 347-27-36. Fax: (48 58) 347-26-94., E-mail: kd@chem.pg.gda.pl.
† Gdansk University of Technology.
‡ Department of Histology and Immunology, Medical University of Gdansk.
§ Intercollegiate Faculty of Biotechnology, Medical University of Gdansk.

10.1021/jm021067v CCC: $25.00 © 2003 American Chemical Society
Published on Web 02/19/2003

Scheme 1

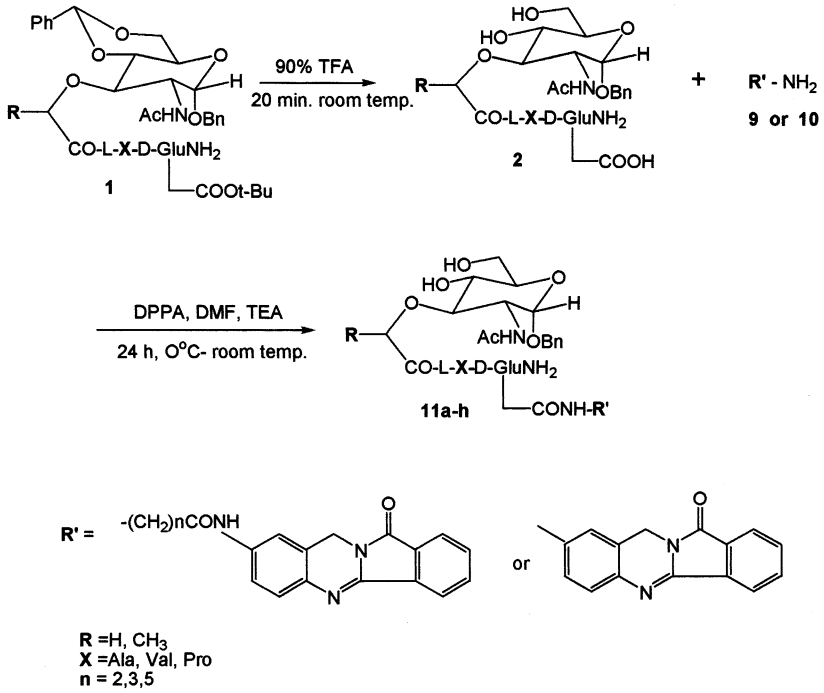


Table 1. MDP or nor-MDP Conjugates of Batracylin **11a**−**h**

compd[a]	R	X	*n*	molecular formula	mp (°C)	yield (%)
11a	CH_3	Ala		$C_{41}H_{48}N_7O_{11}$ (814.0)	223−227	50
11b	CH_3	Ala	2	$C_{44}H_{52}N_8O_{12}$ (884.0)	192−199	52
11c	H	Ala		$C_{43}H_{50}N_8O_{12}$ (870.0)	127−133	55
11d	H	Ala	5	$C_{46}H_{56}N_8O_{12}$ (912.0)	134−138	56
11e	CH_3	Val		$C_{43}H_{52}N_7O_{11}$ (842.0)	196−202	54
11f	CH_3	Val	3	$C_{47}H_{59}N_8O_{12}$ (927.0)	211−216	57
11g	H	Val	5	$C_{47}H_{58}N_8O_{12}$ (926.0)	224−229	58
11h	CH_3	Pro	5	$C_{48}H_{61}N_8O_{12}$ (941.0)	144−151	56

[a] ^{1}H NMR spectra of all compounds were in compliance with the expected ones. Elemental analyses (C, H, N) of all compounds agreed within ±0.4% of theoretical values.

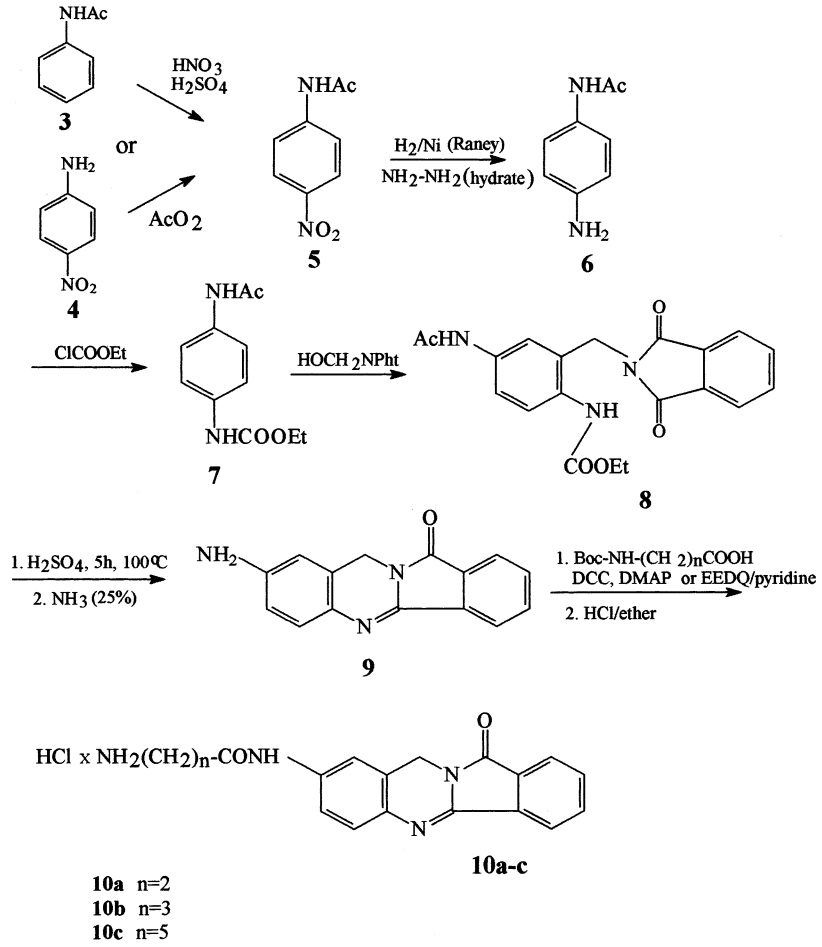
the carboxyl group with 90% TFA at room temperature to give compounds **2**, followed by a formation of an amide bond at the C-terminus with the BAT **9** or with its derivatives **10a**−**c**. The acylation was performed using DPPA (diphenyl azidophosphate) as a coupling agent in the presence of TEA (when using hydrochloride). Treating compounds **1** with 90% TFA for 20 min caused removal of the protecting *tert*-butyl and 4,6-benzylidene groups. There is no earlier information on this acidolysis of compounds **1**. The final products **11a**−**h** 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. Yields and melting points of the products are collected in Table 1.

Batracylin **9** was synthesized by our modified method (Scheme 3). There are several methods for the synthesis of batracylin (BAT). Kabbe[13] proposed the condensation of 2,5-diaminobenzylamine with phthalic anhydride. The reaction involves heating of substrates in dioxane for 4 h under mild conditions, and the yield of BAT was 56%. However, the preparation of 2,5-diaminobenzylamine is difficult. This substance is very unstable and is not commercially available. Roservear and Wilshere[16] described a BAT synthesis using ethyl *N*-(4-acetamidophenyl)carbamate, in which the reaction with *N*-(hydroxymethyl)phthalimide, according to the Czerniak−Einhorn reaction, provides ethyl [4-acetamido)-2-(phthalimidomethyl)phenyl]carbamate **8** (Scheme 2).

The Czerniak−Einhorn reaction is an electrophilic substitution, where the proton donor *N*-(hydroxymethyl)phthalimide is the electrophilic reagent. The reaction takes place in the presence of sulfuric acid. After acid hydrolysis of acetyl and urethane protecting groups and because of alkalinization with ammonia to promote cyclization, BAT was obtained. The *N*-(hydroxymethyl)-phthalimide is commercially available, but the synthesis of unsymmetrically protected phenylenediamine [ethyl 4-(acetamido)phenylcarbamate] consists of many steps and is difficult.[16] This method is the most common method for BAT synthesis in which *p*-nitroaniline or acetanilide is used as the substrate. In this method we modified the reduction of the nitro group of compound **5** using Raney nickel and 100% hydrazine hydrate (Scheme 2). The third method of BAT synthesis involves catalytic reduction of 8-nitroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one in the presence of glacial acetic acid.[15] Thorough analysis of the above-referred methods of BAT synthesis served as basis for some of our modifications. However, the question arises of why unsymmetrical protection was, up to now, used for BAT synthesis.

In our modified method, 1,4-phenylenediamine **12** was used with symmetrically protecting acetyl or urethane groups (Scheme 3). A symmetrically protected 1,4-phenylenediamine derivative could undergo the Czerniak−Einhorn reaction, and after hydrolysis of the protecting groups, BAT would result. The *N*,*N*′-diacetyl-1,4-phenylenediamine **13** or *N*,*N*′-diethoxycarbonyl-1,4-

Scheme 2



phenylenediamine **14** were reacted with *N*-(hydroxymethyl)phthalimide under standard conditions of the Czerniak−Einhorn reaction. Protecting groups present in the product mixture were hydrolyzed by the method described.[16] Concentrated sulfuric acid was used as the hydrolyzing agent for 5 h at 100 °C. After alkalinization with NH_3, an aqueous solution of BAT in the form of crystals was obtained. The yields after crystallization with DMF were 65−70% (for acetyl groups) and 75−80% (for urethane groups). The advantage of the method consists of elimination of two steps carried out in previously exploited methods and elimination of the difficult and troublesome reduction of the nitro group to the amino group. The synthesis of BAT via this modified route (Scheme 3) now appears to be feasible on a multigram scale. Batracylin derivatives [*N*-(*N*^{n}-Boc-amino acid)-BAT] and hydrochloride salts **10a**−**c** were obtained making use of known methods of amide bond formation: DCC and DMAP in anhydrous methylene chloride (**10b**,**c**); EEDQ and anhydrous pyridine in anhydrous methylene chloride (**10a**).

Results and Discussion

The low water solubility of BAT limits its oral administration. Toxicity of BAT, especially when rats are tested, reduces its usefulness as a chemotherapeutic agent when high doses must be used. It is very probable that BAT can be very toxic for the human organism. Therefore, syntheses of BAT analogues with improved therapeutic characteristics are undertaken. We hoped that combining strong antitumor BAT with immunomodulators, e.g., MDP or nor-MDP, would improve its pharmacological properties. Our synthesized conjugates are more soluble in water than BAT alone. All the final compounds **11a**−**h** were tested for their cytotoxicity in the National Cancer Institute (NCI, Bethesda, MD) screening system based on 60 human tumor lines.[26−28] This primary antitumor screen is designed to discover selective, disease-specific drugs. The synthesized conjugates did not exhibit cytotoxic activity at 10^{-4}−10^{-8} M. The results were contrary to what we had expected.

The influence of conjugates of MDP or nor-MDP with BAT was also tested in vitro on the growth of the Ab melanoma cells. These investigations were carried out at the Department of Histology and Immunology, Medical University of Gdansk, Poland. Bomirski melanoma variant Ab cells were maintained by serial passage in hamsters using the suspension method of tumor transplantation. The natural history and characteristics of that melanoma are given by Bomirski[30] and Slominski.[31] Batracylin (BAT) alone did not influence the proliferation of Ab melanoma cells. Two of the examined conjugates **11c** and **11e** inhibited the proliferation of Ab melanoma cells in vitro compared with batracylin or the solvent. Additional post-hoc tests revealed that the inhibiting effect exerted by **11c** and **11e** is mainly dependent on the two highest concentrations of the analogues: 0.05 and 0.1 mg/mL (Table 2 and Figure 1). The reductive effect of low doses of the used conjugates on the growth of the tumor cells could not be clearly

Scheme 3

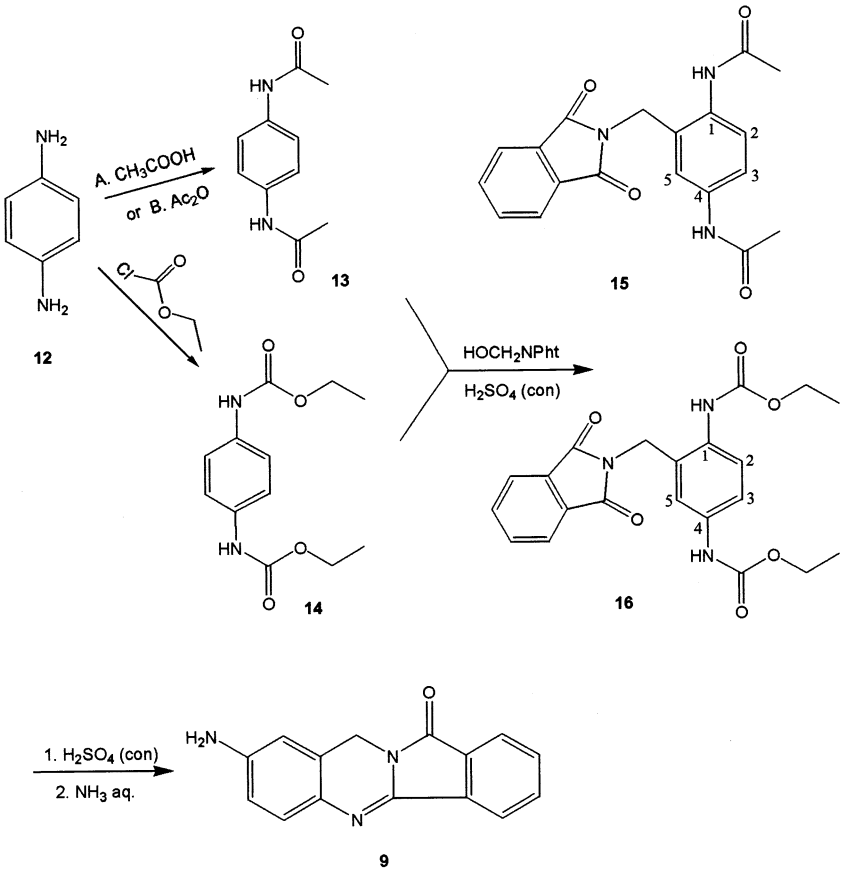


Table 2. ANOVA Test: Between-Group Comparison of the Serial Dilutions of the Examined Conjugates

compared pairs	*F*	*P*
BAT/DMSO	1.152	0.3435
11d/DMSO	1.907	0.3012
11h/DMSO	10.492	0.0835
11e/DMSO	305.18	0.0032
11c/DMSO	363.37	0.0027
BAT/**11d**	1.159	0.3942
BAT/**11h**	1.349	0.3819
BAT/**11e**	249.14	0.0039
BAT/**11c**	292.76	0.0033

elucidated because all the experiments were performed in vitro against Ab melanoma cells alone. MDP most visibly affects the immune cells, so the MDP fragment of the conjugates might indirectly block the tumor growth by an activation of the immune system. However, only further in vivo tests on animals could verify such hypothesis. The investigations will be carried out at the Medical University of Gdansk, Poland.

Experimental Section

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

^{1}H NMR spectra were measured in DMSO solutions with Varian 500 and 200 MHz NMR spectrometers. 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 volume) were used for TLC development: n-BuOH−H_2O−AcOH (4:1:1) (A), $CHCl_3$−MeOH (4:1) (B), CH_2Cl_2−$(CH_3)_2CO$ (10:1) (C), CH_2Cl_2−$(CH_3)_2CO$ (50:50) (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. ESI mass spectrometry was performed on a Mariner spectrometer. Benzyl 1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl(or normuramyl)-L-alanyl(valyl or prolyl)-D-isoglutaminate **1** was prepared as described.[24,25] *N*-(4-Nitrophenyl)acetamide **5** is commercially available and cheap.

***N*-(4-Aminophenyl)acetamide (6).** An amount of 3.6 g (20 mmol) of *N*-(4-nitrophenyl)acetamide **5** was hydrogenated with Raney Ni in 300 mL of dioxane in the presence of 2 mL of 100% hydrazine hydrate for 3 h. The reaction mixture was filtered off, and the catalyst was rinsed with 100 mL of hot dioxane. The filtrates were combined and the solvent was evaporated to give 2.70 g of 4-aminoacetanilide (90%): mp 161−162 °C (lit.[32] mp 162 °C); ^{1}H NMR ($CDCl_3$) δ 2.1 (s, 3H, $COCH_3$), 3.25 (br s, 2H, NH_2−), 6.52 (d, J = 8.8 Hz, 2H, protons of aromatic), 7.24 (d, J = 8.8 Hz, 2H, protons of aromatic), 9.45 (br s, 1H, −NH−CO−).

Ethyl *N*-(4-Acetamidophenyl)carbamate (7). This was prepared from *N*-(4-aminophenyl)acetamide **6** as a solid (78%): mp 210−212 °C (lit.[33] mp 202.5 °C); ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7 Hz, 3H, $-CH_2CH_3$), 1.98 (s, 3H, $COCH_3$), 4.3 (q, J = 7 Hz, 2H, $-CH_2CH_3$), 6.52 (d, J = 8.8 Hz, 2H, protons of aromatic), 7.24 (d, J = 8.8 Hz, 2H, protons of aromatic), 8.99 (br s, 1H, NH−COOEt), 9.8 (br s, 1H, NH−COMe).

Ethyl *N*-[4-Acetamido-2-(phthalimidomethyl)phenyl]-carbamate (8). This was prepared from ethyl *N*-(4-acetamidophenyl)carbamate **7** as a solid (75%): mp 251−254 °C (lit.[16] mp 258−260 °C); ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7 Hz, 3H, $COOCH_2CH_3$), 1.94 (s, 3H, $COCH_3$), 4.10 (q, J = 6.8 Hz, 2H, $COOCH_2CH_3$), 4.72 (s, 2H, CH_2N), 7.15 (brs, 1H, C_3-aromatic), 7.2 (d, J = 8 Hz, 1H, C_6-aromatic), 7.64 (dd, J = 2 Hz and J = 8 Hz, 1H, C_5-aromatic), 8.9 (br s, 1H, $NHCOOC_2H_5$), 9.83 (br s, 1H, $NHCOCH_3$). Anal. ($C_{20}H_{19}N_3O_5$) C, H, N.

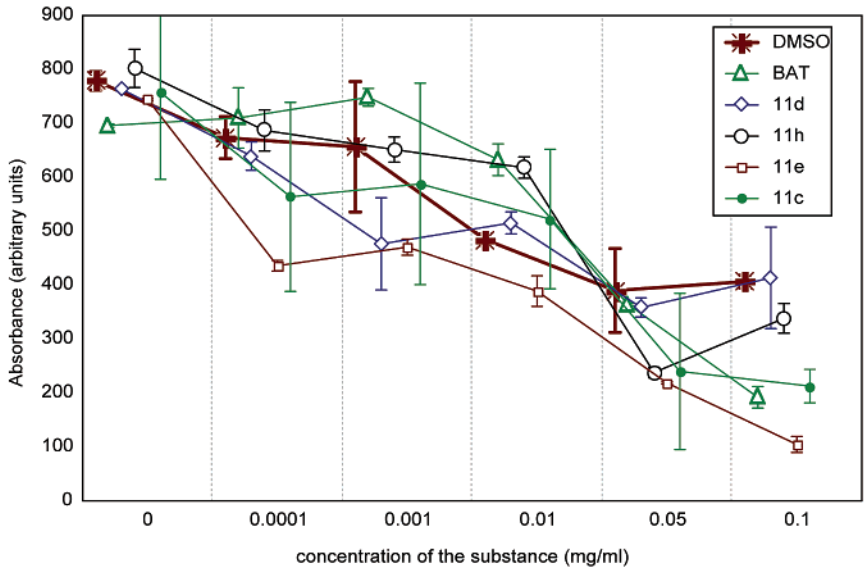


Figure 1. In vitro activity of the conjugates **11c**−**e**,**h** on the growth of the Ab melanoma cells.

8-Aminoisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (BAT) (9). This was prepared from ethyl *N*-[4-acetamido-2-(phthalimidomethyl)phenyl]carbamate **8** as a yellow solid (75%): mp 275−278 °C (lit. mp 270 °C[16] and 287−288 °C[13]); ^{1}H NMR (DMSO-d_6) δ 4.8 (s, 2H, B10), 5.55 (s, 2H, NH_2), 6.4 (s, 1H, B9), 6.48 (d, J = 8.2 Hz, B7), 7.15 (d, J = 8.2 Hz, B6), 7.67 (t, J = 7.4 Hz, 1H, B3), 7.74 (t, J = 7.4 Hz 1H, B2), 7.83 (d, J = 7.4 Hz, 1H, B4), 7.92 (d, J = 7.4 Hz, 1H, B1). Anal. ($C_{15}H_{11}N_3O$) C, H, N.

***N*-(3-Aminopropanoyl)batracylin Hydrochloride (10a).** To a stirred solution of 0.283 g (1.5 mmol) of Boc-β-alanine in 170 mL of anhydrous methylene chloride, cooled to 4 °C, 0.72 g (3 mmol) of EEDQ and 0.37 g (1.5 mmol) of batracylin **9** in dry pyridine (0.6 mL) were added. The stirring was continued for 2 h at 4 °C and then at room temperature for 3 days (TLC in system C or D). After evaporation of the solvents under reduced pressure, the residue was purified using radial chromatography in solvent D and *N*-(3-(1,1-dimethylethoxycarbonylamino)propanoyl)batracylin in 60% (0.28 g) yield was obtained: mp 214−216 °C; ^{1}H NMR (DMSO) δ 1.36 (s, 9H, Boc), 2.74 (t, J = 6.8 Hz, 2H, −NHCH$_2$ *CH*$_2$CONH-), 3.08 (q, J = 6.8 Hz, 2H, −NH*CH*$_2$CH$_2$CONH−), 4.86 (s, 2H, B10), 6.47 (brs, 1H, Boc−*NH*), 7.34 (d, J = 8.8 Hz, 1H, B6), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.88 (t, J = 7.4 Hz, 1H, B3), 7.80 (t, J = 7.4 Hz 1H, B2), 7.89 (d, J = 7.4 Hz, 1H, B4), 8.0 (d, J = 7.4 Hz, 1H, B1), 10.2 (s, 1H, B8-NH).

The obtained 0.2 g of *N*-(3-(1,1-dimethylethoxycarbonylamino)propanoyl)batracylin was poured into a saturated solution of hydrogen chloride in diethyl ether (10 mL) and left for 2 h at 0 °C. Then the solution was evaporated and the residue was washed several times with ethyl ether. The yellow hydrochloride salt of compound **10a** was obtained: yield 0.2 g (92%); mp 258 °C (dec); ESI (MeOH) *m*/*z* 321.2 (MH)$^+$; ^{1}H NMR (DMSO) δ 2.75 (t, J = 6.8 Hz, 2H, −NHCH$_2$*CH*$_2$CONH−), 3.10 (t, J = 6.8 Hz, 2H, −NH*CH*$_2$CH$_2$CONH−), 4.90 (s, 2H, B10), 7.34 (d, J = 8.8 Hz, 1H, B6), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.88 (t, J = 7.4 Hz, 1H, B3), 7.80 (t, J = 7.4 Hz 1H, B2), 7.90 (d, J = 7.4 Hz, 1H, B4), 7.90 (bs, 3H, NH_3^+), 8.0 (d, J = 7.4 Hz, 1H, B1), 10.3 (s, 1H, B8-NH).

***N*-(6-Aminohexanoyl)batracylin Hydrochloride (10c).** To a stirred solution of 0.5 g (2 mmol) of compound **9** in 290 mL of anhydrous methylene chloride cooled to 0 °C, 0.92 g (4 mmol) of 6-(1,1-dimethylethoxycarbonylamino)hexanoic acid, 0.83 g (4 mmol) of DCC, and 0.04 g of DMAP ((dimethylamino)-pyridine) were added. The stirring was continued for 4 h at 0 °C and then at room temperature for 4 days. The reaction was monitored with TLC in system solvent D. The side product of dicyclohexylurea was removed by filtration. The solvent was evaporated under reduced pressure, the yellow residue was purified with radial chromatography and eluted with system solvent D, and *N*-(6-(1,1-dimethylethoxycarbonylamino)hexanoyl)batracylin in 75% (0.52 g) yield was obtained: mp 187−190 °C; ^{1}H NMR (DMSO) δ 1.22−1.42 (m, 4H, −NHCH$_2$-CH$_2$*CH*$_2$CH$_2$CH$_2$CONH−), 1.36 (s, 9H, Boc), 1.57 (q, J = 7 Hz, 2H, −NHCH$_2$*CH*$_2$CH$_2$*CH*$_2$CH$_2$CONH−), 2.3 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$(CH$_2$)$_2$*CH*$_2$CONH−), 2.88 (q, J = 7 Hz, 2H, −NH*CH*$_2$CH$_2$(CH$_2$)$_2$CH$_2$CONH−), 4.9 (s, 2H, B10), 6.8 (brs, 1H, Boc−*NH*), 7.34 (d, J = 8.8 Hz, 1H, B6), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.88 (t, J = 7.4 Hz, 1H, B3), 7.80 (t, J = 7.4 Hz 1H, B2), 7.89 (d, J = 7.4 Hz, 1H, B4), 8.0 (d, J = 7.4 Hz, 1H, B1), 10.1 (s, 1H, B8-NH).

The obtained 0.2 g of *N*-(6-(1,1-dimethylethoxycarbonylamino)hexanoyl)batracylin was poured into a saturated solution of hydrogen chloride in diethyl ether (10 mL) and left for 2 h at 0 °C. Then the solution was evaporated and the residue was washed several times with ethyl ether. The yellow hydrochloride salt of compound **10c** was obtained: yield 0.19 g (90%); ESI (MeOH) *m*/*z* 363.2 (MH)$^+$; ^{1}H NMR (DMSO) δ 1.30−1.40 (m, 2H, −NHCH$_2$CH$_2$*CH*$_2$CH$_2$CH$_2$CONH−), 1.50−1.65 (m, 4H, −NHCH$_2$*CH*$_2$CH$_2$*CH*$_2$CH$_2$CONH−), 2.35 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$(CH$_2$)$_2$*CH*$_2$ CONH−), 2.75 (t, J = 7 Hz, 2H, −NH*CH*$_2$CH$_2$(CH$_2$)$_2$CH$_2$ CONH), 4.9 (s, 2H, B10), 7.38 (d, J = 8.8 Hz, 1H, B6), 7.57 (d, J = 8.6 Hz, 1H, B7), 7.62 (s, 1H, B9), 7.78 (t, J = 7.3 Hz, 1H, B3), 7.82 (t, J = 7.3 Hz 1H, B2), 7.90 (d, J = 7.4 Hz, 1H, B4), 7.90 (bs, 3H, NH_3^+), 8.05 (d, J = 7.4 Hz, 1H, B1), 10.20 (s, 1H, B8-NH).

The *N*-(4-aminobutanoyl)batracylin **10b** was obtained in the same manner. *N*-(4-(1,1-dimethylethoxycarbonylamino)butanoyl)batracylin: mp 222−224 °C; yield 82%; ^{1}H NMR (DMSO) δ 1.36 (s, 9H, Boc), 1.65 (q, J = 7 Hz, 2H, −NHCH$_2$*CH*$_2$CH$_2$-CONH−), 2.3 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$*CH*$_2$CONH−), 2.96 (q, J = 7 Hz, 2H, −NH*CH*$_2$CH$_2$CH$_2$CONH−), 4.9 (s, 2H, B10), 6.9 (brs, 1H, Boc−*NH*), 7.34 (d, J = 8.8 Hz, 1H, B6), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.88 (t, J = 7.4 Hz, 1H, B3), 7.80 (t, J = 7.4 Hz 1H, B2), 7.89 (d, J = 7.4 Hz, 1H, B4), 8.0 (d, J = 7.4 Hz, 1H, B1), 10.1 (s, 1H, B8-NH).

The hydrochloride salt of *N*-(4-aminobutanoyl)batracylin **10b**, mp 255 °C (dec), was obtained in 95% yield; ESI (MeOH) *m*/*z* 335.3 (MH)$^+$; ^{1}H NMR (DMSO) δ 1.65 (q, J = 7 Hz, 2H, −NHCH$_2$*CH*$_2$CH$_2$CONH−), 2.3 (t, J = 7 Hz, 2H, −NHCH$_2$-CH$_2$*CH*$_2$CONH−), 2.98 (t, J = 7.2 Hz, 2H, −NH*CH*$_2$CH$_2$CH$_2$-CONH−), 4.9 (s, 2H, B10), 7.34 (d, J = 8.8 Hz, 1H, B6), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.88 (t, J = 7.4 Hz, 1H, B3), 7.80 (t, J = 7.4 Hz 1H, B2), 7.91 (d, J = 7.4 Hz, 1H, B4), 7.91 (bs, 3H, NH_3^+), 8.0 (d, J = 7.4 Hz, 1H, B1), 10.18 (s, 1H, B8-NH).

General Procedure for the Synthesis of 1-*O*-Benzyl-*N*-acetylmuramyl(or normuramyl)-L-alanyl(valyl or prolyl)-D-isoglutamine (2). A total of 2 mmol of compound **1** in 4 mL of 90% trifluoroacetic acid was left at room temperature

for 20 min. Then the solution was evaporated under reduced pressure, and the residue was washed several times with ethyl ether to give **2** as a white powder.

1-*O*-Benzyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (2a): yield 1.08 g (93%); mp 88−92 °C; ^{1}H NMR (DMSO) δ 1.22 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.25 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.7 (s, 3H, AcMur), 1.74 (m, 1H, βCH_2-isoGln), 1.92 (m, 1H, βCH_2-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.6 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10.8 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1(m, 1H, αCH-isoGln), 4.3 (m, 2H, αCH-Mur, αCH-Ala), 4.45 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.75 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH_2-isoGln), 7.4 (m, 5H, Ph), 7.66 (d, J = 6.8 Hz, 1H, NH-Ala), 8.12 (d, J = 8.4 Hz, 1H, NH-Mur), 8.26 (d, J = 8.4 Hz, 1H, NH-isoGln), 10.05 (s, 1H, COOH).

1-*O*-Benzyl-*N*-acetyl-normuramyl-L-alanyl-D-isoglutamine (2b): yield 1.08 g (95%); mp 63−66 °C; ^{1}H NMR (DMSO) δ 1.20 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.83 (s, 3H, AcMur), 1.68 (m, 1H, βCH_2-isoGln), 1.95 (m, 1H, βCH_2-isoGln), 2.20 (t, J = 7.4 Hz, 2H, γCH_2-isoGln), 3.32−3.45 (m, 3H, H−C3,4,5-Mur), 3.50 (d, J = 5.8 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10 Hz, 1H, H−C6-Mur), 3.85 (m, 1H, H−C2-Mur), 4.08 (d, J = 15.8 Hz, 1H, OCH_2CO), 4.15 (m, 1H, αCH-isoGln), 4.20 (d, J = 15.8 Hz, 1H, OCH_2CO), 4.30 (m, 2H, αCH-Mur, αCH-Ala), 4.44 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.67 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.70 (d, J = 3.3 Hz, 1H, H−C1-Mur), 5.65 (br, 1H, OH−C4-Mur), 7.05 and 7.30 (2s, 2H, NH_2-isoGln), 7.35 (m, 5H, Ph), 8.05 (d, J = 6.6 Hz, 1H, NH-Ala), 8.18 (d, J = 8.8 Hz, 1H, NH-Mur), 8.25 (d, J = 8.8 Hz, 1H, NH-isoGln), 10.0 (s, 1H, COOH).

1-*O*-Benzyl-*N*-acetylmuramyl-L-valyl-D-isoglutamine (2c): yield 1.15 g (94%); mp 106−110 °C; ^{1}H NMR (DMSO) δ 0.84 (d, J = 6.8 Hz, 3H, CH_3-Val), 0.87 (d, J = 6.8 Hz, 3H, CH_3-Val), 1.18 (d, J = 6.7 Hz, 3H, CH_3-Mur), 1.80 (s, 3H, AcMur), 1.69 (m, 1H, βCH_2-isoGln), 1.93 (m, 1H, βCH_2-isoGln), 1.98 (m, 1H, βCH-Val), 2.06 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.6 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10.8 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.02 (m, 1H, αCH-Val), 4.1(m, 1H, αCH-isoGln), 4.3 (m, 1H, αCH-Mur), 4.44 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.70 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.80 (d, J = 3.4 Hz, 1H, H−C1-Mur), 5.5 (br, 1H, OH−C4-Mur), 7.08 and 7.32 (2s, 2H, NH_2-isoGln), 7.34−7.4 (m, 5H, Ph), 8.11 (d, J = 8.4 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.35 (d, J = 8.0 Hz, 1H, NH-Val), 10.2 (s, 1H, COOH).

1-*O*-Benzyl-*N*-acetylnormuramyl-L-valyl-D-isoglutamine (2d): yield 1.13 g (95%); mp 66−70 °C; ^{1}H NMR (DMSO) δ 0.84 (d, J = 6.8 Hz, 3H, CH_3-Val), 0.87 (d, J = 6.8 Hz, 3H, CH_3-Val), 1.18 (d, J = 6.7 Hz, 3H, CH_3-Mur), 1.80 (s, 3H, AcMur), 1.69 (m, 1H, βCH_2-isoGln), 1.93 (m, 1H, βCH_2-isoGln), 1.98 (m, 1H, βCH-Val), 2.06 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.6 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10.8 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.02 (m, 1H, αCH-Val), 4.08 (d, J = 15.8 Hz, 1H, OCH_2CO), 4.1 (m, 1H, αCH-isoGln), 4.20 (d, J = 15.8 Hz, 1H, OCH_2CO), 4.3 (m, 1H, αCH-Mur), 4.44 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.70 (d, J = 12.4 Hz, 1H, CH_2-Ph), 4.80 (d, J = 3.4 Hz, 1H, H−C1-Mur), 5.5 (br, 1H, OH−C4-Mur), 7.08 and 7.32 (2s, 2H, NH_2-isoGln), 7.34−7.4 (m, 5H, Ph), 8.11 (d, J = 8.4 Hz, 1H, NH-Mur), 8.22 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.35 (d, J = 8.0 Hz, 1H, NH-Val), 10.2 (s, 1H, COOH).

1-*O*-Benzyl-*N*-acetylmuramyl-L-prolyl-D-isoglutamine (2e): yield 1.16 g (92%); mp 69−74 °C; ^{1}H NMR (DMSO) δ 1.23 (d, J = 6.4 Hz, 3H, CH_3-Mur), 1.67−2.07 (m, 4H, Pro-3, Pro-4), 1.73 (m, 1H, βCH_2-isoGln), 1.78 (s, 3H, AcMur), 2.01(m, 1H, βCH_2-isoGln), 2.21 (m, 2H, γCH_2-isoGln), 3.35−3.51 (m, 3H, H−C3,4,5-Mur), 3.52 and 3.64 (m, 2H, Pro-5), 3.50 (d, J = 8.3 Hz, 1H, H−C6-Mur), 3.62 (d, J = 10.5 Hz, 1H, H−C6-Mur), 4.19 (dt, J = 5.2 Hz, J = 7.8 Hz, 1H, αCH-isoGln), 4.32 (m, 1H, Pro-2), 4.34 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.58 (t, J = 5.9 Hz, OH−C6-Mur), 4.65 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.83 (d, J = 3.4 Hz, 1H, H−C1-Mur), 5.38 (d, J = 6.9 Hz, OH−C4-Mur), 7.03 and 7.17 (2s, 2H, NH_2-isoGln), 7.24−7.38 (m, 5H, Ph), 7.92 (d, J = 8.3 Hz, 1H, NH-Mur), 8.32 (d, J = 7.9 Hz, 1H, NH-isoGln), 10.4 (s, 1H, COOH).

General Procedure for the Synthesis of *N*-[1-*O*-Benzyl-*N*-acetyl(muramyl or normuramyl)-L-(alanyl, valyl, or prolyl)-D-γ-isoglutaminyl)-*N*^{n}-amino acid]batracylin (11a−h). To a stirred solution of compounds **2** (1 mmol) and **9** or **10** (1.1 mmol) in anhydrous DMF (5 mL), cooled to 0 °C, DPPA (1.1 mmol) in DMF (5 mL) was added followed by the addition of TEA (2.2 mmol) in DMF (2 mL) at 0 °C for 3 h and then 24 h at room temperature. After evaporation of the solvent, the reaction mixture was purified using radial chromatography and preparative TLC in solvent system D to afford compounds **11a**−**h**. The yield and melting points of **11a**−**h** are given in Table 1.

***N*-[1-*O*-Benzyl-*N*-acetylmuramyl-L-alanyl-D-γ-isoglutaminyl]batracylin (11a):** ^{1}H NMR (DMSO) δ 1.22 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.25 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.74 (m, 1H, βCH_2-isoGln), 1.78 (s, 3H, AcMur), 1.92 (m, 1H, βCH_2-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1(m, 1H, αCH-isoGln), 4.3 (m, 2H, αCH-Mur, αCH-Ala), 4.45 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.74 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH_2-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.66 (d, J = 6.8 Hz, 1H, NH-Ala), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.1(s, 1H, B8-NH). Anal. ($C_{41}H_{48}N_7O_{11}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylmuramyl-L-alanyl-D-γ-isoglutaminyl]-(*N*^{2}-aminoalanyl)batracylin (11b):** ^{1}H NMR (DMSO) δ 1.22 (d, J = 7.3 Hz, 3H, CH_3-Mur), 1.25 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.74 (m, 1H, βCH_2-isoGln), 1.80 (s, 3H, AcMur), 1.90 (m, 1H, βCH_2-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 2.67 (t, J = 6.8 Hz, 2H, −NHCH$_2$ *CH*$_2$CONH−), 3.08 (q, J = 6.8 Hz, 2H, −NH*CH*$_2$CH$_2$CONH−), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.65 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1 (m, 1H, αCH-isoGln), 4.3 (m, 2H, αCH-Mur, αCH−Ala), 4.42 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.74 (d, J = 12.2 Hz, 1H, CH_2-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH_2-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.66 (d, J = 6.8 Hz, 1H, NH-Ala), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.1 (s, 1H, B8-NH). Anal. ($C_{44}H_{52}N_8O_{12}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylnormuramyl-L-alanyl-D-γ-isoglutaminyl]batracylin (11c):** ^{1}H NMR (DMSO) δ 1.25 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.72 (m, 1H, βCH_2-isoGln), 1.74 (s, 3H, AcMur), 1.94 (m, 1H, βCH_2-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH_2-isoGln), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.64 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1 (m, 1H, αCH-isoGln), 4.12 (d, J = 15.2 Hz, 1H, OCH_2CO), 4.2 (d, J = 15.2 Hz, 1H, OCH_2-CO), 4.3 (m, 2H, αCH-Mur, αCH-Ala), 4.45 (d, J = 12 Hz, 1H, CH_2-Ph), 4.75 (d, J = 12 Hz, 1H, CH_2-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH_2-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.66 (d, J = 6.8 Hz, 1H, NH−Ala), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.1 (s, 1H, B8-NH). Anal. ($C_{43}H_{50}N_8O_{12}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylnormuramyl-L-alanyl-D-γ-isoglutaminyl]-(*N*^{5}-aminocaproyl)batracylin (11d):** ^{1}H NMR (DMSO) δ 1.2−1.42 (m, 2H, −NHCH$_2$CH$_2$*CH*$_2$ CH$_2$CH$_2$CONH-), 1.25 (d, J = 6.8 Hz, 3H, CH_3−Ala), 1.57 (q, J = 7 Hz, 4H, −NHCH$_2$*CH*$_2$ CH$_2$*CH*$_2$CH$_2$CONH-), 1.72 (m, 1H, βCH_2-isoGln),

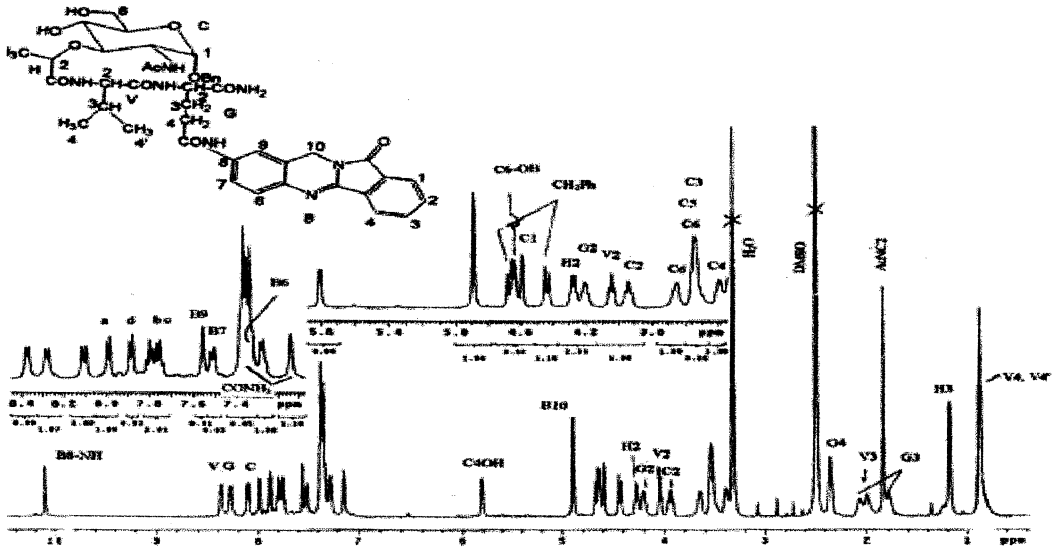


Figure 2. The 500 MHz ^{1}H NMR spectrum of *N*-(1-*O*-benzyl-*N*-acetylmuramyl-L-valyl-D-γ-isoglutaminyl)batracylin **11e** in DMSO.

1.82 (s, 3H, AcMur), 1.90 (m, 1H, βCH$_2$-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 2.32 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$-(CH$_2$)$_2$ *CH$_2$*CONH-), 2.89 (q, J = 7 Hz, 2H, −NH*CH$_2$*CH$_2$(CH$_2$)$_2$-CH$_2$CONH-), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.58 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.67 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1 (m, 1H, αCH-isoGln), 4.14 (d, J = 15.4 Hz, 1H, OCH$_2$CO), 4.2 (d, J = 15.4 Hz, 1H, OCH$_2$CO), 4.3 (m, 2H, αCH-Mur, αCH−Ala), 4.46 (d, J = 12 Hz, 1H, CH$_2$-Ph), 4.72 (d, J = 12 Hz, 1H, CH$_2$-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH$_2$-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.66 (d, J = 6.8 Hz, 1H, NH−Ala), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.1(s, 1H, B8-NH). Anal. (C$_{46}$H$_{56}$N$_8$O$_{12}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylmuramyl-L-valyl-D-γ-isoglutaminyl]batracylin (11e):** ^{1}H NMR (DMSO) δ 0.88 and 0.89 (2d, J = 6.3 Hz, 6H, −CHCH(*CH$_3$*)$_2$CO−), 1.19 (d, J = 6.7 Hz, 3H, CH$_3$-Mur), 1.80 (m, 1H, βCH$_2$-isoGln), 1.85 (s, 3H, AcMur), 2.01 (m, 1H, −CH*CH*(CH$_3$)$_2$ CO),2.08 (m, 1H, βCH$_2$-isoGln), 2.36 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 3.41 (dt, J = 6.3 Hz, J = 8.5 Hz, 1H, H−C4-Mur), 3.55 (m, 3H, H−C3,5,6-Mur), 3.56 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.67 (m,1H, H−C6-Mur), 3.95 (dt, J = 3.5 Hz, J = 10.3 Hz, 1H, H−C2-Mur), 4.05 (t, J = 7.5 Hz, 1H, -*CH*CH(CH$_3$)$_2$CO-), 4.21 (dt, J = 5.3 Hz, J = 8.7 Hz, 1H, αCH-isoGln), 4.29 (q, J = 6.8 Hz, 1H, αCH-Mur), 4.45 (d, J = 12.2 Hz, 1H, CH$_2$-Ph), 4.65 (t, J = 5.6 Hz, 1H, OH−C6-Mur), 4.68 (d, J = 12.2 Hz, 1H, CH$_2$-Ph), 4.60 (d, J = 3.5 Hz, 1H, H−C1-Mur), 4.90 (s, 2H, B-10), 5.82 (d, J = 6.3 Hz, 1H, OH−C4-Mur), 7.15 and 7.37 (2s, 2H, NH$_2$-isoGln), 7.37 (d, J = 8.6 Hz, 1H, B6), 7.25−7.4 (m, 5H, Ph), 7.52 (d, J = 8.6 Hz, 1H, B7), 7.57 (s, 1H, B9), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.81 (t, J = 7.4 Hz, 1H, B2), 7.89 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 9.6 Hz, 1H, NH-Mur), 8.0 (d, J = 7.4 Hz, 1H, B1), 8.29 (d, J = 7.9 Hz, 1H, NH-isoGln), 8.38 (d, J = 7.9 Hz, 1H, NH-Val), 10.11 (s, 1H, B8-NH). Anal. (C$_{43}$H$_{52}$N$_7$O$_{11}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylmuramyl-L-valyl-D-γ-isoglutaminyl](*N*^{3}-aminobutanoyl)batracylin (11f):** ^{1}H NMR (DMSO) δ 0.88 and 0.89 (2d, J = 6.3 Hz, 6H, −CHCH(*CH$_3$*)$_2$CO−), 1.20 (d, J = 7.3 Hz, 3H, CH$_3$-Mur), 1.64 (q, J = 7 Hz, 2H, −NHCH$_2$ *CH$_2$*CH$_2$CONH−), 1.68 (m, 1H, βCH$_2$-isoGln), 1.80 (s, 3H, AcMur), 1.93 (m, 1H, βCH$_2$-isoGln), 2.0 (m, 1H, −CH*CH*(CH$_3$)$_2$-CO−), 2.1 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 2.32 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$*CH$_2$*CONH−), 2.94 (q, J = 7 Hz, 2H, −NH*CH$_2$*CH$_2$CH$_2$CONH−), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.56 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.67 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1 (m, 1H, αCH-isoGln), 4.06 (t, J = 7.5 Hz, 1H, −*CH*CH(CH$_3$)$_2$CO−), 4.3 (q, J = 6.8 Hz, 1H, αCH-Mur), 4.45 (d, J = 12.5 Hz, 1H, CH$_2$-Ph), 4.70 (d, J = 12.5 Hz, 1H, CH$_2$-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH$_2$-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.38 (d, J = 8 Hz, 1H, NH-Val), 10.1 (s, 1H, B8-NH). Anal. (C$_{47}$H$_{59}$N$_8$O$_{12}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylnormuramyl-L-valyl-D-γ-isoglutaminyl](*N*^{5}-aminocaproyl)batracylin (11g).** ^{1}H NMR (DMSO) δ 0.88 and 0.9 (2d, J = 6.3 Hz, 6H, −CHCH(*CH$_3$*)$_2$-CO−), 1.2−1.4 (m, 2H, −NHCH$_2$CH$_2$*CH$_2$*CH$_2$CH$_2$CONH−), 1.56 (q, J = 7 Hz, 4H, −NHCH$_2$*CH$_2$*CH$_2$*CH$_2$*CH$_2$CONH−), 1.71 (m, 1H, βCH$_2$-isoGln), 1.85 (s, 3H, AcMur), 1.90 (m, 1H, βCH$_2$-isoGln), 2.0 (m, 1H, −CH*CH*(CH$_3$)$_2$CO−), 2.1 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 2.3 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$-(CH$_2$)$_2$*CH$_2$*CONH−), 2.86 (q, J = 7 Hz, 2H, −NH*CH$_2$*CH$_2$(CH$_2$)$_2$-CH$_2$ CONH−), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.55 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.67 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.04 (t, J = 7.5 Hz, 1H, −*CH*CH(CH$_3$)$_2$CO−), 4.1 (m, 1H, αCH-isoGln), 4.12 (d, J = 15 Hz, 1H, OCH$_2$CO), 4.22 (d, J = 15 Hz, 1H, OCH$_2$CO), 4.3 (m, 1H, αCH-Mur), 4.45 (d, J = 12 Hz, 1H, CH$_2$-Ph), 4.72 (d, J = 12 Hz, 1H, CH$_2$-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH$_2$-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.4 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.76 (t, J = 7.4 Hz, 1H, B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 8.38 (d, J = 8 Hz, 1H, NH-Val), 10.1 (s, 1H, B8-NH). Anal. (C$_{47}$H$_{58}$N$_8$O$_{12}$) C, H, N.

***N*-[1-*O*-Benzyl-*N*-acetylmuramyl-L-prolyl-D-γ-isoglutaminyl](*N*^{5}-aminocaproyl)batracylin (11h):** ^{1}H NMR (DMSO) δ 1.2−1.42 (m, 2H, −NHCH$_2$CH$_2$*CH$_2$* CH$_2$CH$_2$CONH−), 1.22 (d, J = 7.3 Hz, 3H, CH$_3$-Mur), 1.65 and 1.96 (m, 2H, C3-Pro), 1.56 (q, J = 7 Hz, 4H, −NHCH$_2$*CH$_2$*CH$_2$*CH$_2$*CH$_2$CONH−), 1.68 (m, 1H, βCH$_2$-isoGln), 1.75 (s, 3H, AcMur), 1.80 (m, 2H, C4-Pro), 1.92 (m, 1H, βCH$_2$-isoGln), 2.1 (t, J = 7.8 Hz, 2H, γCH$_2$-isoGln), 2.32 (t, J = 7 Hz, 2H, −NHCH$_2$CH$_2$(CH$_2$)$_2$*CH$_2$*CONH−), 2.88 (q, J = 7 Hz, 2H, −NH*CH$_2$*CH$_2$(CH$_2$)$_2$CH$_2$CONH−), 3.32−3.55 (m, 3H, H−C3,4,5-Mur), 3.48 and 3.60 (m, 2H, C5-Pro), 3.54 (d, J = 5.7 Hz, 1H, H−C6-Mur), 3.62 (d, J = 10.7 Hz, 1H, H−C6-Mur), 3.8 (m, 1H, H−C2-Mur), 4.1 (m, 1H, αCH-isoGln), 4.3 (m, 1H, αCH-Mur), 4.31 (m, 1H, C2-Pro), 4.42 (d, J = 12.5 Hz, 1H, CH$_2$-Ph), 4.75 (d, J = 12.5 Hz, 1H, CH$_2$-Ph), 4.76 (d, J = 3.4 Hz, 1H, H−C1-Mur), 4.9 (s, 2H, B-10), 5.5 (br, 1H, OH−C4-Mur), 7.05 and 7.35 (2s, 2H, NH$_2$-isoGln), 7.36 (d, J = 8.6 Hz, 1H, B6), 7.38−7.44 (m, 5H, Ph), 7.5 (d, J = 8.6 Hz, 1H, B7), 7.56 (s, 1H, B9), 7.76 (t, J = 7.4 Hz, 1H,

Table 3. ^{1}H NMR Data of *N*-(1-*O*-Benzyl-*N*-acetylmuramyl-L-valyl-D-γ-isoglutaminyl)batracylin **11e**

fragment structure	assignment	δ (ppm)	multiplicity and *J* (Hz)	ROE
GlcN	C1	4.60	d 3.5	CH2ϕ
	C2	3.95	dt 3.5, 10.3	
	C3	3.55	m	H2
	C4	3.41	dt 6.3, 8.5	dt 6.3; 8.5
	C5	3.55	m	m
	C6	3.55, 3.67	m, m	m, m
	C4-OH	5.82	d 6.3	d 6.3
	C6-OH	4.65	t 5.6	t 5.6
	C2-NH	8.12	d 9.6	d 9.6
	N-Ac	1.85	s	s
Bz	CH2	4.45, 4.68	d, d 12.5	C1, ϕ
	ϕ	7.25−7.4	m	CH2
Khp	H2	4.29	q 6.8	C3, V2-NH
	H3	1.19	d 6.7	
Val	V2	4.05	t 7.5	G2-NH
	V3	2.01	m	
	V4	0.88, 0.89	d, d 6.3	
	V2-NH	8.38	d 7.9	H2
Glu	G2	4.21	dt 5.3, 8.7	CONH2
	G3	1.80, 2.08	m, m	
	G4	2.36	t 7.8	B8-NH
	G2-NH	8.29	d 7.9	V2
	CONH2	7.15, 7.37	s, s	G2
B	B1 (B4)	8.00	d 7.4	
	B2 (B3)	7.81	t 7.4	
	B3 (B2)	7.76	t 7.4	
	B4 (B1)	7.89	d 7.4	
	B6	7.37	d 8.6	
	B7	7.52	d 8.6	
	B8-NH	10.11	s	B7, B9, G4
	B9	7.57	s	B10
	B10	4.90	s	B9

B3), 7.8 (t, J = 7.4 Hz, 1H, B2), 7.9 (d, J = 7.4 Hz, 1H, B4), 8.12 (d, J = 8.3 Hz, 1H, NH-Mur), 8.2 (d, J = 7.4 Hz, 1H, B1), 8.26 (d, J = 8.3 Hz, 1H, NH-isoGln), 10.1(s, 1H, B8-NH). Anal. ($C_{48}H_{61}N_8O_{12}$) C, H, N.

***N,N′*-Diacetyl-1,4-phenylenediamine (13). Method A.** This was prepared from 1,4-phenylenediamine **12** and glacial acetic acid, as a solid (84.7%): mp 308−312 °C (lit.[29] mp 255−257 °C).

Method B. This was prepared from 1,4-phenylenediamine **12**, acetic anhydride, and pyridine, as a solid (92%): mp 310−313 °C (lit.[29] mp 312−315 °C); ^{1}H NMR (DMSO) δ 2.01 (s, 6H, 2CH_3), 7.48 (s, 4H, protons of aromatic), 9.84 (s, 2H, 2NH).

***N,N′*-Diethoxycarbonyl-1,4-phenylenediamine (14).** This was prepared from 1,4-phenylenediamine **12**, as colorless crystals (89%): mp 193−196 °C (lit.[33] mp 196−196.5 °C); ^{1}H NMR (DMSO) δ 1.25 (t, J = 6.6 Hz, 3H, CH_3), 4.05 (q, J = 6.6 Hz, 2H, $-CH_2CH_3$), 7.4 (s, 4H, protons of aromatic), 9.8 (s, 2H, 2NH).

***N*-[2-(Phthalimidomethyl)-1,4-acetyl]phenylenediamine (15).** An amount of 0.96 g (5 mmol) of *N*,*N*′-diacetyl-1,4-phenylenediamine **13** dissolved in 5 mL of concentrated sulfuric acid at 50 °C and an amount of 1.78 g (10 mmol) of *N*-(hydroxymethyl)phthalimide in portions were added for 1 h. After the reagent had dissolved, the solution was stirred at 50 °C for 3 h. The reaction mixture was poured onto ice and mixed with 50 mL of ethanol. The precipitate formed was collected by filtration. The yield of crystals was 1.13 g (64.2%): mp 258−262 °C; ^{1}H NMR (DMSO) δ 1.95 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.65 (s, 2H, CH_2 protons of phthalimidomethylenediamine), 7.16 (s, 1H, C5), 7.18 (d, J = 8.5 Hz, 1H, C2), 7.62 (d, J = 8.5 Hz, 1H, C3), 7.84−7.96 (m, 4H, protons of phthalic group), 9.58 (brs, 1H, NH), 9.84 (s, 1H, NH). Anal. ($C_{19}H_{17}N_3O_4$) C, H, N.

Ethyl *N*-{[2-(Phthalimidomethyl)-4-ethoxycarbonylamino]phenyl}carbamate (16). An amount of 8 g (17.3 mmol) of compound **15** was dissolved in 20 mL of concentrated sulfuric acid at 50 °C and was combined with 6.14 g of *N*-(hydroxymethyl)phthalimide, and the mixture was stirred for 3 h. The reaction mixture was poured onto ice and mixed with 250 mL of chloroform. The undissolved solids were filtered out. The chloroform layer was evaporated to dryness. Crystallization from acetic acid−water gave 7.1 g (54%) of ethyl *N*-{[2-(phthalimidomethyl)-4-ethoxycarbonylamino]phenyl}carbamate: mp 197−202 °C; ^{1}H NMR (DMSO) δ 1.15 (t, J = 7 Hz, 3H, CH_3), 1.25 (t, J = 7 Hz, 3H, CH_3), 4.05 (q, J = 6.8 Hz, 2H, $-CH_2CH_3$), 4.10 (q, J = 6.8 Hz, 2H, $-CH_2CH_3$), 4.7 (s, 2H, CH_2 protons of phthalimidomethylene group), 7.1 (s, 1H, C5), 7.18 (d, J = 8.5 Hz, 1H, C2), 7.42 (d, J = 8.5 Hz, 1H, C3), 7.86−7.94 (m, 4H, protons of phthalic group), 8.98 (brs, 1H, NH), 9.46 (s, 1H, NH). Anal. ($C_{21}H_{21}N_3O_6$) C, H, N.

8-Aminoisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (BAT) (9) Prepared by Our Method. Method A. This was prepared from 1.13 g of *N*-[2-(phthalimidomethyl)-1,4-acetyl]-phenylenediamine **15** and recrystallized from DMF to give a yellow solid (0.56 g, 70%): mp 268−270 °C.

Method B. This was prepared from 1 g of ethyl *N*-{[2-(phthalimidomethyl)-4-ethoxycarbonylamino]phenyl]}-carbamate **16** and recrystallized from DMF to give a yellow solid (0.48 g, 80%): mp 269−272 °C; ^{1}H NMR (DMSO-d_6) δ 4.8 (s, 2H, B10), 5.55 (s, 2H, NH_2), 6.4 (s, 1H, B9), 6.48 (d, J = 8.2 Hz, B7), 7.15 (d, J = 8.2 Hz, B6), 7.67 (t, J = 7.4 Hz, 1H, B3), 7.74 (t, J = 7.4 Hz 1H, B2), 7.83 (d, J = 7.4 Hz, 1H, B4), 7.92 (d, J = 7.4 Hz, 1H, B1). Anal. ($C_{15}H_{11}N_3O$) C, H, N.

In Vitro Activity on the Growth of the Ab Melanoma Cells. Proliferation Test. In all tests, cells were incubated for 12 h in an atmosphere of 5% CO_2 at 37 °C on 96 plastic flat-bottomed well plates (Corning, Science Products) in triplicate. Ab melanoma cells were dispensed as 1×10^4 cells per well in 100 μL of F10 medium with 5% heat-inactivated fetal calf serum (Gibco, Life Technologies Inc.). The cultures were incubated with the following final concentrations of batracylin and its derivatives: 0, 0.0001, 0.001, 0.01, 0.05, and 0.1 mg/mL. Since the examined substances were dissolved in DMSO, additional control cultures were performed only with the serial dilutions of the solvent.

Colorimetric MTT Assay. After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co.) was added to all the wells for a final concentration of 1 mg/mL and the plates were incubated at 37 °C in an atmosphere of 5% CO_2 for an additional 4 h. Then the reaction was stopped by addition of 100 μL of 2-propanol. Optical density was read at 570 nm on the automated plate reader (FL600, Bio-Tec). The results were obtained and analyzed in arbitrary units in absorbance mode using the computer program Kineticalc 4 (Bio-Tec).

Statistical Analysis. Since the results satisfied the normal distribution, statistical analysis was based on ANOVA tests.

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. We are grateful to Danuta Laskowska for her skillful technical assistance.

Appendix

Table 3 and Figure 2 show additional ^{1}H NMR data of **11e**.

References

(1) Adam, A.; Lederer, E. Muramylpeptides: immunomodulators, sleep factors and vitamins. *Med. Res.* **1984**, *4*, 111−152.
(2) Baschang, G. Muramylpeptides and lipopeptides: studies towards immunostimulants. *Tetrahedron* **1989**, *45*, 6331−6360.
(3) Dzierzbicka, K.; Gozdowska, M.; Kołodziejczyk, A. M. Influence of immunomodulators of the type MDP on secretion and activity of cytokines. *Postepy Biochem*. **1998**, *44* (3), 216−219.
(4) 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.

(5) Plowman, J.; Paull, K. D.; Atassi, G.; Harrison, S. D.; Dykes, D. J.; Kabbe, H. J.; Narayanan, V. L.; Yoder, O. C. Preclinical antitumor activity of batracylin (NSC 320846). *Invest. New Drugs* **1988**, *6*, 147−153.
(6) Waud, W. R.; Harrison, S. D.; Gilbert, K. S.; Laster, W. R.; Griswold, D. P. Antitumor drug cross-resistance in vivo in a cisplatin-resistant murine P388 leukemia. *Cancer Chemother. Pharmacol.* **1991**, *27*, 456−463.
(7) Mucci-LoRusso, P.; Polin, L.; Bissery, M. C.; Valeriote, F.; Plowman, J.; Luk, G. D.; Corbett, T. H. Activity of batracylin (NSC 320846) against solid tumors of mice. *Invest. New Drugs* **1989**, *7*, 295−306.
(8) Stevens, G. J.; Meegalla, S. K.; Makhey, D.; LaVoie, E. J.; McQueen, Ch. A. Genotoxicity of batracylin and several structural analogs. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, 158.
(9) Stevens, G. J.; McQueen, Ch. A. Species variation in the genotoxicity of batracylin. *Toxicol. Appl. Pharmacol.* **1994**, *126*, 39−44.
(10) El-Hawari, M.; Stoltz, M. L.; Pallas, F. Species differences in the toxic responses to batracylin (NSC 320846). *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 626.
(11) Ames, M. M.; Mathiesen, D. A.; Reid, J. M. Differences in *N*-acetylation of the experimental antitumor agent batracylin in the mouse and the rat. *Invest. New Drugs* **1991**, *9*, 219−225.
(12) Stevens, G. J.; LaVoie, E. J.; McQueen, Ch. A. The role of acetylation in the mutagenicity of the antitumor agent, batracylin. *Carcinogenesis* **1996**, *17*, 115−119.
(13) Kabbe, H. J. Heterocycles from diamines and diacyl compounds. *Justus Liebigs Ann. Chem*. **1978**, 398−404.
(14) Luo, Y.; Ren, Y. F.; Chou, T. Ch.; Chen, A. Y.; Yu, Ch.; Liu, L. F.; Cheng, C. C. A structure−activity relationship study of batracylin analogues. *Pharm. Res.* **1993**, *10* (6), 918−923.
(15) Meegalla, S. K.; Stevens, G. J.; McQueen, Ch. A.; Chen, A. Y.; Yu, Ch.; Liu, L. F.; Barrows, L. R.; LaVoie, E. J. Synthesis and pharmacological evaluation of isoindolo[1,2-*b*]quinazolinone and isoindolo[2,1-*a*]benzimidazole derivatives related to the antitumor agent. *J. Med. Chem*. **1994**, *37*, 3434−3439.
(16) Roservear, J.; Wilshire, F. K. A comparison of the reactions of some ethyl *N*-arylcarbamates with those of the corresponding acetanilides. II Amidomethylation with *N*-hydroxymethylphthalimide. *Aust. J. Chem*. **1990**, *43*, 339−353.
(17) Stella, V.; Al-Razzak, L. A.; Kabbe, H. J. U.S. Patent 4,980,343, 1990.
(18) Lerchen, H. G.; Piel, N.; Lorentzen, J. P.; Antonicek, H. P.; Kabbe, H. J. Fucose-marked batracylin glycopeptide conjugates as antitumor agents. European Patent Office No. 0 501 250 A1, 1992; *Chem. Abstr.* **1993**, *118*, 60133k.
(19) Lerchen, H. G.; Von Dem Bruch, K.; Petersen, U.; Baum-Gartem, J.; Piel, N.; Antonicek, H. P.; Weichel, W.; Sperzel, M.; Bremm, K.; Dieter, K. Preparation of carbohydrate-modified cytostatic agents. Patent No. PCT/EP96/01279, 1996; *Chem. Abstr.* **1997**, *126*, 31574q.
(20) 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.
(21) 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. *Pol. J. Chem*. **1997**, *71*, 767−773.
(22) 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*. **1999**, *56*, 20−24.
(23) Sosnowska, D.; Myśliwski, A.; Dzierzbicka, K.; Kołodziejczyk, A. The in vitro effect of new muramyl peptide derivatives on cytotoxic activity of NK (Natural Killer) cells from hamsters bearing Ab Bomirski melanoma. *Biotherapy* **1997**, *10*, 161−168.
(24) 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 analogs. *Int. J. Pept. Protein Res.* **1977**, *9*, 249−257.
(25) Gozdowska, M. Ph.D. Thesis, Gdansk University of Technology, Gdansk, Poland, 1998.
(26) 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.
(27) 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.
(28) Information from NCI, Bethesda MD.
(29) Pfeiffer, F. R.; Case, F. H. The preparation of some pyrido and pyridyl derivatives of phenazine and quinoxaline. *J. Org. Chem*. **1966**, *31*, 3384−3390.
(30) Bomirski, A.; Slominski, A.; Bigda, J. The natural history of a family of transplantable melanomas in hamsters. *Cancer Metastasis Rev.* **1988**, *7*, 95−118.
(31) Slominski, A.; Paus, R. Bomirski melanoma: a versatile and powerful model of pigment cell and melanoma research. *Int. J. Oncol.* **1993**, *2*, 221−228.
(32) Giffney, C. J.; O'Connor, Ch. J. Spectrophotometric determination of basicity constants. Part II. Acetanilides. *J. Chem. Soc., Perkin Trans. 2* **1975**, 706−712.
(33) Schiff, H.; Ostrogovich, A. Uramide, urethane und oxamathane der *p*- und *m*-phenylendiamine. *Justus Liebigs Ann. Chem*. **1896**, *293*, 371−388.

JM021067V