VOL. 55 NO. 3, MAR. 2002 THE JOURNAL OF ANTIBIOTICS pp. 308 — 314

New Amidino-benzimidazolyl Derivatives of Tylosin and Desmycosin

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(Received for publication October 3, 2001)

New amidino-benzimidazolyl derivatives of antibiotics tylosin and desmycosin are prepared in the reaction of corresponding amidino-substituted *o*-phenylendiamine with tylosin respectively desmyicosin on the 20-C aldehyde group. The reaction was carried out in absolute ethanol in the presence of *p*-benzoquinone. On this way are prepared:

20-[5-(*N*-isopropylamidino)-2-benzimidazolyl]tylosin hydrochloride **9**, 20-[5-(2-imidazolinyl)-2-benzimidazolyl]tylosin hydrochloride **10**, 20-[5-(*N*-morpholinylamidino)-2-benzimidazolyl]tylosin hydrochloride **11**, 20-[5-(*N*-isopropylamidino)-2-benzimidazolyl]desmycosin hydrochloride **12**, 20-[5-(2-imidazolinyl)-2-benzimidazolyl]desmycosin hydrochloride **13**, 20-[5-(*N*-morpholinylamidino)-2-benzimidazolyl]desmycosin hydrochloride **14**.

Their antimicrobial activity was tested on a series of microorganisms.

The use of tylosin in veterinary medicine for the treatment and prevention of serious respiratory illness among farm animals has been accepted[1,2]. The microbial spectrum of this important antibiotic includes Gram-positive-bacteria and *Mycoplasma* species[3]. In order to expand this antibiotic spectrum, a program of chemical modification and evaluation of novel derivatives of tylosin was undertaken, using tylosin-related intermediates as starting substances[4~6]. Investigations showed that C-20 aldehyde group could be readily modified with retention of antibacterial activity[7]. MATSUBARA[8] used reductive amination of the C-20 aldehyde group of tylosin like macrolides to synthesize a series of derivatives having C-20 secondary or tertiary amino functions, but members of this series of C-20 macrolides showed no improvement in antimicrobial activity. KIRST[1] studied a lot of transformations on C-20 aldehyde group of tylosin and desmycosin. One of the first derivatives tested, 20-deoxo-20-(heptamethylenamino)desmycosin showed excellent activity against *P. multocida*. KIRST[9] described a lot of other transformations on the aldehyde group of tylosin and desmycosin. Decarbonylated tylosin and desmycosin, resulted, for example, in the reduction of microbial activity. Recently KIRST[10] described chemical modifications on the other parts of the molecule combined with the modification of the aldehyde in order to incorporate anticipated pharamacokinetic benefits as observed in tilmicosin and other aldehyde modified macrolides. The goal of these efforts was to find ways to inhibit resistant bacteria. Recently the aldol condensation reaction of tricarbonyliron complexes was used to prepare, building blocks for the synthesis of carbomycin B/tylosin macrolide antibiotics and fluorinated analogs[11].

On the other hand the benzimidazole nucleus is found in the variety of naturally occurring compounds such as vitamin B_{12} and its derivatives[12] and is also a key feature in cardiotonic agents such as pimobenden[13], adibenden[14], potential antitumor agents[15] and antiulcer drugs[16].

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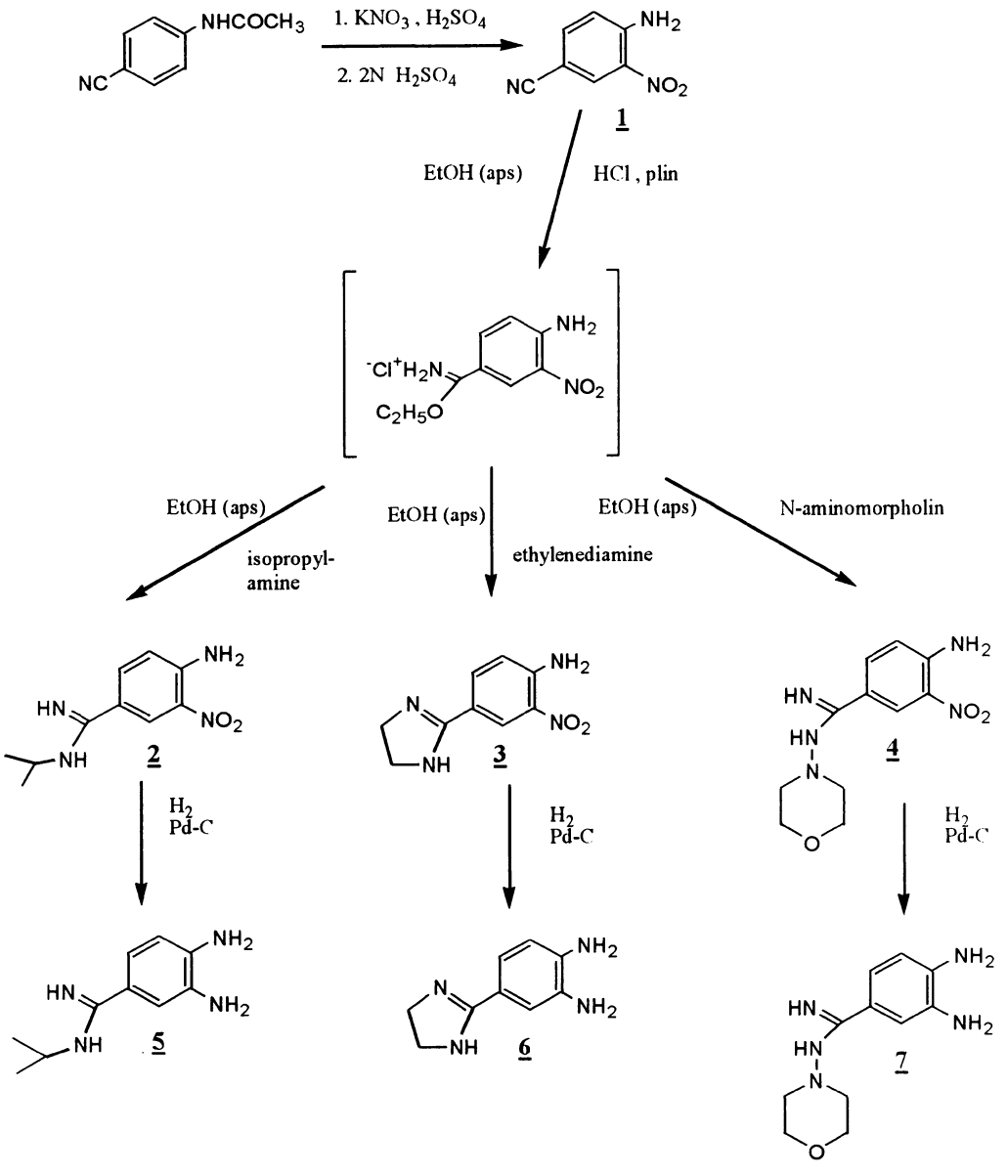
Amidino compounds are widely investigated on their biological activity too. TIDWELL[17] synthesized a series of analogues of 1,5-bis-(4-amidino-phenoxy)pentane (pentamidine) for screening against a rat model of *Pneumocystis carinii* pneumonia (PCP). In addition to their activity against PCP, the synthesized compounds were also evaluated for affinity for DNA. KUMAR[18] reported about the synthesis of some dicationic amidines of diarylpyridines, which showed modest anti HIV-1 activity and selectivity in primary limphocytes. Activity in a rat model of *Pneumocystis carinii* pneumonia (PCP) was described for bis-cationic amidines from carbazole series[19]. Amidinic isolated compounds showed antiparasitic activity[20]. Some dicationic amidino bis-benzimidazoles were found to have potent fungicidal activity[21]. A series of amidino substituted carbazoles, furans and benzimidazoles possess antimicrobial activity against a wide range of eucaryotic pathogens and show inhibitory and fungicidal activities against *Candida albicans* and *Cryptococcus neoformans*. Selected compounds were also found to be active against *Aspergillus fumigatus*, *Fusarium solani* and *Candida* species other than *C. albicans*[22].

Recently synthesized di-cations of bis-amidino benzimidazolyl substituted diphenylfurans inhibited HIV-1 infection[23]. WANG found that a series of an aromatic di-cations with an amidine-phenyl-furan-benzimidazole-

Scheme 1. Amidino substituted *o*-phenylenediamines.



amidine structure could recognize specific sequences of DNA by binding in the minor groove of DNA as a dimer[24].

Finally, two amidino-benzimidazoles were introduced into clinical use known as Hoechest's medicals, Hoechst 33258 and Hoechst 33342[25].

Results and Discussion

The known biological activities of tylosin and their derivatives, as well as well-known different biological activities of a number of amidino-substituted benzimidazoles, prompted us to synthesize the compounds in title[21]. The multiple synthesis of amidino-benzimidazolyl derivatives of tylosin and desmycosin started, with corresponding 4-amidino or 4-(*N*-substituted)-amidino-1,2-phenylene diamines according to the Scheme 1.

In the first step of the reaction 4-acetamidobenzonitrile was nitrated and hydrolized into 4-amino-3-nitrobenzonitrile (**1**). The cyano group of compound (**1**) reacted in a Pinner reaction[26] in apsolute EtOH with dry HCl gas to give an intermediate imidoester. Reaction of imidoester hydrochloride with isopropylamine, ethylendiamine or *N*-aminomorpholine in dry EtOH gave the corresponding 4-amino-3-nitro-*N*-substituted benzimidazoles (**2**), (**3**) and (**4**), which were catalytically reduced into 4-*N*-substituted-amidino-1,2-phenylene diamine (**5**), (**6**) and (**7**), and used later in the condensation with tylosin and desmycosin. Compounds (**5**), (**6**) and (**7**) reacted with C-20 aldehyde group of tylosin to produce amidino substituted tylosines (**8**~**11**), while C-20 aldehyde group of desmycosin gave compounds (**12**~**14**) according the Scheme 2.

Scheme 2. Synthesis scheme of amidino-benzimidazolyl tylosines and desmicosines.

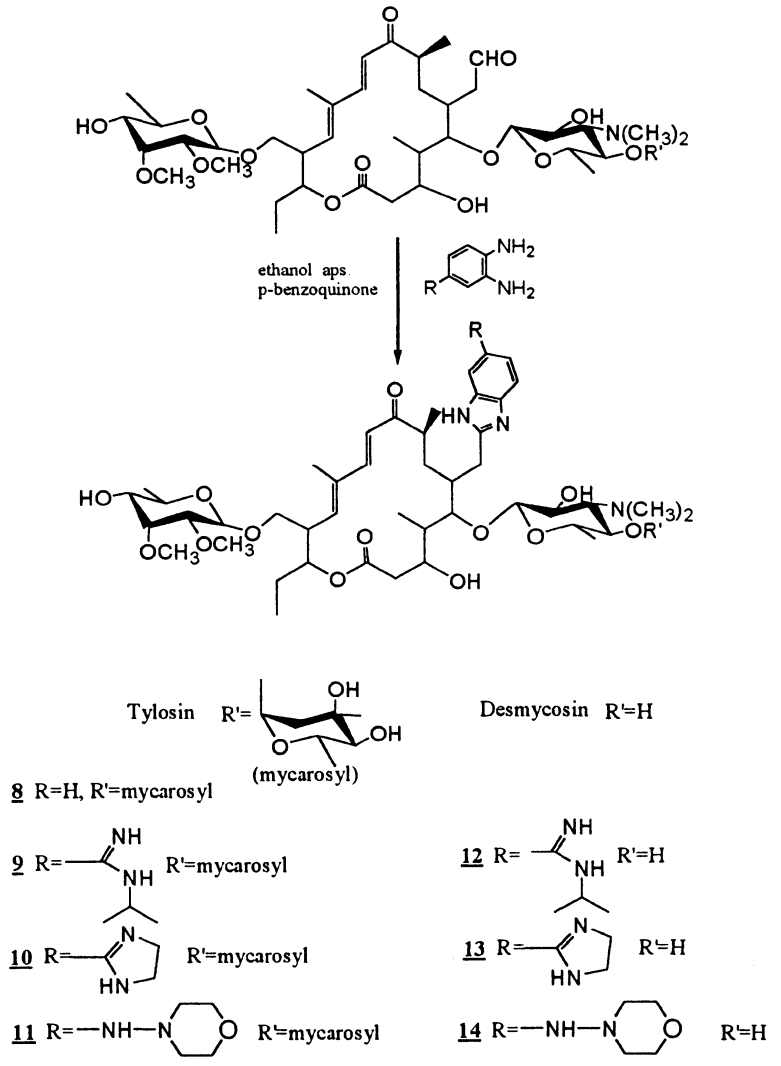


Table 1. Antimicrobial *in vitro* activities of new compounds compared with that of tylosin.

	MIC (μg/ml)						
Test organism	**9**	**10**	**11**	**12**	**13**	**14**	**Tylosin**
S. aureus / B 0329	16	8	32	8	8	32	0,5
S. aureus / iMLS / B 0538	32	32	64	16	32	64	1
S. aureus / cMLS / B 0330	>64	>64	>64	>64	>64	>64	>64
S. aureus / M / B 0331	32	16	64	8	16	64	1
S. pneumoniae / B 0541	8	4	8	1	2	8	<=0,125
S. pneumoniae / cMLS / B 0328	>64	>64	>64	>64	>64	>64	>64
S. pneumoniae / M / B 0326	4	2	8	4	8	16	<=0,125
S. pyogenes / B 0542	2	1	4	0,5	1	2	<=0,125
S. pyogenes / iMLS / B 0543	8	8	16	2	2	8	<=0,125
S. pyogenes / cMLS / B 0544	>64	>64	>64	2	>64	4	>64
M. chatarralis / B 0324	16	16	32	2	4	8	0,25
H. influenzae / B 0529	>64	64	>64	16	16	>64	8
E. faecalis / B 0004	16	16	32	8	16	32	0,25
E. coli / B 0001	>64	>64	>64	>64	>64	>64	>64
S. cerevisiae / B0332	>64	>64	>64	>64	>64	>64	>64
S. pneumoniae / B 0627	>64	>64	>64	>64	>64	>64	>64
S. pneumoniae / B 0633	>64	>64	>64	>64	>64	>64	>64

In Vitro Activity

The antimicrobial activities of new compounds are compared with that of tylosin. As shown in Table 1, all compounds show general decrease in activity, except compound **12** and **14** on *S. pyogenes*/cMLS/B0544. Best activity overall among tested macrolides was that of **12** which showed especially great increase in activity on constitutive resistance of *S. pyogenes*, as mentioned before. Compound **13** and **10** showed moderate activity on *S. pyogenes*/B0542, which was better then of other molecules but less then of **12**. New desmicosin derivatives are generally more active then corresponding new tylosin derivatives.

The resulting compounds (**8**~**14**) were identified by ^{1}H and ^{13}C NMR spectra, as well as, by molecular ion from the mass spectra.

Experimental

Physico-chemical Determination

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on Varian Gemini 300 and Bruker Avance DPX 300 and Bruker Avance DRX 500 spectrophotometers with TMS as internal standard. Mass spectra were recorded on Varian MAT 311A with EI and FAB type of ionization or Micromass Platform LCZ with ESI type of ionization.

In Vitro Evaluation

Minimal inhibitory concentrations were determinate on automatic robotic TECAN System. Briefly, stock solutions of substances were 5 mg/ml DMF, and from these working solutions in concentration range from 64 μg/ml to 0.05 μg/ml were made. After 24 hours incubation on 37°C, the optical density (λ=630 nm) was determined.

4-Amino-3-nitrobenzonitrile (**1**)

This compound was prepared from 4-acetamidobenzonitrile (10 g, 0.062 mol) and KNO_3 (13 g, 0.13 mol) in concentrated sulphuric acid by the method described earlier[27]. It was obtained 9.22 g (91%) light yellow crystals mp 158~160°C (lit[27] mp 160~161°C)

4-Amino-3-nitro-(*N*-isopropyl)benzamidine (**2**)

Amidino derivative (**2**) was prepared from the corresponding nitrile (**1**) by modification of the Pinner reaction[27]. The cyano derivative (**1**) (5.06 g, 0.031 mol) was suspended in abs. EtOH. The solution was than cooled to 0°C and saturated with dry HCl gas. After returning to room temperature the solution was allowed to stir until the disappearance of the nitrile band in IR spectrum (~2250 cm^{-1}) (about three days). The imido ester hydrochloride intermediate was then precipitated from the solution by either cooling or by the addition of diethylether. The imido ester was collected, dried, then suspended in dry EtOH and

stirred in success of isopropylamine (26.3 g, 0.31 mol) under nitrogen during 72 hours. The amidine was isolated by the removal of the solvent and addition of dry diethylether. Yellow solid precipitated which was filtered off and dried. It was obtained 7.07 g (88%) yellow solid mp>300°C. ^{1}H NMR (δ) (DMSO-d_6): 8.43 (d, 1H, NH), 8.42 (s, 1H, NH), 8.04 (s, 1H, $H_{arom.}$), 7.68 (d, 1H, J=8.98 Hz, $H_{arom.}$), 7.10 (d, 2H, J=8.98 Hz, $H_{arom.}$), 3.97 (m, 1H, CH), 2.47 (s, 2H, NH_2), 1.12 (m, 6H, CH_3).

4-Amino-3-nitro-(*N*-(2-imidazolinyl)]benzamidine (**3**)

Compound (**3**) was obtained from (**1**) (5.35 g, 0.033 mol) and ethylenediamine (6.6 ml, 0.098 mol) on the way described for preparation of (**2**). It was obtained 6.31 g (93%) yellow solid mp 300°C. ^{1}H NMR (DMSO-d_6): 8.74 (t, 1H, NH), 8.20 (s, 1H, $H_{arom.}$), 7.87 (d, 1H, J=9.31 Hz, $H_{arom.}$), 7.13 (d, 1H, J=8.98 Hz, $H_{arom.}$), 3.916 (s, 4H, CH_2).

4-Amino-3-nitro-(*N*-morpholinyl)benzamidine (**4**)

Compound (**4**) was prepared from (**1**) (2.55 g, 0.018 mol) and *N*-aminomorpholine (17 ml, 0.175 mol) on the way described for preparation of (**2**). It was obtained 3.40 g (74%) yellow crystals mp >300°C. ^{1}H NMR (DMSO-d_6): 8.52 (s, 1H, NH), 8.08 (s, 1H, $H_{arom.}$), 7.72 (d, 1H, J=8.98 Hz, $H_{arom.}$), 7.11 (d, 1H, J=8.31 Hz, $H_{arom.}$), 3.727 (m, 4H, CH_2), 2.860 (m, 4H, CH_2).

4-(*N*-Isopropyl)amidino-1,2-phenylene Diamine (**5**)

The compound (**5**) was prepared by the method described earlier[27]. A solution of (**2**) (7.07 g, 0.027 mol) in methanol (200 ml) and 10% Pd-C (0.71 g, 0.0067 mol) was hydrogenated until the required quantity of H_2 was taken up. The solution was filtered through sinter to remove the catalyst and methanol was removed under reduced pressure. The resulting light violet collored solid was triturated with a small amount of MeOH and collected by filtration to afford 4.93 g (83%) crystals mp 227~231°C. ^{1}H NMR (DMSO-d_6): 8.91 (d, 1H, NH), 8.81 (s, 1H, NH), 6.82 (d, 1H, J=7.99 Hz, $H_{arom.}$), 6.78 (s, 1H, $H_{arom.}$), 6.55 (d, 1H, J=7.98 Hz, $H_{arom.}$), 5.45 (s, 2H, NH_2), 4.87 (s, 2H, NH_2), 3.94 (m, 1H, CH), 1.18 (d, 6H, CH_3).

4-[*N*-(2-Imidazolinyl)]amidino-1,2-phenylene-diamine (**6**)

Compound (**6**) was prepared on the way described for (**5**) from (**3**) (2.15 g, 0.009 mol) and 10% Pd-C (0.22 g, 0.0023 mol). It was obtained 1.83 g (60%) light brown crystals mp 213~217°C. ^{1}H NMR (DMSO-d_6): 9.81 (s, 1H, NH), 7.08 (d, 1H, J=8.31 Hz, $H_{arom.}$), 6.97 (m, 1H, $H_{arom.}$), 6.56 (d, 1H, J=8.31 Hz, $H_{arom.}$), 5.78 (s, 2H, NH_2), 4.86 (s, 2H, NH_2), 3.83 (s, 4H, CH_2).

4-[*N*-(*N*-Morpholinyl)]amidino-1,2-phenylene-diamine (**7**)

Compound (**7**) was prepared on the way described for (**5**) from (**4**) (3.42 g, 0.0014 mol) and 10% Pd-C (0.35 g, 0.032 mol). The yield was 2.74 g (67%) light brown crystals mp 269~274°C. ^{1}H NMR (DMSO-d_6): 10.44 (s, 1H, NH), 9.15 (s, 1H, NH), 6.87 (d, 1H, J=7.98 Hz, $H_{arom.}$), 6.82 (s, 1H, $H_{arom.}$), 6.56 (d, 1H, J=7.98 Hz, $H_{arom.}$), 5.56 (s, 2H, NH_2), 4.89 (s, 2H, NH_2), 3.71 (s, 4H, CH_2), 2.82 (s, 4H, CH_2).

20-(2-Benzimidazolyl)tylosin Hydrochloride (**8**)

Compound (**8**) was prepared using the method described earlier[19]. A solution of 0.92 g (0.001 mol) tylosin, 0.108 g (0.001 mol) *p*-benzoquinone and 0.192 g (0.001 mol) 1,2-phenylenediamine in EtOH (18 ml) was stirred at reflux for 4 hours (under nitrogen). The reaction mixture was cooled to room temperature and, ethylether was added and the resulting solid was filtered off. It was obtained 0.6 g (59%) light yellow solid, mp 250~253°C. ^{1}H NMR (δ) (DMSO-d_6): 12.3 (s, 1H, $NH_{imidaz.}$), 7.42 (d, 1H, J=7.56 Hz, $H_{arom.}$), 7.34 (d, 1H, J=7.15 Hz, $H_{arom.}$), 7.11~7.18 (m, 1H, $H_{arom.}$).

20-[5-(*N*-isopropylamidino)-2-benzimidazolyl]tylosin Hydrochloride (**9**)

Compound (**9**) was prepared on the way described for (**8**), from tylosin (0.916 g, 0.001 mol), compound (**5**) (0.19 g, 0.001 mol) and 1,4-benzoquinone (0.11 g, 0.001 mol). The yield was 0.48 g, (44%) light yellow crystals mp 202~207°C. ^{1}H NMR (δ) (DMSO-d_6): 13.08 (s, 1H, $NH_{imid.}$), 9.51 (s, 1H, $NH_{amid.}$), 8.99 (m, 1H, $NH_{amid.}$), 7.78 (s, 1H, $H_{arom.}$), 7.63 (d, 1H, J=8.28 Hz, $H_{arom.}$), 7.52 (d, 1H, J=8.33 Hz, $H_{arom.}$). ^{13}C (DMSO): 111.08, 115.33, 114.52, 122.12, 137.13, 141.09. MS *m/z* 1087 (M−HCl $C_{56}H_{90}N_5O_{16}$).

20-[5-(2-imidazolinyl)-2-benzimidazolyl]tylosin Hydrochloride (**10**)

A protocol similar to that described above was employed the condensation of tylosin (0.92 g, 0.001 mol), compound (**6**) (0.18 g, 0.001 mol) and 1,4-benzoquinone (0.11 g, 0.001 mol) to give 0.84 g (78%) light violet powder mp 202~206°C. ^{1}H NMR (δ) (DMSO-d_6): 13.11 (s, 1H, $NH_{imid.}$), 10.56 (m, 1H, $NH_{imid.}$), 7.82 (m, 1H, $H_{arom.}$), 7.68 (m, 2H, $H_{arom.}$). ^{13}C (DMSO): 111.82, 112.65, 115.72, 118.22, 118.91, 119.86, 122.54. MS *m/z* 1071 (M−HCl $C_{55}H_{85}N_5O_{16}$).

20-[5-(*N*-Morpholinylamidino)-2-benzimidazolyl]tylosin Hydrochloride (**11**)

A protocol similar to that described above was employed for the condensation of tylosin (0.92 g, 0.001 mol),

compound (**7**) (0.23 g, 0.001 mol) and 1,4-benzoquinone (0.11 g, 0.001 mol) to give 0.86 g (76%) light violet powder mp 195~201°C. ^{1}H NMR (δ) (DMSO-d_6): 13.30 (s, 1H, $NH_{imid.}$), 13.19 (s, 1H, $NH_{amid.}$), 7.90 (s, 1H, $NH_{morph.}$), 7.87 (s, 1H, $H_{arom.}$), 7.62 (m, 2H, $H_{arom.}$). ^{13}C (DMSO): 11.28, 115.53, 117.60, 117.98, 122.16. MS *m/z* 1130 (M−HCl $C_{57}H_{90}N_6O_{17}$).

20-[5-(*N*-Isopropylamidino)-2-benzimidazolyl]desmycosin Hydrochloride (**12**)

Compound (**12**) was prepared from desmycosin (3.1 g, 0.004 mol) (which was prepared by the acid hydrolysis of tylosin; tylosin (5 g, 0.0054 mol) was dissolved in 0.2 N HCl (100 ml) and stirred for 4 hours on the room temperature). Chloroform (35 ml) was added in the reaction mixture. pH of the reaction mixture was on 8.5 by the addition of 10% NaOH. A chloroform layer was separated and to the water was added chloroform (30 ml) and extracted. The chloroform extracts were combined, washed with the solution of $NaHCO_3$ and dried on Na_2CO_3. The chloroform extract is filtered off, reduced under the vacuum until dryness. It was obtained 4.0 g (95%) white crystals mp 121~124°C, on the way described above, compound (**5**) (0.77 g, 0.004 mol) and 1,4-benzoquinone (0.432 g, 0.004 mol) to give 1.35 g (36%) light violet powder mp 200~205°C. ^{1}H NMR (δ) (DMSO-d_6): 13.15 (s, 1H, $NH_{imidaz.}$), 8.35 (s, 1H, $NH_{amid.}$), 7.77 (s, 1H, $NH_{amid.}$), 7.62 (s 1H, $H_{arom.}$), 7.52 (d, 1H, J=7.27 Hz, $H_{arom.}$), 7.12 (m, 1H, $H_{arom.}$). ^{13}C (DMSO): 158.09, 141.06, 118.28, 118.13, 117.94, 115.52, 112.69, 112.44. MS *m/z* 979 (M $C_{49}H_{78}N_5O_{13}Cl$).

20-[5-(2-Imidazolinyl)-2-benzimidazolyl]desmycosin Hydrochloride (**13**)

A protocol similar to that described above was employed for the condensation on desmycosin (0.77 g, 0.001 mol), compound (**6**) (0.18 g, 0.001 mol) and 1,4-benzoquinone (0.11 g, 0.001 mol) to give 0.67 g, (73%) light brown powder mp 206~210°C. ^{1}H NMR (δ) (DMSO-d_6): 13.35 (s, 1H, $NH_{imid.}$), 12.85 (s, 1H, $NH_{imid.}$), 8.14 (m, 1H, $H_{arom.}$), 7.83 (m, 1H, $H_{arom.}$), 7.70 (m, 1H, $H_{arom.}$). ^{13}C (DMSO): 112.64, 112.71, 115.72, 119.90, 138.27, 141.78. MS *m/z* 927 (M−HCl $C_{48}H_{73}N_5O_{13}$).

20-[5-(*N*-Morpholinylamidino)-2-benzimidazolyl] desmycosin Hydrochloride (**14**)

A protocol similar to that described above was employed for the condensation of desmycosin (1.54 g, 0.002 mol), compound (**7**) (0.47 g, 0.002 mol) to give 0.72 g (37%) light brown powder mp 210~214°C. ^{1}H NMR (δ) (DMSO-d_6): 13.20 (s, 1H, $NH_{imid.}$), 7.99 (s, 1H, $NH_{amid.}$), 7.87 (s, 1H, $NH_{morph.}$), 7.63 (m, 2H, $H_{arom.}$), 7.13 (m, 1H, $H_{arom.}$). ^{13}C (DMSO): 111.27, 115.52, 117.47, 122.13, 137.41. MS *m/z* 986 (M−HCl $C_{50}H_{78}N_6O_{14}$).

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