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Synthesis and anthelmintic potential of a novel series of 2-mercaptobenzimidazolopeptides

RAJIV DAHIYA* and RAJWINDER KAUR

Department of Pharmaceutical Chemistry, Rajiv Academy for Pharmacy, Mathura - 281 001(India)

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ABSTRACT

A novel series of 1,2-disubstituted benzimidazole derivatives was synthesized by coupling 2-mercaptobenzimidazol-1-acetic acid with various amino acid methyl ester hydrochlorides/dipeptides/ tripeptides using dicyclohexylcarbodiimide as the coupling agent and triethylamine as the base. All newly synthesized compounds were characterized by spectral as well as elemental analysis and evaluated for their anthelmintic potential. Compounds 6, 7 and 10 were found to exhibit potent activity against three earthworm species *M. konkanensis*, *P. corethruses* and *Eudrilus* sp. in comparison to mebendazole at 2 mg/ml dose level. Hydrolyzed benzimidazolopeptide analogs were found to be more potent than corresponding ester derivatives.

Key words: Benzimidazoles, peptides, amino acids, coupling, anthelmintic activity.

INTRODUCTION

Benzimidazole nucleus is of vital importance due to its involvement as a core ring system in numerous medicinal drugs[1]. Benzimidazole analogs exhibit a wide array of bioactivities including antimicrobial, anti-inflammatory, antiprotozoal, antitumour, anthelmintic and DHFR inhibitory activity[2-8]. 2-Mercaptobenzimidazole congeners have already proved their potential as potent antimycobacterial and antimicrobial agents[9,10]. Moreover, literature is enriched with reports on incorporation of amino acids/peptides into the aromatic/heterocyclic moieties resulting in compounds with potent bioactivities with fewer side effects[11,12]. Thus, keeping in view the therapeutic potential of benzimidazoles as well as taking advantage of biodegradability and biocompatibility of peptides and in continuation of our synthetic efforts on bioactive benzimidazolopeptide[13,14] and other aromatic analogs[15-19], a novel series of 2-mercaptobenzimidazol-1-acetyl amino acids and peptides was synthesized with an anticipation to get potent agents of more therapeutic efficacy with less toxicity.

2-Mercaptobenzimidazole was agitated with ethylchloroacetate in presence of PEG 400 and anhydrous K_2CO_3 to give ethyl-2-mercaptobenzimidazol-1-acetate which on alkaline hydrolysis afforded 2-mercaptobenzimidazol-1-acetic acid[1].

Dipeptides Boc-Thr-Pro-OMe, Boc-Try-nitro(Arg)-OMe and Boc-Gly-Gly-OMe were prepared by coupling of Boc-protected amino acids Boc-Thr-OH, Boc-Try-OH and Boc-Gly-OH with respective amino acid methyl ester hydrochlorides Pro-OMe.HCl, niro(Arg)-OMe.HCl and Gly-OMe.HCl using dicyclohexylcarbodiimde (DCC) as coupling agent and N-methylmorpholine (NMM) as base according to the Bodanzsky and Bodanzsky procedure with certain modifications[20]. Similarly, tripeptide Boc-Ile-Thr-Try-OMe was prepared by coupling deprotected dipeptide Boc-Ile-Thr-OH with Try-OMe.HCl. Dipeptide and tripeptide units were deprotected at amino end using trifluoroacetic acid (TFA) prior to coupling[21].

Compound 1 was coupled with amino acid methyl ester hydrochlorides Pro-OMe.HCl, Phe-OMe.HCl, His-OMe.HCl and deprotected di/

tripeptides using DCC and triethylamine (TEA) to yield novel 2-mercaptobenzimidazol-1-acetyl amino acids/peptide methyl esters (2-8). Selected ester derivatives[4,7-8] were further hydrolyzed using lithium hydroxide to get respective free acids[9-11] Fig. 1.

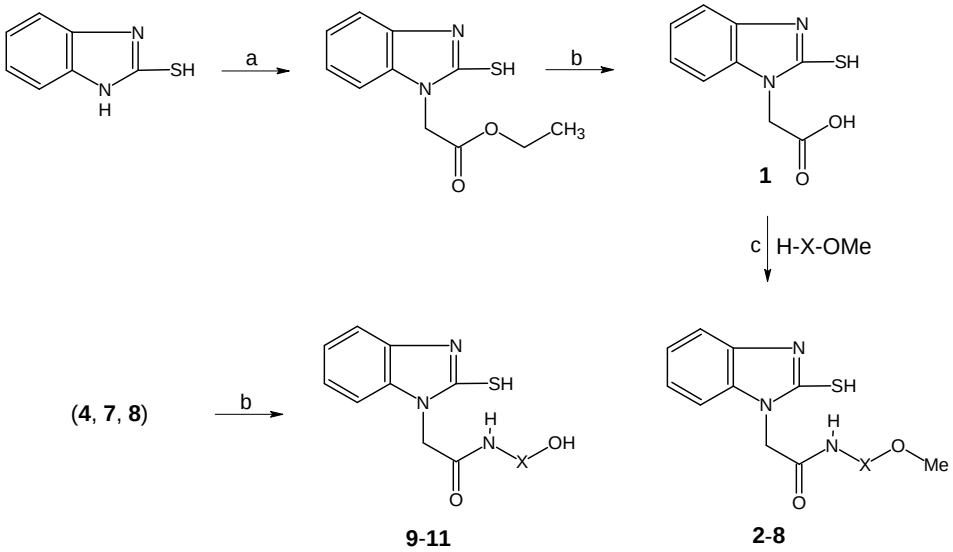
Structures of all the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectra. Elemental analysis of the novel compounds was performed for carbon, hydrogen and nitrogen content. Physical characterization data of all synthesized analogs is compiled in Table 1.

MATERIAL AND METHODS

Melting points were determined by open capillary method and are uncorrected. L-amino acids, di-tert-butylpyrocarbonate (Boc_2O), DCC, TFA, NMM and TEA were obtained from Spectrochem Limited, Mumbai, India. IR spectra were recorded on Shimadzu 8700 fourier transform infrared spectrophotometer using a thin film supported on KBr pellets/$CHCl_3$ solvent for all synthesized compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer (300 MHz) using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were recorded on Jeol JMS DX 303 Mass spectrometer operating at 70 eV. Elemental analysis of all compounds were performed on Elementar vario EL III. Purity of all the compounds was checked by TLC on precoated silica gel G plates.

Preparation of 2-mercaptobenzimidazol-1-acetic acid[1]

2-Mercaptobenzimidazole (3.75 g, 25 mmol) was added to PEG 400 (4 ml). To this mixture, anhydrous K_2CO_3 (8 g) in dry $CHCl_3$ (200 ml) and ethylchloroacetate (3 ml) were added. The reaction mixture was agitated for a period of 5 h. Separated inorganic solids were filtered and the solvent was evaporated to get ethyl-2-mercaptobenzimidazol-1-acetate. To a solution of 10 mmol of ester in THF/H_2O (1:1, 36 ml), LiOH (0.36 g, 15 mmol) was added at 0 °C. After stirring the mixture at RT for 1 h, acidification to pH 3.5 was done using H_2SO_4 (0.5 mol/l). The aqueous layer was extracted with Et_2O (3× 25 ml) and combined organic extracts were dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get pure compound 1.



X = L-Pro (**2**), L-Phe (**3**), L-His (**4**), L-Thr-L-Pro (**5**), L-Try-L-nitro(Arg) (**6**), Gly-Gly (**7**), L-Ile-L-Thr-L-Try (**8**), L-His (**9**), Gly-Gly (**10**), L-Ile-L-Thr-L-Try (**11**).

a = $ClCH_2COOC_2H_5$, PEG 400, anhyd. K_2CO_3, $CHCl_3$, RT, 5 h
b = LiOH, THF:H_2O (1:1), RT, 1 h
c = DCC, TEA, $CHCl_3$, RT, 24 h

Fig. 1. Synthetic pathway for novel 1,2-disubstituted benzimidazole analogs

1: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.56 (1H, br. s, -OH, -COOH), 6.50-7.48 (1H, d, *J* = 7.8 Hz, H-δ, benzimidazole (bzi)), 7.44-7.42 (1H, d, *J* = 7.6 Hz, H-*η*, bzi), 7.40-7.37 (1H, t, H-*ζ*, bzi), 7.32-7.29 (1H, t, H-*ε*, bzi), 4.95 (2H, s, CH_2, acetyl), 3.28 (1H, br. s, -SH) ppm; ^{13}C NMR ($CDCl_3$, 300 MHz): δ 165.2 (C=O, -COOH), 165.0 (C-*β*, bzi), 139.5 (C-*γ*, bzi), 133.2 (C-β, bzi), 124.5, 123.2, 120.9 (3C, C-*ζ*, C-*ε* and C-*δ*, bzi), 111.4 (C-*η*, bzi), 51.1 (CH_2, acetyl) ppm.

General procedure for synthesis of 2-mercaptobenzimidazol-1-acetyl amino acid and peptide derivatives[2-11]

Compound 1 (2.1 g, 10 mmol) was dissolved in DMF (35 ml) and added to a mixture of L-amino acid methyl ester hydrochloride/ deprotected dipeptide/tripeptide methyl ester (10 mmol) in DMF (45 ml) to which TEA (2.9 ml) was previously added at 0 °C with stirring. To the above mixture, DCC (2.1 g, 10 mmol) was added and stirring was done for 24 h. After 24 h, the reaction mixture was filtered and water was added in equal proportions and finally aqueous layer was washed with Et_2O (3×40 ml). The organic layer was dried over anhydrous Na_2SO_4 followed by evaporation in vacuum. The crude product was dissolved in chloroform and washed with 10 % HCl, saturated $NaHCO_3$ solutions and water (25 ml each) followed by evaporation in vacuum. Compounds **4**, **7** and **8** were further subjected to alkaline hydrolysis using LiOH to get corresponding acid derivatives **9**-**11**.

2-Mercaptobenzimidazol-1-acetyl-proline methyl ester[2]

^{13}C NMR ($CDCl_3$, 300 MHz): δ 174.0 (C=O, pro), 165.6 (C-*β*, bzi), 161.3 (C=O, acetyl), 140.1 (C-*γ*, bzi), 136.8 (C-*β*, bzi), 126.0, 122.9, 121.2 (3C, C-*ζ*, C-*ε* and C-δ, bzi), 113.5 (C-*η*, bzi), 58.7 (C-*a*, pro), 55.1 (CH_2, acetyl), 52.9 (-OCH_3, ester), 45.4 (C-*δ*, pro), 28.9 (C-*β*, pro), 24.2 (C-*γ*, pro) ppm.

2-Mercaptobenzimidazol-1-acetyl-phenylalanine methyl ester[3]

Table 1. Physical characterization of compounds 2-11

Compd.	Mol. formula (Mol. wt.)	m.p. (°C)	Yield (%)	R_f value#	% Analysis [calcd. (found)] C	H	N	S
1.	$C_9H_8N_2O_2S$ (208)	189-190	91	0.85	51.91 (51.89)	3.87 (3.82)	13.45 (13.46)	15.40 (15.43)
2.	$C_{15}H_{17}N_3O_3S$ (319)	120-122	69	0.72	56.41 (56.38)	5.36 (5.38)	13.16 (13.20)	10.04 (9.98)
3.	$C_{19}H_{19}N_3O_3S$ (369)	-	67	0.80	61.77 (61.72)	5.18 (5.22)	11.37 (11.38)	8.68 (8.68)
4.	$C_{16}H_{17}N_5O_3S$ (359)	102-103	88	0.67	53.47 (53.44)	4.77 (4.80)	19.49 (19.52)	8.92 (9.89)
5.	$C_{19}H_{24}N_4O_5S$ (420)	-	72	0.79[1]	54.27 (54.25)	5.75 (5.75)	13.32 (13.33)	7.62 (7.65)
6.	$C_{27}H_{31}N_9O_6S$ (609)	165-166	70	0.61[1]	53.19 (53.22)	5.13 (5.13)	20.68 (20.75)	5.26 (5.25)
7.	$C_{14}H_{16}N_4O_4S$ (336)	148-149	81	0.48[1]	49.99 (50.02)	4.79 (4.77)	16.66 (16.69)	9.53 (9.52)
8.	$C_{31}H_{38}N_6O_6S$ (622)	-	76	0.65	59.79 (59.80)	6.15 (6.19)	13.50 (13.52)	5.15 (5.21)
9.	$C_{15}H_{15}N_5O_3S$ (345)	75-77	69	0.59	52.17 (52.15)	4.38 (4.44)	20.28 (20.29)	9.28 (9.25)
10.	$C_{13}H_{14}N_4O_4S$ (322)	110-112	78	0.67[1]	48.44 (48.45)	4.38 (4.42)	17.38 (17.40)	9.95 (9.92)
11.	$C_{30}H_{36}N_6O_6S$ (608)	-	72	0.76	59.20 (59.25)	5.96 (5.99)	13.81 (13.82)	5.27 (5.23)

#($CHCl_3$:MeOH / 9:1), [1] ($CHCl_3$:MeOH / 8:2)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95-7.93 (1H, δ, *J* = 7.75 Hz, H-*δ*, bzi), 7.59-7.56 (1H, t, H-*ζ*, bzi), 7.32-7.30 (1H, δ, *J* = 7.7 Hz, H-*η*, bzi), 7.21-7.17 (1H, t, H-*ε*, bzi), 7.13-7.08 (1H, t, H-*π*, phe), 6.99-6.84 (4H, m, H-*m* and H-*o*, phe), 6.96 (1H, br. s, -NH, phe), 4.82 (2H, s, C$\underline{H}_2$, acetyl), 4.72-4.67 (1H, q, H-*a*, phe), 3.52 (3H, s, -OC$\underline{H}_3$, ester), 3.28 (1H, br. s, -SH), 2.83-2.81 (2H, δ, *J* = 5.7 Hζ, H-*β*, phe) ppm.

2-Mercapto-5-methoxybenzimidazol-1-acetyl-histidine methyl ester[4]

^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.12 (1H, br. s, -NH, imidazole (imz)), 7.93-7.91 (1H, δ, *J* = 7.8 Hz, H-*δ*, bzi), 7.76-7.74 (1H, δ, *J* = 7.95 Hζ, H-*β*, imz), 7.62-7.59 (1H, t, H-*ζ*, bzi), 7.52 (1H, s, H-δ, imz), 7.33-7.31 (1H, δ, *J* = 7.65 Hζ, H-*η*, bzi), 7.23-7.19 (1H, t, H-*ε*, bzi), 6.99 (1H, br. s, -NH, his), 4.89-4.85 (1H, q, H-*α*, phe), 4.80 (2H, s, C$\underline{H}_2$, acetyl), 3.54 (3H, s, -OC$\underline{H}_3$, ester), 3.27 (1H, br. s, -SH), 2.80-2.78 (2H, δ, *J* = 5.65 Hz, H-*β*, his) ppm.

2-Mercaptobenzimidazol-1-acetyl-threonyl-proline methyl ester[5]

IR ($CHCl_3$): 3342 (m/br, -OH str, thr), 3135 (m, -NH str, amide), 3072 (w, -CH str, bzi), 2997-2989 (m, -CH str, cyclic CH_2, pro), 2955, 2869 (m, -CH str, asym and sym, CH_3), 2926, 2853 (m, -CH str, asym and sym, CH_2), 2582 (w, S–H str), 1744 (s, -C=O str, ester), 1666, 1640 (s, -C=O str, 3° and 2° amide), 1581, 1489 (m, skeletal bands, bzi), 1533 (m, -NH bend, 2° amide), 1463 (m, -CH bend (scissoring), CH_2), 1269 (s, C–O str, ester), 875, 832 (s, -CH bend, out-of-plane (oop), bzi), 668 (m/br, -OH bend, oop, thr) cm^{-1}.

2-Mercaptobenzimidazol-1-acetyl-tryptophanyl-nitro(arginine) methyl ester[6]

MASS: m/z (rel. int.) 31 (7), 33 (5), 46 (13), 59 ($MeOCO^+$, 18), 90 (14), 91 (25), 116 (9), 117 (15), 130 (18), 131 (19), 145 (22), 163 (34), 191 (70), 349 (57), 377 (base peak, 100), 550 (16), 578 (M-31, 28), 609 (M^+, 7), 610 (4), 611 (2).

2-Mercaptobenzimidazol-1-acetyl-glycyl-glycine methyl ester[7]

^{13}C NMR ($CDCl_3$, 300 MHz): δ 176.2, 170.7 (2C, C=O, gly-1 and gly-2), 167.6 (C=O, acetyl), 164.5 (C-*β*, bzi), 140.0, 136.2 (2C, C-*γ* and C-*β*, bzi), 125.8, 123.5, 120.9 (3C, C-*ζ*, C-*ε* and C-*δ*, bzi), 113.1 (C-*η*, bzi), 54.5 (C$\underline{H}_2$, acetyl), 49.7 (-OC$\underline{H}_3$, ester), 41.4, 39.6 (2C, C-*α*, gly-2 and gly-1) ppm.

2-Mercaptobenzimidazol-1-acetyl-isoleucyl-threonyl-tryptophan methyl ester[8]

IR ($CHCl_3$): 3493 (m, -NH str, indole ring), 3338 (m/br, -OH str, thr), 3136-3129 (m, -NH str, amide), 3074, 3069-3066 (w, -CH str, rings), 2953, 2951, 2867 (m, -CH str, asym and sym, CH_3), 2925, 2853, 2849 (m, -CH str, asym and sym, CH_2), 2586 (w, S–H str), 1748 (s, -C=O str, ester), 1665, 1643 (s, -C=O str, 3° and 2° amide), 1584, 1493-1488 (m, skeletal bands, rings), 1539-1535, 1529 (m, -NH bend, 2° amide), 1465 (m, -CH bend (scissoring), CH_2), 1273 (s, C–O str, ester), 878-875, 832, 829 (s, -CH bend, oop, rings), 665 (m/br, -OH bend, oop, thr) cm^{-1}; MASS: m/z (rel. int.) 15 (5), 17 (8), 29 (11), 31 (9), 33 (6), 57 (19), 59 ($MeOCO^+$, 21), 90 (12), 91 (28), 116 (11), 130 (19), 163 (29), 191 (67), 276 (45), 304 (base peak, 100), 377 (52), 405 (76), 563 (14), 591 (M-31, 33), 622 (M^+, 5), 623 (2).

2-Mercapto-5-methoxybenzimidazol-1-acetyl-histidine[9]

^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.62 (2H, br. s, -NH, imz and –OH, -COOH), 8.05-8.03 (1H, δ, *J* = 8.0 Hz, H-*β*, imz), 7.92-7.90 (1H, δ, *J* = 7.75 Hz, H-*δ*, bzi), 7.74 (1H, br. s, -NH, his), 7.65 (1H, s, H-*δ*, imz), 7.60-7.57 (1H, t, H-*ζ*, bzi), 7.32-7.30 (1H, δ, *J* = 7.7 Hz, H-*η*, bzi), 7.22-7.19 (1H, t, H-*ε*, bzi), 5.16-5.13 (1H, q, H-*α*, phe), 4.75 (2H, s, C$\underline{H}_2$, acetyl), 3.26 (1H, br. s, -SH), 2.91-2.89 (2H, δ, *J* = 5.7 Hz, H-*β*, his) ppm.

2-Mercaptobenzimidazol-1-acetyl-glycyl-glycine[10]

^{13}C NMR ($CDCl_3$, 300 MHz): δ 181.2, 174.8 (2C, C=O, gly-2 and gly-1), 168.9 (C=O, acetyl), 164.4 (C-*β*, bzi), 141.1, 137.5 (2C, C-*γ* and C-*β*, bzi), 126.4, 123.0, 120.1 (3C, C-*z*, C-*ε* and C-*δ*, bzi), 112.6 (C-*η*, bzi), 55.1 (C$\underline{H}_2$, acetyl), 41.7, 39.2 (2C, C-*α*, gly-2 and gly-1) ppm.

2-Mercaptobenzimidazol-1-acetyl-isoleucyl-threonyl-tryptophan[11]

IR ($CHCl_3$): 3489 (m, -NH str, indole ring), 3335 (m/br, -OH str, thr), 3288-2524 (m/br, -OH str, -COOH), 3133-3126 (m, -NH str, amide), 3075,

3067-3063 (w, -CH str, rings), 2955, 2952, 2866 (m, -CH str, asym and sym, CH_3), 2922, 2852, 2849 (m, -CH str, asym and sym, CH_2), 2584 (w, S–H str), 1710 (s, -C=O str, -COOH), 1667, 1644, 1641 (s, -C=O str, 3° and 2° amide), 1588, 1492-1489 (m, skeletal bands, rings), 1539-1532, 1527 (m, -NH bend, 2° amide), 1406 (m, -C-O-H bend, -COOH), 877-872, 835, 829 (s, -CH bend, oop, rings), 669 (m/br, -OH bend, oop, thr) cm^{-1}; MASS: m/z (rel. int.) 15 (7), 17 (5), 29 (14), 33 (8), 45 (14), 57 (19), 90 (14), 91 (25), 116 (15), 130 (28), 163 (22), 191 (66), 276 (39), 304 (base peak, 100), 377 (44), 405 (69), 563 (M-45, 24), 591 (M-17, 15), 608 (M^+, 7).

Anthelmintic activity

All synthesized compounds[2-11] were subjected to anthelmintic activity studies[22] were performed against three different species of earthworms *Megascoplex konkanensis* (ICARBC 211), *Pontoscotex corethruses* (ICARBC 408) and *Eudrilus* sp. (ICARBC 042) at 2 mg/ml using standard drug - mebendazole. The mean paralyzing and death times were calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive. The results of anthelmintic activity studies are compiled in Table 2. Experimental details of anthelmintic activity procedures are given in our previously published reports[13].

RESULTS AND DISCUSSION

Synthesis of 2-mercaptobenzimidazol-1-acetic acid (1) was accomplished with good yield (> 90 %). Presence of free carboxylic group in structure of compound 1 was clearly indicated by singlets at 165.2 and 7.56 ppm corresponding to carbonyl and hydroxyl portions of -COOH group in ^{13}C and ^1H NMR spectra. Moreover, appearance of broad singlet at 3.28 ppm in ^1H NMR spectra confirmed presence mercapto group (-SH) in compound 1. All benzimidazolopeptide derivatives[2-11] were synthesized successfully and DCC was found to be a good coupling agent providing 67-88 % yield of synthesized compounds. IR spectra of newly synthesized peptide derivatives showed characteristic Amide I and Amide II bands of the -CO-NH- moieties at 1667-1640 and 1539-1527 cm^{-1} which clearly indicated the completeness of coupling reaction. This fact was further confirmed by appearance of singlets at 7.74-6.96 and 181.2-170.7 ppm corresponding to -CO-NH- moiety in ^1H and ^{13}C NMR spectra of compounds[2-11]. Presence of bands at 3288-2524, 1748 cm^{-1} (for -C(=O)-OH moiety) in IR spectra and broad singlet at 10.62 ppm (for -COOH moiety) in ^1H NMR spectra of compounds[9-11] and disappearance of strong bands at 1748, 1268 cm^{-1} corresponding to C=O and C–O moiety (ester) in IR spectra and singlets at 49.7, 3.54 ppm (for $-OCH_3$ moiety) in ^{13}C and ^1H NMR spectra of compounds 4, 7 and 8 further confirmed accomplishment of hydrolysis reaction. Moreover, mass spectrum of ester and corresponding acid derivatives showed characteristic fragment ion peaks (M"31 and $MeOCO^+$ - m/z 59) and (M"17, M"45 and $COOH^+$ - m/z 45) along with respective molecular ion peak (M^+) and elemental analysis afforded values (± 0.06), consistent with molecular composition of synthesized compounds. All the synthesized compounds[2-11] possessed moderate to good activity against earthworm species. Analysis of anthelmintic activity data suggested that benzimidazolodipeptide analogs[5-7] and possessed[10] more activity in comparison to tripeptides 8 and 11 which in turn, exhibited more activity than amino acid derivatives 2-4 and 9. Further, compound 7 and its hydrolyzed derivative 10 showed better activity against all three earthworm species, in comparison to standard drug- mebendazole and compound 6 displayed anthelmintic activity comparable to reference drug. *Eudrilus* sp. was found to be less sensitive towards the newly synthesized benzimidazolopeptide derivatives, in comparison to other two species *M. konkanensis* and *P. corethruses*. From comparison of anthelmintic activity data, it was concluded that hydrolyzed peptide derivatives 9-11 displayed better activity in comparison to corresponding ester derivatives 4, 7 and 8. On passing toxicity tests, these compounds may prove good candidates for clinical studies and can be new anthelmintic agents of future.

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Table 2: Anthelmintic activity data for compounds 2-11

Comp.	*M. konkanesis*		*P. coerthruses*		*Eudrilus sp.*	
	Mean paralyzing time (min)*	**Mean death time (min)***	**Mean paralyzing time (min)**	**Mean death time (min)***	**Mean paralyzing time (min)**	**Mean death time (min)**
2.	31.18 ± 0.22	45.50 ± 0.50	38.57 ± 0.28	47.26 ± 0.22	32.25 ± 0.73	44.34 ± 0.66
3.	33.16 ± 0.78	48.54 ± 0.42	36.65 ± 0.44	49.22 ± 0.37	33.67 ± 0.92	45.27 ± 0.80
4.	29.04 ± 0.52	44.05 ± 0.84	35.17 ± 0.83	46.18 ± 0.42	29.73 ± 0.42	39.06 ± 0.31
5.	15.26 ± 0.12	25.29 ± 0.60	19.25 ± 0.22	32.11 ± 0.15	16.49 ± 0.32	27.08 ± 0.52
6.	13.32 ± 0.41	23.02 ± 0.25	17.58 ± 0.13	29.48 ± 0.28	14.09 ± 0.58	24.11 ± 0.62
7.	08.39 ± 0.41	16.22 ± 0.43	14.02 ± 0.24	24.02 ± 0.72	12.55 ± 0.41	22.10 ± 0.60
8.	18.17 ± 0.30	27.09 ± 0.76	24.08 ± 0.18	33.23 ± 0.50	19.39 ± 0.35	26.52 ± 0.11
9.	26.40 ± 0.82	43.24 ± 0.92	32.61 ± 0.96	45.39 ± 0.20	27.82 ± 0.83	35.24 ± 0.65
10.	07.54 ± 0.42	15.08 ± 0.22	13.47 ± 0.40	22.12 ± 0.28	11.24 ± 0.84	19.55 ± 0.36
11.	17.08 ± 0.63	25.43 ± 0.25	22.11 ± 0.34	33.12 ± 0.73	18.30 ± 0.26	26.36 ± 0.45
Control	-	-	-	-	-	-
Meben-dazole	13.37 ± 0.64	22.50 ± 0.53	17.45 ± 1.03	29.51 ± 1.20	13.54 ± 0.45	24.05 ± 0.62

*Data are given as mean ± S.D. (n=3)

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