ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2016, *61*(1), 15-22

SYNTHESIS AND ANTINOCICEPTIVE ACTIVITY OF MEPERIDINE-LIKE BENZIMIDAZOLE DERIVATIVES

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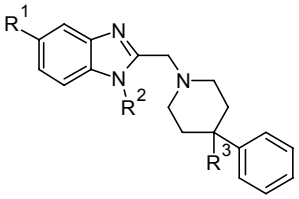
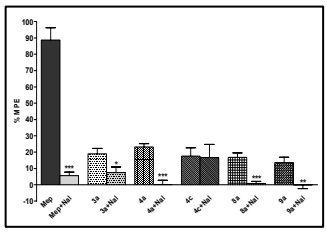
Received August 6, 2015

A series of novel benzimidazole derivatives have been prepared and characterized by IR, ^{1}H-NMR spectroscopic data and elemental analysis. All the final compounds were screened for their antinociceptive activities with tail flick test. Among the synthesized compounds 3a, 4a, 4c, 8a, 9a exhibited significant antinociceptive activity. Compound 9a was found to have the highest antinociceptive activity at both 60 minutes and 120 minutes. Additionally, compounds 3a, 4a, 8a and 9a showed naloxone-reversible antinociceptive activity.





INTRODUCTION

Meperidine (a) is a narcotic analgesic which binds to opioid receptors, particularly μ receptor. The 4-phenylpiperidine series of compound meperidine possesses a rapid onset and short duration of action because of esterase hydrolysis.[1-4] Plasma esterase hydrolyses the ester bond to give the inactive 4-carboxylic acid derivative (b). One of the normeperidine (c) metabolite is produced by N-demethylation of meperidine (Fig. 1). Meperidine is not recommended for long term use due to its neurotoxic active metabolite, normeperidine.[1-6] Additionally, meperidine has some limitations in clinical use such as addiction, tolerance, respiratory depression and euphoria like other opioid agonists.[1-4] Therefore, development of a new, safer and high potent meperidine derivative is still a great deal of interest for many researchers. Benzimidazole nucleus is an important pharmacophore and has various biological activities such as antiulcer, antihypertensive, antifungal, antihelminthic, anticancer, and antihistamine. Pimobendan, Mebendazole, Astemizole, Omeprazole, Candesartan, Enviradine are some of the commercially available benzimidazole-based drugs.[7-9] Additionally, Gaba and co-worker describe that benzimidazole scaffold has emerged as a pharmacophore of choice for designing analgesic and anti-inflammatory agents.[10] Identically to benzimidazoles, benzothiazoles can serve as unique and versatile scaffolds for drug design. In order to search more effective, safer and long duration meperidine derivatives, our group synthesized a series of novel benzimidazole/benzothiazole derivatives which have 4-phenylpiperidine ring with carboxylic acid or cyano residue (Fig. 1 d,e). Synthesized compounds were characterized and tested according to their antinociceptive activity on subject mice by tail-flick test.

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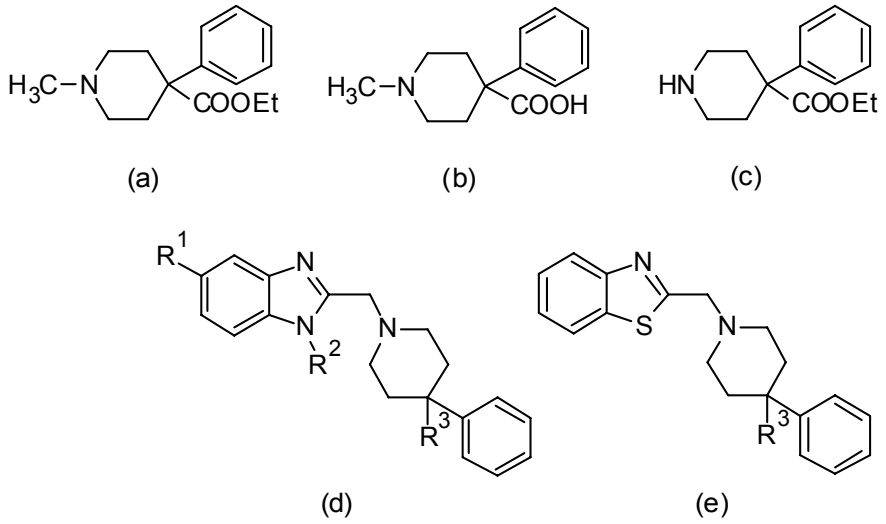
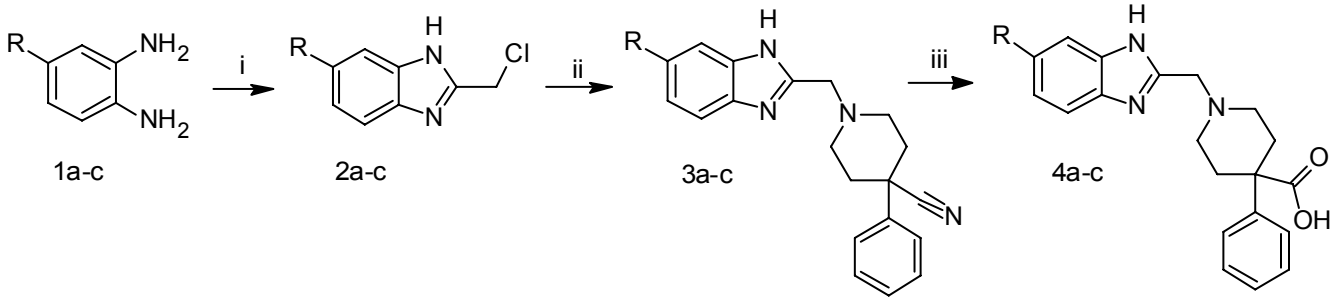


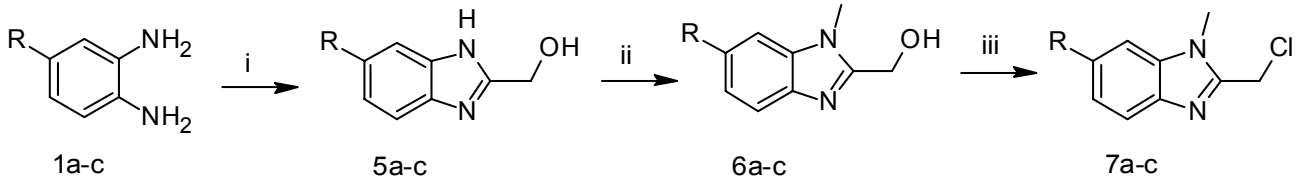
Fig. 1 – Chemical structures of meperidine (a), 4-carboxylic acid derivatives of meperidine (b), normeperidine (c), and synthesized compounds (d, e). R^1: H, CH_3, Cl; R^2: H, CH_3; R^3: CN, COOH.

RESULTS AND DISCUSSION

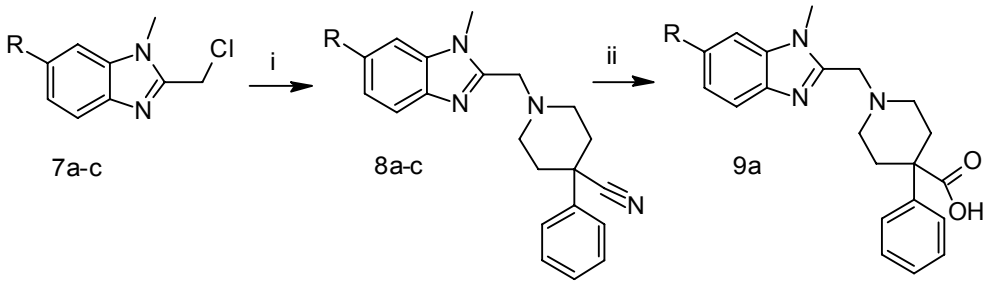
The synthetic routes for the synthesized compounds are outlined in Schemes 1-4. The general synthesis of benzimidazole derivatives involves condensation of 1,2-diaminobenzene derivatives with appropriate acid (Scheme 1, 2). 2-Chloromethylbenzothiazole was readily prepared by reaction of 2-aminobenzenethiol and chloroacetylchloride under microwave irradiation as shown in Scheme 4. 2-Chloromethylbenzimidazol/2-Chloromethylbenzothiazole derivatives were than reacted with 4-cyano-4-phenylpiperidine to obtain carbonitrile derivatives. Subsequent hydrolysis of the cyano group under acidic condition afforded acid derivatives (Scheme 1, 3, 4). The structure of the compounds was elucidated by IR, ^{1}H-NMR spectral data and elemental analysis.



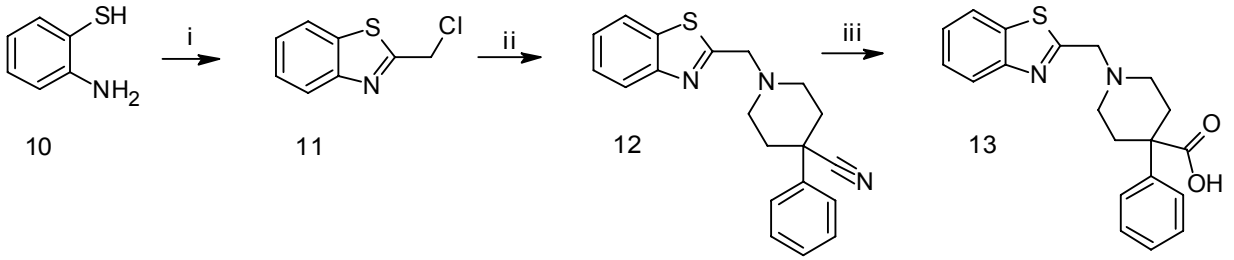
Scheme 1 – Reagents: (i) chloroacetic acid, 5N HCl, reflux 3h then sodium bicarbonate; (ii) 4-cyano-4-phenylpiperidine hydrochloride, triethylamine, tetrahydrofurane, reflux 6h; (iii) N,N-dimethylformamide/ water / H_2SO_4 (1:1:2), reflux 2h then sodium bicarbonate. R: H (a), CH_3 (b), Cl (c).



Scheme 2 – Reagents: (i) glycolic acid, 5N HCl, reflux 5h then sodium bicarbonate; (ii) dimethylsulfate, sodium hydroxide, reflux 10 h; (iii) thionyl chloride, dichloromethane, reflux 5h. R: H (a), CH_3 (b), Cl (c).



Scheme 3 – Reagents: (i) 4-cyano-4-phenylpiperidine hydrochloride, triethylamine, tetrahydrofurane, reflux 6h; (ii) N,N-dimethylformamide/water/H_2SO_4 (1:1:2), reflux 2h then sodium bicarbonate. R: H (a), CH_3 (b), Cl (c).



Scheme 4 – Reagents: (i) chloroacetyl chloride, microwave irradiation, 10 min, (ii) 4-cyano-4-phenylpiperidine hydrochloride, triethylamine, tetrahydrofurane, reflux 6h; (iii) N,N-dimethylformamide/water/H_2SO_4 (1:1:2), reflux 2h then sodium bicarbonate.

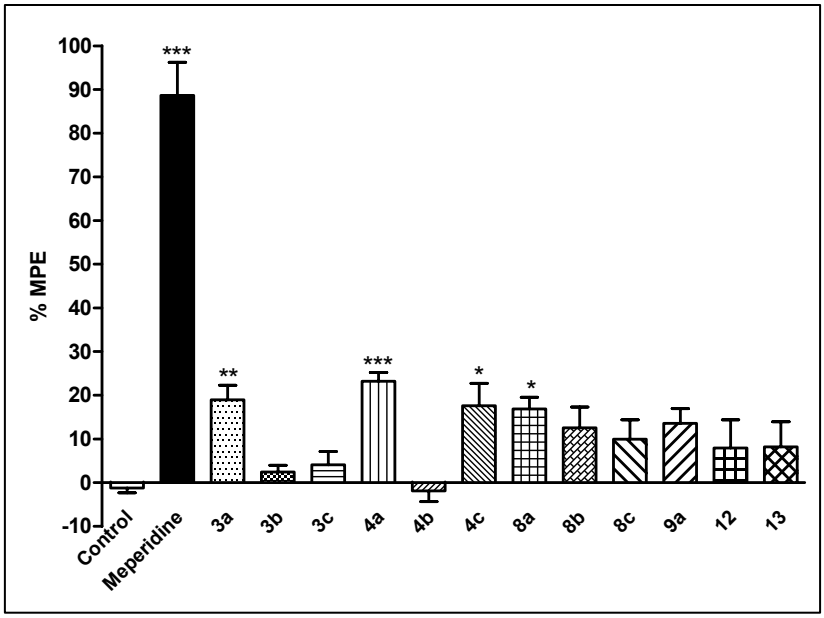


Fig. 2 – **% MPE values at the 30th minute.** *p<0.05, **p<0.01, ***p<0.0001; vs control (oneway ANOVA, post hocDunnet) There is a statistically significant increase in the maximum possible analgesic effect in the meperidine, 3a, 4a, 4c and 8a groups at the 30th minute while compared with the control group.

In this preliminary study, the antinociceptive activity of the title compounds was tested with tail flick test (Figs. 2-4). The tail-flick test is used to assess opioid analgesic activity[21] and first described by D'Amour and Smith in 1941.[20] The standard meperidine exerts a significant analgesic effect (88.63%) and the synthesized compounds 3a, 4a, 4c and 8a showed moderate analgesic effect (18.94%, 23.21%, 17.56%, 16.87% respectively) at 30 minutes. In 4a group, significant analgesic effect is observed only at 30th minute and this effect is finalized earlier than meperidine and other test chemicals. After 60 minutes, analgesic activity of meperidine was decreased (26.99%). Analgesic activities of 3a, 4c and 8a are slightly increased at 60th minute, 21.31%, 18.62% and 21.71%, respectively. Especially compound 9a exerts 2 fold analgesic effects at 60 minutes by comparison with the 30 minutes. The maximum analgesic effect was observed at 60 min after administration of

compound 9a (29.30%). Analgesic effect of 3a, 4c and 8a groups are similar with meperidine group in 30th and 60th minutes and this effect is finalized at 120 minutes. Our results showed that compounds 3a, 4a, 4c, 8a and 9a have central antinociceptive activity. The % MPE produced by nonsubstitut-edbenzimidazole derivatives were significantly higher than in the other compounds in tail-flick test. Additionally, an interaction with naloxone was also studied in tail flick method for its mechanism of central analgesic action. The antinociceptive effect of compounds 3a, 4a, 8a and 9a were reversed by naloxone (Figs. 5-7). The central analgesic action seems to be mediated through opioid receptors. The effect of compound 4c was not significantly blocked with naloxone. Therefore, analgesic effect of 4c may result from different mechanism of action.

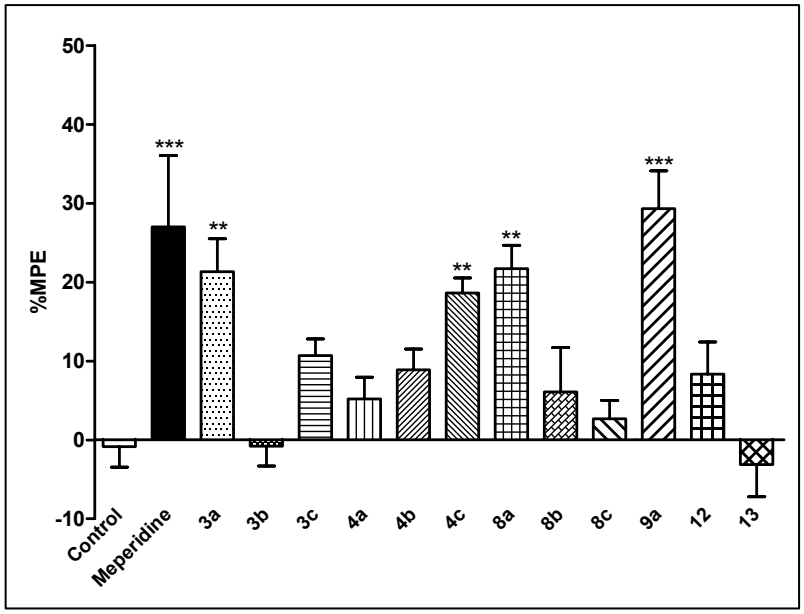


Fig. 3 – **% MPE values at the 60th minute.** **p<0.01, ***p<0.0001; vs control (oneway ANOVA, post hocDunnet). There is a statistically significant increase in the maximum possible analgesic effect in the meperidine, 3a, 4c, 8a and 9a groups at the 60th minute while compared with the control group.

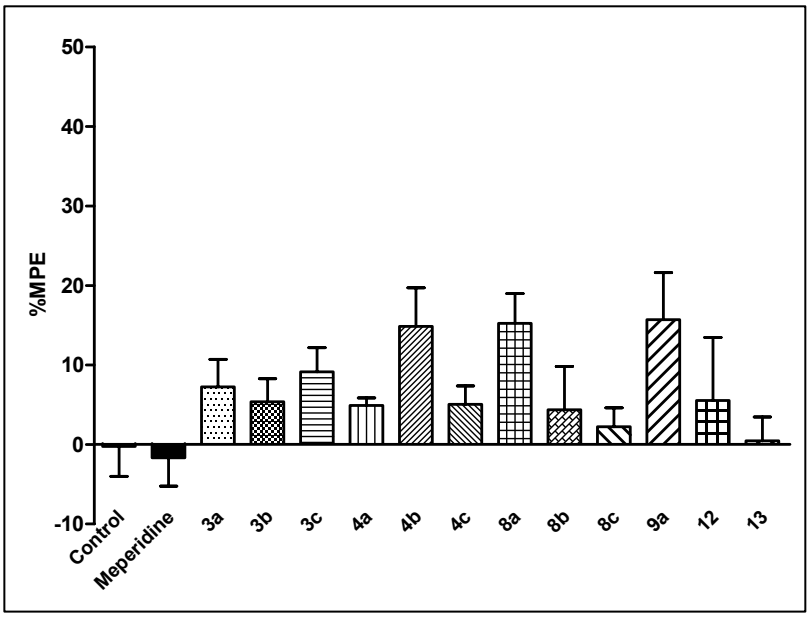


Fig. 4 – **% MPE values at the 120th minute.** There is no statistically significant difference in the maximum possible analgesic effect in any group at the 120th minute while compared with the control group.

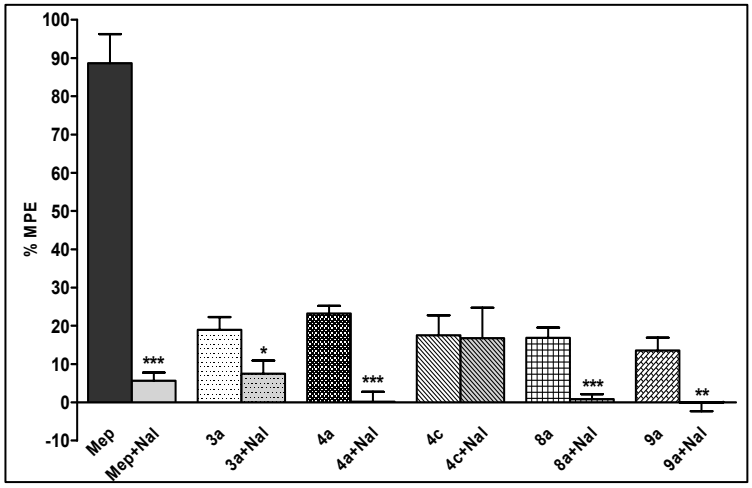


Fig. 5 – **% MPE values at the 30th minute with or without naloxone.***p<0.05, **p<0.01, ***p<0.0001; *vs group without naloxone (Student's t test). There is a statistically significant decrease in the maximum possible analgesic effect in the meperidine, 3a, 4a, 8a and 9a groups at the 30th minute while compared with the group pretreated with naloxone.

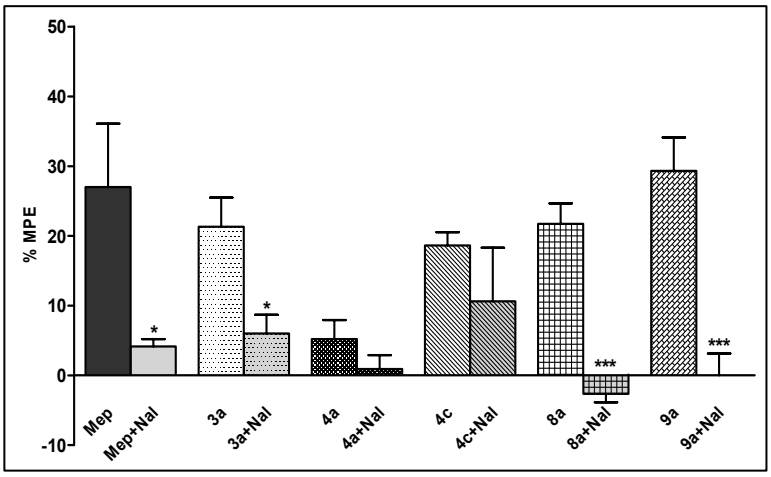


Fig. 6 – **% MPE values at the 60th minute with or without naloxone.** *p<0.05, ***p<0.0001; *vs group without naloxone (Student's t test). There is a statistically significant decrease in the maximum possible analgesic effect in the meperidine, 3a, 8a and 9a groups at the 60th minute while compared with the group pretreated with naloxone.

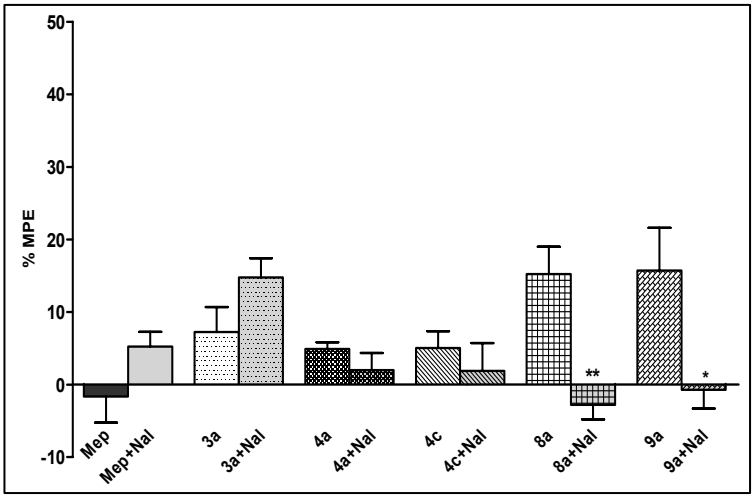


Fig. 7 – **% MPE values at the120th Minute with or without naloxone.** *p<0.05, **p<0.01; *vs group without naloxone (Student's t test). There is a statistically significant decrease in the maximum possible analgesic effect in the 8a and 9a groups at the 120th minute while compared with the group pretreated with naloxone.

EXPERIMENTAL

Chemistry

All chemicals and solvents were purchased locally from MerckAG and Aldrich Chemicals. The microwave reaction was carried out in a MicroSYNTH Microwave Lab station (Milestone S.r.l.). Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectra were recorded on Perkin Elmer Spectrum 400 FT-IR and FT-NIR spectrometers with a Universal ATR sampler. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR spectrometer at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). Microanalyses for C, H, and N were performed on a Leco-932 at Faculty of Pharmacy, Ankara University, Ankara, Turkey, and they were within the range of ±0.4% of the theoretical value. The synthesis of 2a, 2b, 2c, 5a, 5b, 5c, 6a, 6b, 6c, 7a, 7b, 7c, 8a and 11 were previously reported.[11-19]

General procedure for the preparation of benzimidazole derivatives (2a-c, **5a-c)**

The mixture of appropriate 1,2-diaminobenzene derivatives (0.1 mol) and appropriate carboxylic acid derivatives (0.15 mol) in 100 ml 5N HCl were heated to reflux and stirred for 3-5 hours. After the reaction mixture was cooled to the room temperature and neutralized with sodium bicarbonate. The precipitate formed was filtered by suction filtration, washed with water and dried. (Yields; 2a, 93%; 2b, 80%; 2c, 69%; 5a, 47%; 5b, 88%; 5c, 43%)

General procedure for the preparation of N-methyl-2-hydroxymethylbenzimidazole derivatives (6a-c)

A mixture of 2-hydroxymethybenzimidazole derivatives (5a-c, 0.023 mol), dimethyl sulfate (2.37 ml, 0.025 mol), NaOH (1g, 0.025 mol in 3 ml water) in methanol (15 ml) was refluxed for 10 h at 100 ^{o}C. The mixture was diluted with water (15 ml) and extracted with chloroform (3x30 ml). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The precipitate formed was crystallized from water. (Yields; 6a, 48%; 6b, 35%; 6c, 13%).

General procedure for the preparation of N-methyl-2-chloromethylbenzimidazole derivatives (7a-c)

The mixture of appropriate N-methyl-2-hydroxymethyl-benzimidazole derivatives (6a-c, 0.01 mol), thionyl chloride (0.1 mol) in 30 ml dichloromethane were heated to reflux and stirred for 5 hours. The solvent was evaporated, and the residue was subsequently used without further purification. (Yields; 7a, 50%; 7b, 95%; 7c, 93%)

2-Chloromethyl-1,3-benzothiazole (11)

The mixture of 2-aminothiophenol (0.85 ml, 8 mmol) 2-chloroacetyl chloride (0.95 ml, 12 mmol) in acetic acid (15 ml) was irradiated in a microwave reactor for 10 min at 120 ^{o}C. After cooling, the mixture was poured onto crushed ice (100 ml), basified with 5N NaOH. The precipitate formed was filtered by suction filtration, washed with water and dried. (Yield, 74%)

General procedure for the preparation of carbonitrile derivatives (3a-c, **8a-c**, **12)**

The mixture of appropriate chloromethyl derivatives (5 mmol), triethylamine (1.54 ml, 11mmol) and 4-cyano-4-phenylpiperidine hydrochloride (1.11 g, 5 mmol) in 30 ml tetrahydrofurane were heated to reflux and stirred for 6 hours. After the mixture was cooled, the organic salts were filtered off, the solvent was evaporated, and the residue purified by crystallization with the ethanol.

General procedure for the preparation of carboxylic acid derivatives (4a-c, **9a**, **13)**

Appropriate carbonitrile derivatives (1 mmol) were added to (6 ml) of N,N-dimethylformamide/water/ sulphuric acid mixture (1:1:2). After stirring at room temperature for 10 minutes, the mixture was refluxed for 2 hours, subsequently cooling to the room temperature; the reaction mixture was neutralized with sodium bicarbonate. The precipitate formed was filtered by suction filtration, washed with water, dried, and crystallized from the ethanol.

1-[(1H-Benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile **(3a).** Yield 39%; m.p. 150-151 ^{o}C; IR (ν, cm^{-1}): 3600 (N-H), 2290 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 12.24 (1H, s, NH), 7.51-7.11 (9H, m, aromatic H), 3.81 (2H, s, CH_2), 3.02-2.99 (2H, m, piperidine), 2.52-2.45 (2H, m, piperidine), 2.13-2.04 (4H, m, piperidine). Anal. Calc. for $C_{20}H_{20}N_4$. 1/2H_2O: C, 73.82; H, 6.50; N, 17.22. Found: C, 73.73; H, 6.26; N, 16.89%.

1-[(1H-Benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carboxylic acid **(4a).** Yield 75%; m.p. 280 ^{o}C (decomp.); IR (ν, cm^{-1}): 3482-3400 (N-H, O-H), 1684 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 12.24 (1H, s, NH), 7.52-7.12 (9H, m, aromatic H), 3.69 (2H, s, CH_2), 2.80-2.77 (2H, m, piperidine), 2.45-2.42 (2H, m, piperidine), 2.29-2.23 (2H, m, piperidine), 1.90-1.83 (2H, m, piperidine). Anal. Calc. for $C_{20}H_{21}N_3O_2$. 1/3 H_2O: C, 70.36; H, 6.40; N, 12.31. Found: C, 70.26; H, 6.64; N, 12.30%.

1-[(5-Methyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile **(3b).** Yield 48%; m.p. 90-91 ^{o}C; IR (ν, cm^{-1}): 3500 (N-H), 2250 (C≡N); ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.50-7.06 (8H, m, aromatic H), 3.97 (2H, s, CH_2), 3.07-3.05 (2H, m, piperidine), 2.82-2.77 (2H, m, piperidine), 2.46 (3H, s, CH_3), 2.25-2.13 (4H, m, piperidine). Anal. Calc. for $C_{21}H_{22}N_4$. 2.3H_2O: C, 67.72; H, 7.22; N, 15.04. Found: C, 67.96; H, 6.83; N, 14.78%.

1-[(5-Methyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carboxylic acid **(4b).** Yield 55%; m.p. 292 ^{o}C (decomp.); IR (ν, cm^{-1}): 3400-2972 (N-H, O-H), 1584 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.40-6.94 (8H, m, aromatic H), 3.66 (2H, s, CH_2), 2.80-2.77 (2H, m, piperidine), 2.45-2.42 (2H, m, piperidine), 2.38 (3H, s, CH_3), 2.28-2.23 (2H, m, piperidine), 1.90-1.84 (2H, m, piperidine). Anal. Calc. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.35; H, 6.48; N, 11.65%.

1-[(5-Chloro-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile **(3c).** Yield 52%; m.p. 119 ^{o}C; IR (ν, cm^{-1}): 3300-2800 (N-H), 2234 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 12.49 (1H, s, NH), 7.62-7.15 (8H, m, aromatic H), 3.82 (2H, s, CH_2), 3.03-3.00 (2H, m, piperidine), 2.50-2.46 (2H, m, piperidine), 2.16-2.04 (4H, m, piperidine). Anal. Calc. for $C_{20}H_{19}ClN_4$. 1/3 C_2H_5OH: C, 67.78; H, 5.78; N, 15.30. Found: C, 68.07; H, 5.76; N, 14.94%.

1-[(5-Chloro-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carboxylic acid **(4c).** Yield 60%; m.p. 286 ^{o}C (decomp.); IR (ν, cm^{-1}): 3400-2940 (N-H, O-H), 1599 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.56-7.16 (8H, m, aromatic H), 3.80 (2H, s, CH_2), 2.88-2.86 (2H, m, piperidine), 2.48-2.45 (2H, m, piperidine), 2.40-2.37 (2H, m, piperidine), 1.92-1.89 (2H, m, piperidine). Anal. Calc. for $C_{20}H_{20}ClN_3O_2$.1/3 H_2O: C, 63.91; H, 5.54; N, 11.18. Found: C, 63.68; H, 5.59; N, 11.11%.

1-[(1-Methyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile **(8a).** Yield 72%; m.p. 173 ^{o}C; IR (ν, cm^{-1}): 2250 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ,

ppm): 7.61-7.15 (9H, m, aromatic H), 3.88 (2H, s, CH_2), 3.86 (3H, s, CH_3), 3.02-2.99 (2H, m, piperidine), 2.47-2.44 (2H, m, piperidine), 2.13-2.10 (2H, m, piperidine), 2.04-1.98 (2H, m, piperidine). Anal. Calc. for $C_{21}H_{22}N_4$: C, 76.33; H, 6.71; N, 16.96. Found: C, 76.76; H, 6.58; N, 16.78%.

***1-[(1-Methyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carboxylic acid* (9a).** Yield 82%; m.p. 270 ^oC (decomp.); IR (ν, cm^{-1}): 3590-3200 (O-H), 1685 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.65-7.21 (9H, m, aromatic H), 4.26 (2H, s, CH_2), 3.84 (3H, s, CH_3), 3.20-3.16 (2H, m, piperidine), 2.80-2.75 (2H, m, piperidine), 2.55-2.50 (2H, m, piperidine), 2.10-2.00 (2H, m, piperidine). Anal. Calc. for $C_{21}H_{23}N_3O_2.1/2H_2SO_4.2H_2O$: C, 58.05; H, 6.50; N, 9.67; S, 3.69. Found: C, 58.50; H, 6.51; N, 9.70; S, 3.72%.

***1-[(1,5-Dimethyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile* (8b).** Yield 34%; m.p. 134-135 ^oC; IR (ν, cm^{-1}): 2230 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.54-7.31 (8H, m, aromatic H), 3.84-3.81 (5H, m, CH_3, CH_2), 3.01-2.98 (2H, m, piperidine), 2.46-2.40 (5H, m, piperidine, CH_3), 2.13-2.10 (2H, m, piperidine), 2.03-1.99 (2H, m, piperidine). Anal. Calc. for $C_{22}H_{24}N_4.1/2H_2O$: C, 74.76; H, 7.13; N, 15.85. Found: C, 74.65; H, 7.02; N, 15.55%.

***1-[(5-Chloro-1-methyl-1H-benzimidazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile* (8c).** Yield 26%; m.p. 163-164 ^oC; IR (ν, cm^{-1}): 2200 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.71-7.18 (8H, m, aromatic H), 3.88-3.84 (5H, m, CH_3, CH_2), 3.01-2.98 (2H, m, piperidine), 2.47-2.44 (2H, m, piperidine), 2.13-1.97 (4H, m, piperidine). Anal. Calc. for $C_{21}H_{21}ClN_4$: C, 69.13; H, 5.80; N, 15.36. Found: C, 69.40; H, 5.66; N, 15.24%.

***1-[(1,3-Benzothiazol-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile* (12).** Yield 72%; m.p. 82 ^oC; IR (ν, cm^{-1}): 2238 (C≡N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.06 (1H, d, aromatic H), 7.95 (1H, d, aromatic H), 7.58-7.38 (7H, m, aromatic H), 4.08 (2H, s, CH_2), 3.13-3.10 (2H, m, piperidine), 2.64-2.57 (2H, m, piperidine), 2.20-2.17 (2H, m, piperidine), 2.11-2.04 (2H, m, piperidine). Anal. Calc. for $C_{20}H_{19}N_3S$: C, 72.04; H, 5.74; N, 12.60; S, 9.62. Found: C, 72.33; H, 5.57; N, 12.51; S, 9.57%.

***1-[(1,3-Benzothiazol-2-yl)methyl]-4-phenylpiperidine-4-carboxylic acid* (13).** Yield 66%; m.p. 209-210 ^oC; IR (ν, cm^{-1}): 1684 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.04 (1H, d, aromatic H), 7.92 (1H, d, aromatic H), 7.49-7.24 (7H, m, aromatic H), 3.93 (2H, s, CH_2), 2.90-2.87 (2H, m, piperidine), 2.49-2.46 (2H, m, piperidine), 2.42-2.36 (2H, m, piperidine), 1.89-1.84 (2H, m, piperidine). Anal. Calc. for $C_{20}H_{20}N_2O_2S.1/4\ H_2O$: C, 67.30; H, 5.79; N, 7.85; S, 8.98. Found: C, 67.34; H, 5.63; N, 7.85; S, 8.96%.

Biological activity

Animals

Male Balb-c mice weighing 24 to 41 g were obtained from Gazi University Laboratory Animals and Experimental Research Center, Ankara and housed in plastic cages with water and food provided ad libitum. They were kept on a 12-h/12-h light–dark cycle and acclimatized to the test chamber before testing. The study was approved by the Gazi University Local Ethics Committee for Animal Experiments (G.Ü.ET-12.074)

Experimental groups

Mice were randomly divided into twenty groups, each comprising 6–7 animals, and treated according to the predetermined schedule. Experimental groups were named as following: Control, Meperidine, Naloxone+Meperidine, 3a, 3b, 3c, 4a, 4b, 4c, 8a, 8b, 8c, 9a, 12, 13, 3a+Naloxone, 4a+Naloxone, 4c+Naloxone, 8a+Naloxone and 9a+Naloxone**.** Meperidine, naloxone and all test chemicals dissolved in dimethyl sulfoxide (DMSO).

Analgesic activity

Nociception was evaluated by the radiant heat tail-flick test modified from D'Amour and Smith's method.[20] Each rat was placed in a Plexiglas test chamber to be immobilized. The tail of the rat protruded through the caudal hole. The tail-flick apparatus (Tail Flick Unit Cat.No. 7360; Ugo Basile; Italy) generated a beam of radiant heat which was focused on the underside of the tail, 2 cm from the tip. The intensity of the heat source was set at 75, which allowed the basal tail-flick latency to be controlled approximately 3 s. A cut-off latency of 10s was used to avoid tissue damage to the tail. The latency of reflexive removal of the tail from the heat was measured by a photocell timing circuit to the nearest 0.1 s. Nociception was assessed as the duration between onset of the thermal stimulus to the skin of tail and the tail-flick response. At the study day, basal tail-flick latencies (baseline) were measured for each mouse and then saline, meperidine (40 mg/kg) or test chemicals (40 mg/kg) applied subcutaneously at the 0.005 ml/g body weight. Naloxone (2 mg/kg, sc) was injected 15 minutes before meperidine or test chemicals at the same volume. Naloxone application is only performed for the test chemicals that showed high analgesic activity. After injections tail-flick latencies (post-treatment) were measured in 30, 60 and 120 minutes. The analgesic activity was expressed as the percentage of maximum possible effect (%MPE), calculated by the following formula:

$$\%MPE = (\text{Post-treatment latency}) - (\text{Baseline latency}) / (\text{Cut-off time}) - (\text{Baseline latency}) \times 100$$

Acute toxicity

Mice were followed for the morbidity and mortality until 48 hours after the first injection.

Statistical analysis

Data were expressed as "mean ± standart error of the mean". The groups without naloxone were assessed with one-way ANOVA followed by Dunnet test; the groups with or without naloxone for each chemicals were compared with *Student's* t test. p values less than 0.05 were considered as statistically significant.

CONCLUSIONS

Meperidine is a synthetic opioid analgesic used in the treatment of moderate to severe pain. Prolonged meperidine use may increase the risk of toxicity from the normeperidine. Additionally, meperidine can be abused similar to other opioid agonists. This led us to investigate the novel and safer meperidine derivatives. In this study, we synthesized meperidine like benzimidazole derivatives and tested their antinociceptive activity with tail flick test. Among the synthesized compounds 3a, 4a, 4c, 8a, 9a exhibited significant antinociceptive activity. Additionally, compounds

3a, 4a, 8a and 9a showed naloxone-reversible antinociceptive activity. The analgesic action seems to be mediated through opioid receptors. In conclusion, these compounds appear to provide a starting point for the design and development of the new and more active antinociceptive agents. Further studies are in progress.

Acknowledgements: This study was supported by Gazi University BAP. (Project Number 02/2012-31).

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