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ÜMİDE DEMİR ÖZKAY

ÖZGÜR DEVRİM CAN

NAZLI TURAN

BETÜL KAYA ÇAVUŞOĞLU

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Synthesis and antinociceptive activities of some novel benzimidazole-piperidine derivatives

Ümide DEMİR ÖZKAY[1], **Özgür Devrim CAN**[1,*], **Nazlı TURAN**[1], **Betül KAYA ÇAVUŞOĞLU**[2]
[1]Department of Pharmacology, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

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Abstract: In this study, a series of benzimidazole-piperidine derivatives were synthesized with the objective of developing potent antinociceptive agents. Some 2-(4-substituted-phenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole derivatives were obtained by microwave-supported reaction of an appropriate 2-(4-substituted-phenyl)-1*H*-benzimidazole with 2-(piperidine-1-yl)ethyl chloride. The chemical structures of the compounds were elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectral data. Antinociceptive activity was assessed by conducting hot-plate, paw-pressure, and formalin tests. Morphine (5 mg/kg, i.p) was used as a reference drug. Among the tested compounds **2a**–**2d** and **2f**–**2h** (10 mg/kg) increased the maximum possible effect (MPE)% values calculated for the hot-plate and paw-pressure tests and decreased the paw licking time of rats in the early phase of the formalin test, indicating centrally mediated antinociceptive activities of these derivatives. In the late-phase **2g** and **2h** were the only compounds reducing the paw licking duration. These data show additional peripherally mediated antinociceptive activities for these two derivatives. Falling latencies of animals in the rotarod test did not change upon the administration of test compounds; thus, the observed antinociceptive effects were specific. Predictions obtained by theoretical calculations of ADME (absorption, distribution, metabolism, excretion) properties supported the antinociceptive potential of the tested benzimidazole-piperidine derivatives.

Key words: Benzimidazole, formalin test, hot-plate test, paw-pressure test, piperidine, rotarod

1. Introduction

Heterocyclic systems are frequently preferred structures for the synthesis of novel molecules with pharmacological activity potential. Among them, *N*-based heterocycles are especially important since many of the biologically active compounds such as alkaloids, glycosides, and hormones as well as some of the clinically used drugs carry *N*-containing heterocycles in their chemical structures.[1,2] As one of the *N*-based heterocycles, the piperidine ring system has been shown to possess various pharmacological effects such as antibacterial, antifungal,[3–5] anti-HIV,[6] antileishmanial,[7] anticancer,[8,9] renin inhibitory,[10] diuretic, and natriuretic[11] effects. Another *N*-containing heterocyclic structure, benzimidazole, is also known to have the ability to interact with biomolecules of living systems.[12] There are many benzimidazole derivative drugs with a wide range of biologically activities,[13–16] such as omeprazole (proton pump inhibitor),[17] albendazole (anthelminthic),[18] domperidone (antiemetic, gastroprokinetic),[19] and pimozide (antipsychotic).[20]

Recent studies screening the pharmacological activity capacity of various compounds bearing piperidine rings in their structure have pointed out a notable therapeutic potential of these derivatives on the central ner-

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[*] Correspondence: ozgurdt@anadolu.edu.tr

vous system. Cognitive enhancer,[21,22] anti-Alzheimer,[23] neuroprotective,[24] antidepressant-like,[25] anxiolytic-like,[26] antipsychotic,[27] anticonvulsant,[28,29] antiobesity,[30] antipyretic,[31,32] and wake-promotion activities[21] of various piperidine derivatives have been demonstrated, so far. Antinociception is another pharmacological activity induced by compounds carrying piperidine moiety.[33] Piperidine derivatives have been shown to possess antinociceptive effects against acute nociceptive stimuli.[31,33–35] Furthermore, they have also been reported for their efficacy on chronic neuropathic and inflammatory pain.[36–38] Central mechanisms of pain seem to be involved in the antinociceptive effect of various piperidine-derivative compounds.[33–36] On the other hand, peripheral mechanisms of nociception should not be ruled out since numerous piperidine derivatives have been shown to suppress peripheral inflammation and pain processes.[32,36]

In this study, based on this current literature indicating the therapeutic potential of piperidine derivatives for pain disorders as well as the pharmacological activity potential of the benzimidazole core ring, we designed and synthesized some benzimidazole-piperidine compounds. Then, with the aim of discovering and developing new analgesic drug candidates, we screened the antinociceptive activities of these novel benzimidazole-piperidine derivatives by using some well-known in vivo nociceptive tests.

2. Results and discussion

The synthetic route of title compounds (**2a**–**2h**) is presented in Figure 1. Condensation of 1,2-phenylenediamine with diverse sodium bisulfide adducts of benzaldehydes under microwave conditions gave the 2-(4-substituted-phenyl)-1*H*-benzimidazole derivatives (**1a**–**1h**). In the next step, compounds **1a**–**1h** were reacted with 2-(piperidine-1-yl)ethyl chloride in the presence of NaH under microwave conditions. Some characteristic properties of the intermediate and final compounds are given in Table 1. The synthesized compounds (**2a**–**2h**) were characterized by FT-IR, HRMS, ^{1}H NMR, and ^{13}C NMR spectroscopic methods. In the IR spectra of the compounds, stretching bands belonging to $C = N$ and $C = C$ were observed between 1620 and 1442 cm^{-1}. The out-of-plane deformation bands belonging 1,4-disubstituted benzene were recorded at 861–819 cm^{-1}. HRMS results agreed well with the calculated molecular formula of compounds **2a**–**2h**.

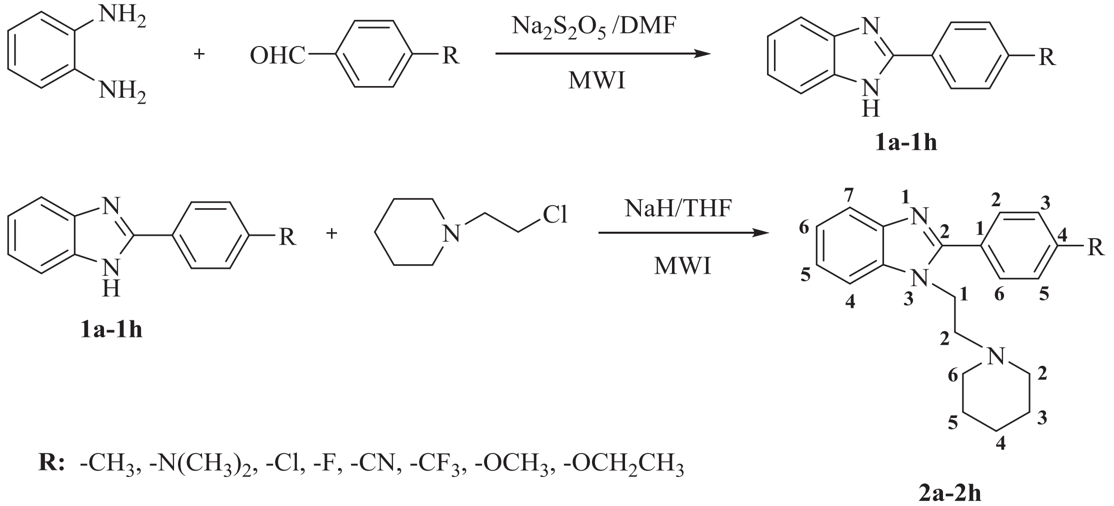

Figure 1. Synthesis of the compounds **2a**–**2h**.

In the ^{1}H NMR spectra of compound **2a**, the 3rd, 4th, and 5th position protons of piperidine were observed as a broad singlet peak at 1.30 ppm while the 2nd and 6th position protons close to nitrogen were

Table 1. The percentage of yields and melting points of the compounds (**1a–1h, 2a–2h**).

Compound	Mp (°C)		Yield (%)	Compound	Mp (°C)	Yield (%)
	Literature	Found				
1a	270–272[39]	274–277	75	**2a**	72–74	69
1b	273–275[40]	275–278	69	**2b**	128–133	74
1c	290–292[39]	287–288	78	**2c**	88–91	80
1d	250–251[41]	253–255	73	**2d**	68–72	76
1e	261–262[39]	261–263	80	**2e**	138–140	72
1f	280–281[42]	281–284	83	**2f**	102–104	66
1g	222–225[39]	223–226	85	**2g**	100–103	65
1h	149–151[43]	152–155	70	**2h**	63–66	70

detected at 2.20 ppm as a broad singlet peak. Methyl group protons attached to the phenyl ring were assigned as a singlet at 2.40 ppm. A triplet peak due to the 1st CH$_2$ protons belonging to the ethyl moiety between piperidine and the benzimidazole ring was observed at 2.56 ppm and the 2nd CH$_2$ protons of this moiety were detected at 4.35 as a triplet. In the aromatic region the 5th and 6th position protons of the benzimidazole ring were assigned at 7.19–7.29 ppm as a multiplet whereas the 4th and 7th position protons of this ring were assigned at 7.60–7.67 as a multiplet. Two doublets belonging to the phenyl ring were detected at 7.37 ppm due to 3rd and 5th position protons and at 7.72 ppm due to 2nd and 6th position protons. In the ^{13}C NMR spectra of compound **2a**, a signal due to methyl carbon was observed at 21.43 ppm. The other aliphatic carbons owing to the piperidine ring and ethyl moiety were detected at 24.23, 25.87, 42.63, 54.67, and 57.78 ppm. The aromatic carbons belonging to benzimidazole and phenyl rings were observed at 111.33, 119.48, 122.27, 122.65, 128.27, 129.61, 129.66, 136.17, 139.67, 143.13, and 153.96 ppm. In the aromatic region, it is difficult to assign a signal to each carbon because of the similar ppm values of carbons.

In the ^{1}H NMR and ^{13}C NMR spectra of the other compounds, similar to the above elucidation, 2nd and 6th position protons of piperidine were observed as a broad singlet between 2.13 and 2.20 ppm, whereas 3rd, 4th, and 5th position protons of piperidine were recorded between 1.24 and 1.35 ppm as a broad singlet. The protons of ethylene between piperidine and benzimidazole were assigned as triplet peaks at 2.53–2.60 ppm and 4.35–4.42 ppm, respectively. The other aromatic and aliphatic protons were observed in the expected regions. In the ^{13}C NMR spectra, the carbon belonging to the 4th position of piperidine was assigned at 24.17–24.28 ppm. The carbons at the 3rd and 5th positions of piperidine were observed between 25.76 and 25.94 ppm, whereas the 2nd and 6th position carbons of piperidine were observed at 54.67–57.72 ppm. The peaks belonging to carbons between piperidine and benzimidazole were recorded at 42.61–57.87 ppm. Aromatic carbons were generally observed at 110.99–164.91 ppm. In the spectra, splitting associated with neighboring atoms was confirmed owing to the presence of fluoro in compound **2d**.

Following the synthesis and structure elucidation, possible antinociceptive activities of the benzimidazole-piperidine derivatives were evaluated by using several well-established nociception assays. In experimental animals, sensation of pain is generally evaluated by monitoring motor responses ranging from spinal reflexes to complex behaviors. In different pain models mechanical, thermal, chemical, or electrical nociceptive stimuli can be used as "noxious stimuli".[44] Based on this knowledge, we examined the antinociceptive activity potential of our novel benzimidazole-piperidine derivatives by using hot-plate, Randall–Selitto paw-pressure, and formalin-induced paw licking tests.

The hot-plate method is used for assessing the response of animals to acute thermal noxious stimuli. Obtained data showed that benzimidazole-piperidine derivative test compounds **2a–2d** and **2f–2h**, administrated at 10 mg/kg doses, induced significant augmentations in the reaction time (maximum possible effect (MPE)%) of rats (Figure 2). Enhancements of the MPE% values demonstrated the antinociceptive effects of these compounds on nociceptive pathways carrying thermal noxious stimuli. Findings of the hot-plate test also suggested a centrally mediated antinociceptive activity profile for these compounds, since this test predominantly measures supraspinally organized nociceptive signaling.[45,46]

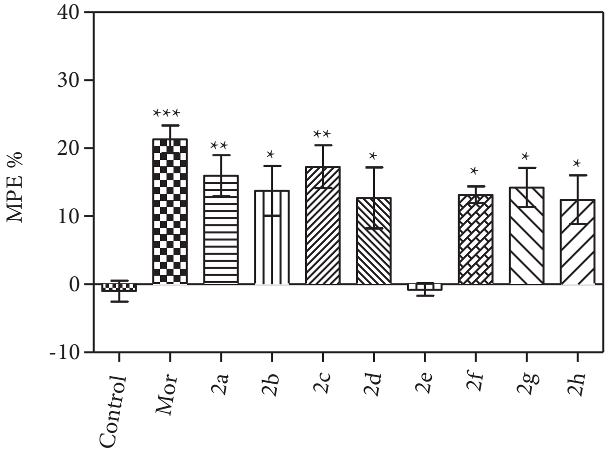

Figure 2. Effects of test compounds (10 mg/kg) and morphine (5 mg/kg) on MPE% values of rats in the hot-plate test. Significance against control group: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are given as mean ± SEM. One-way ANOVA, post hoc Tukey's test, n = 7.

In this study, the reaction of the animals to acute mechanical noxious stimuli was evaluated using the Randall–Selitto paw-pressure test. Obtained results demonstrated that administration of compounds **2a**–**2d** and **2f**–**2h** induced significant increases in the calculated MPE% values of rats (Figure 3), indicating the antinociceptive effects of these compounds on nociceptive pathways carrying mechanical noxious stimuli. Similar to hot-plate tests, results of the paw-pressure tests also suggested centrally mediated antinociceptive activity profiles for the test compounds, since, in the paw-pressure test, pain caused by the compression of the hind paw is centrally mediated and is attributed to the direct stimulation of nociceptor afferent fibers.[44,47]

Antinociceptive efficacy of the compounds against acute chemical noxious stimuli was evaluated using the formalin-induced paw licking test, which is also a well-established method in the elucidation of a compound's mechanism of action at both the peripheral and central levels.[48] The obtained findings showed that compounds **2a**–**2d** and **2f**–**2h** significantly shortened the paw licking time of animals measured in the early phase compared to the control group (Figure 4; Table 2). This finding supported the experimental results of the hot-plate and the paw-pressure tests since the early phase is characterized by "neurogenic pain", which is mediated by direct stimulation of nociceptors in the paw and reflects centrally mediated pain.[48,49]

Among the tested compounds, **2g** and **2h** also decreased the paw licking time of rats measured in the late phase (Figure 5; Table 2). Since the late phase of the formalin test is characterized by "inflammatory pain", which is caused by the release of algogenic substances from damaged local tissues, this second phase reflects peripherally mediated pain.[48,49] Therefore, different from compounds **2a**, **2b**, **2c**, **2d**, and **2f**, the antinociceptive activities of compounds **2g** and **2h** are related to the participation of peripheral mechanisms as well as central ones.

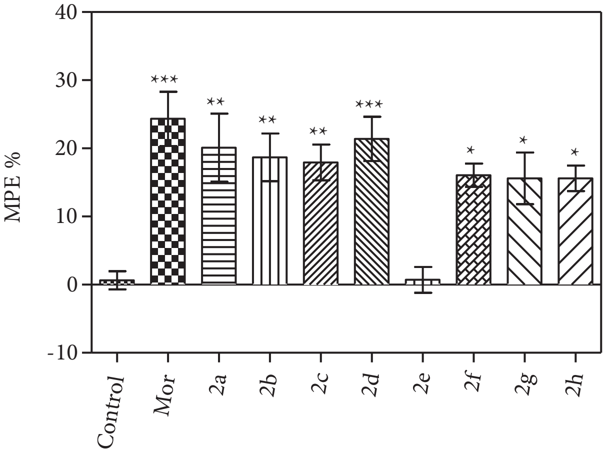

Figure 3. Effects of test compounds (10 mg/kg) and morphine (5 mg/kg) on MPE% values of rats in the paw-pressure test. Significance against control group: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are given as mean ± SEM. One-way ANOVA, post hoc Tukey's test, n = 7.

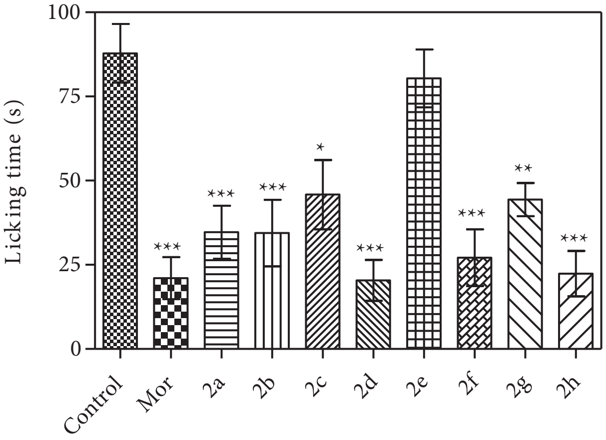

Figure 4. Effects of test compounds (10 mg/kg) and morphine (5 mg/kg) on paw licking time of rats in the early phase of the formalin test. Significance against control Group: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are given as mean ± SEM. One-way ANOVA, post hoc Tukey's test, n = 7.

Table 2. Inhibition % values of experimental groups in the early and late phases of the formalin test.

Treatment	Early phase inhibition %	Late phase inhibition %
Morphine	76.04	80.98
2a	60.52	26.45
2b	60.81	35.60
2c	47.81	35.64
2d	76.81	9.53
2e	8.50	14.52
2f	69.12	41.14
2g	49.44	46.49
2h	74.53	56.28

In all of the nociceptive tests, reference drug morphine sulfate exhibited its antinociceptive efficacy as expected (Figures 2–5 and Table 2).

Data obtained from the rotarod test did not suggest any alteration in the motor coordination of rats, indicating that results of the nociceptive tests are specific. In other words, the observed antinociceptive activities were not affected by any nonspecific sedative or neuromuscular blocker effects caused by the tested compounds.

Theoretically predicted ADME properties of the tested benzimidazole-piperidine derivatives (**2a**–**2h**), namely molecular weight, log P, topological polar surface area (tPSA), number of hydrogen donors and acceptors, volume, and number of rotatable bonds, are presented in Table 3 along with violations of Lipinski's rule.[50,51] This rule suggests that an orally active drug should not possess more than one violation. Hence, according to the data presented in Table 3, all compounds **2a**–**2h** are compatible with Lipinski's rule.

Moreover, it has been determined that the test compounds have ideal lipophilic characters suitable for crossing to the central nervous system. The tPSA values, described to be a predictive indicator of membrane

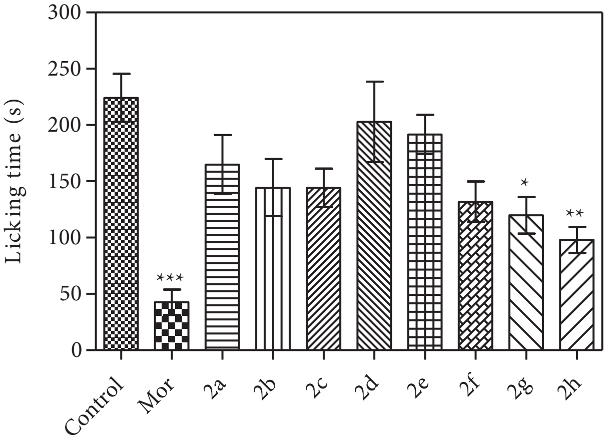

Figure 5. Effects of test compounds (10 mg/kg) and morphine (5 mg/kg) on paw licking time of rats in the late phase of the formalin test. Significance against control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are given as mean ± SEM. One-way ANOVA, post hoc Tukey's test, n = 7.

Table 3. In silico physicochemical parameters of compounds **2a–2h**.

Com	R	Log P	TPSA	MW	nON	nOHNH	nrotb	Volume	Vio
2a	$-CH_3$	5	21.06	319.45	3	0	4	316.77	0
2b	$-N(CH_3)_2$	4.65	24.30	348.49	4	0	5	346.12	0
2c	-Cl	5.23	21.06	339.87	3	0	4	313.75	1
2d	-F	4.71	21.06	323.42	3	0	4	305.14	0
2e	-CN	4.31	44.86	330.44	4	0	4	317.07	0
2f	$-CF_3$	5.45	21.06	373.42	3	0	5	331.51	1
2g	$-OCH_3$	4.61	30.30	335.45	4	0	5	325.75	0
2h	$-OC_2H_5$	4.98	30.30	349.48	4	0	6	342.56	0
Ideal range		≤ 5	≤ 140	≤ 500	≤ 10	≤ 5	≤ 10		≤ 1

log P: log octanol/water partition coefficient; TPSA: total polar surface area; MW: molecular weight; nON: no. of hydrogen acceptors; nOHNH: no. of hydrogen donors; nrotb: no. of rotatable bonds; Vio: violations were calculated using the Molinspiration Calculation of Molecular Properties toolkit.

penetration, are positive (21.06–30.30) and suggest that synthesized compounds **2a–2h** have abilities to pass different membranes and reach the central nervous system. These findings supported the efficacy of these compounds as central antinociceptive agents. On the other hand, participation of peripheral mechanisms in the antinociceptive activities of compounds **2g** and **2h** may be related to alkyloxy substituents (methoxy and ethoxy), which may provide higher ability to these compounds for modifying peripheral nociception mechanisms.

In summary, data acquired from the performed nociceptive tests pointed out centrally mediated antinociceptive actions induced by the compounds **2a**, **2b**, **2c**, **2d**, **2f**, **2g**, and **2h** on nociceptive neuronal pathways carrying mechanical, thermal, and chemical stimuli. Moreover, peripheral mechanisms also seem to contribute to the antinociceptive activities of compounds **2g** and **2h**, as well as central ones. This present study supports the previous literature reporting on the antinociceptive activities of benzimidazole-piperidine derivatives.[31,33–38] Nevertheless, the exact mechanisms of the observed antinociceptive activities need to be clarified with further detailed investigations.

3. Experimental

3.1. Drugs

All of the used chemicals were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA).

3.2. Chemistry

3.2.1. General

Melting points of the synthesized compounds were determined by an MP90 digital melting point apparatus (Mettler Toledo, Columbus, OH, USA) and were uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 300 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) and on a Bruker DPX 75 MHz spectrometer (Bruker Bioscience) in DMSO-d$_{6}$, respectively. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (J) were reported as Hz. The IR spectra were obtained on a Shimadzu IR Affinity-1S (Shimadzu, Tokyo, Japan). HRMS studies were performed on a Shimadzu LCMS-IT-TOF system.

3.2.2. Microwave-assisted synthesis of 2-(4-substituted-phenyl)-1*H*-benzimidazole derivatives (1a–1h)

A mixture of suitable benzaldehyde derivative (0.02 mol), sodium disulfite (3.8 g, 0.02 mol), and DMF (10 mL) was added to a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300, Austria). The resultant mixture was heated under conditions of 240 °C and 10 bar for 5 min. After this period, the vial was cooled down, 1,2-phenylenediamine (2,16 g, 0.02 mol) was added, and then the reaction mixture was kept under the same reaction conditions in the microwave reactor. After TLC screening, the mixture was poured into ice water and the solid was washed with water and dried. The products were crystallized from ethanol.[52–54]

3.2.3. Microwave-assisted synthesis of 2-(4-substituted-phenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole derivatives (2a–2h)

In a vial (30 mL) of microwave synthesis reactor (Anton-Paar Monowave 300), the corresponding 2-(4-substituted-phenyl)-1*H*-benzimidazole derivative (**1a–1h**) (2.5 mmol) was dissolved in THF (10 mL) and NaH (0.072 g, 3 mmol) was added. After the addition of 2-(piperidine-1-yl)ethyl chloride (1 mL), the mixture was heated under conditions of 170 °C and 10 bar for 30 min. After cooling, the mixture was poured into ice water. The resulting precipitate was washed with water and dried. Crystallization of crude product from ethanol gave final compounds **2a–2h**.

3.2.4. 2-(4-Methylphenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2a)

IR (KBr, cm^{-1}): υ_{max} 3049 (aromatic C-H stretching), 2972 (aliphatic C-H stretching), 1610–1448 (C = N and C = C stretching), 1155 (C-N stretching) 827 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d$_{6}$, ppm) δ: 1.30 (6H, br s, piperidine -CH$_{2}$-), 2.20 (4H, br s, piperidine -CH$_{2}$-), 2.40 (3H, s, CH$_{3}$), 2.56 (2H, t, J = 6.5 Hz, -CH$_{2}$-), 4.35 (2H, t, J = 6.5 Hz, -CH$_{2}$-), 7.19–7.29 (2H, m, benzimidazole H$_{5}$, H$_{6}$), 7.37 (2H, d, J = 7.9 Hz, phenyl H$_{3}$, H$_{5}$), 7.60-7.67 (2H, m, benzimidazole H$_{4}$, H$_{7}$), 7.72 (2H, d, J = 8.1 Hz, phenyl

H_2, H_6). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 21.43 (CH_3), 24.23 (CH_2), 25.87 ($2CH_2$), 42.63 (CH_2), 54.67 ($2CH_2$), 57.78 (CH_2), 111.33 (CH), 119.48 (CH), 122.27 (CH), 122.65 (CH), 128.27 (C), 129.61 (2CH), 129.66 (2CH), 136.17 (C), 139.67 (C), 143.13 (C), 153.96 (C). HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{25}N_3$: 320.2121; found: 320.2113.

3.2.5. 2-(4-Dimethylaminophenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2b)

IR (KBr, cm^{-1}): υ_{max} 3082 (aromatic C-H stretching), 2933 (aliphatic C-H stretching), 1606–1442 (C = N and C = C stretching), 1195 (C-N stretching) 819 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 1.35 (6H, br s, piperidine -CH_2-), 2.26 (4H, br s, piperidine -CH_2-), 2.60 (2H, t, J = 6.7 Hz, -CH_2-), 2.99 (6H, s, -$N(CH_3)_2$), 4.35 (2H, t, J = 6.7 Hz, -CH_2-), 6.84 (2H, d, J = 8.9 Hz, phenyl H_2, H_6), 7.15–7.24 (2H, m, benzimidazole H_5, H_6), 7.54–7.62 (2H, m, benzimidazole H_4, H_7), 7.67 (2H, d, J = 8.9 Hz, phenyl H_3, H_5). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 24.28 (CH_2), 25.94 ($2CH_2$), 40.28 ($2CH_3$), 42.77 (CH_2), 54.72 ($2CH_2$), 57.78 (CH_2), 110.99 (CH), 112.07 (2CH), 117.86 (C), 119.03 (CH), 122.01 (CH), 122.10 (CH), 130.53 (2CH), 136.34 (C), 143.29 (C), 151.36 (C), 154.53 (C). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{28}N_4$: 349.2387; found: 349.2373.

3.2.6. 2-(4-Chlorophenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2c)

IR (KBr, cm^{-1}): υ_{max} 3053 (aromatic C-H stretching), 2929 (aliphatic C-H stretching), 1598–1450 (C = N and C = C stretching), 1157 (C-N stretching) 839 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 1.28 (6H, br s, piperidine -CH_2-), 2.17 (4H, br s, piperidine -CH_2-), 2.55 (2H, t, J = 6.3 Hz, -CH_2-), 4.38 (2H, t, J = 6.3 Hz, -CH_2), 7.22-7.32 (2H, m, benzimidazole H_5, H_6), 7.62-7.70 (4H, m, phenyl H_2, H_6, benzimidazole H_4, H7), 7.89 (2H, d, J = 8.6 Hz, phenyl H_3, H_5). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 24.21 (CH_2), 25.83 ($2CH_2$), 42.70 (CH_2), 54.69 ($2CH_2$), 57.80 (CH_2), 111.50 (CH), 119.66 (CH), 122.49 (CH), 122.98 (CH), 129.14 (2CH), 130.08 (C), 131.60 (2CH), 134.90 (C), 136.18 (C), 143.06 (C), 152.80 (C). HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{22}ClN_3$: 340.1575; found: 340.1568.

3.2.7. 2-(4-Fluorophenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2d)

IR (KBr, cm^{-1}): υ_{max} 3089 (aromatic C-H stretching), 2972 (aliphatic C-H stretching), 1606–1454 (C = N and C = C stretching), 1224 (C-N stretching) 840 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 1.29 (6H, br s, piperidine -CH_2-), 2.17 (4H, br s, piperidine -CH_2-), 2.55 (2H, t, J = 6.4 Hz,-CH_2-), 4.36 (2H, t, J =6.4 Hz,-CH_2), 7.24–7.31 (2H, m, benzimidazole H_5, H_6), 7.38–7.44 (2H, m, benzimidazole H_4, H_7), 7.63–7.69 (2H, m, phenyl H_2, H_6), 7.88–7.93 (2H, m, phenyl H_3, H_5). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 24.21 (CH_2), 25.84 ($2CH_2$), 42.61 (CH_2), 54.67 ($2CH_2$), 57.76 (CH_2), 111.43 (CH), 116.10 (phenyl $C_{3,3'}$, d, J = 21.8 Hz), 119.58 (CH), 122.40 (CH), 122.85 (CH), 127.71 (phenyl C_1, d, J = 3.8 Hz), 132.16 (phenyl $C_{2,2'}$, d, J = 9.0 Hz), 136.09 (C), 143.03 (C), 153.03 (C), 163.28 (phenyl C_4, d, J = 245.3 Hz). HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{22}FN_3$: 324.1871; found: 324.1860.

3.2.8. 2-(4-Cyanophenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2e)

IR (KBr, cm^{-1}): υ_{max} 3043 (aromatic C-H stretching), 2931 (aliphatic C-H stretching), 2217 (C≡N), 1614–1446 (C = N and C = C stretching), 1122 (C-N stretching) 848 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d$_6$, ppm) δ: 1.24 (6H, br s, piperidine -CH$_2$-), 2.13 (4H, br s, piperidine -CH$_2$-), 2.53 (2H, t, J = 6.1 Hz, -CH$_2$-), 4.41 (2H, t, J = 6.1 Hz, -CH$_2$-), 7.24–7.35 (2H, m, benzimidazole H$_5$, H$_6$), 7.68–7.72 (2H, m, benzimidazole H$_4$, H$_7$), 8.02–8.10 (4H, m, phenyl H$_2$, H$_6$, phenyl H$_3$, H$_5$). ^{13}C NMR (75 MHz, DMSO-d$_6$, ppm) δ: 24.17 (CH$_2$), 25.78 (2CH$_2$), 42.83 (CH$_2$), 54.71 (2CH$_2$), 57.83 (CH$_2$), 111.71 (CH), 112.45 (C), 118.99 (CN), 119.93 (CH), 122.75 (CH), 123.40 (CH), 130.64 (2CH), 133.00 (2CH), 135.79 (C), 136.30 (C), 143.11 (C), 152.25 (C). HRMS (m/z): [M + H]$^+$ calcd for $C_{21}H_{22}N_4$: 331.1917; found: 331.1902.

3.2.9. 2-[4-(Trifluoromethyl)phenyl]-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2f)

IR (KBr, cm^{-1}): υ_{max} 3080 (aromatic C-H stretching), 2972 (aliphatic C-H stretching), 1620–1442 (C = N and C = C stretching), 1126 (C-N stretching) 861 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d$_6$, ppm) δ: 1.24 (6H, br s, piperidine -CH$_2$-), 2.13 (4H, br s, piperidine -CH$_2$-), 2.54 (2H, t, J = 6.1 Hz, -CH$_2$-), 4.42 (2H, t, J = 6.2 Hz, -CH$_2$), 7.24–7.34 (2H, m, benzimidazole H$_5$, H$_6$), 7.67–7.72 (2H, m, benzimidazole H$_4$, H$_7$), 7.93 (2H, d, J = 8.3 Hz, phenyl H$_2$, H$_6$), 8.10 (2H, d, J = 8 Hz, phenyl H$_3$, H$_5$). ^{13}C-NMR (75 MHz, DMSO-d$_6$, ppm) δ: 24.17 (CH$_2$), 25.76 (2CH$_2$), 42.76 (CH$_2$), 54.70 (2CH$_2$), 57.87 (CH$_2$), 111.65 (CH), 119.86 (CH), 122.66 (CH), 123.26 (CH), 125.93 (phenyl C$_{2,2'}$, q, J = 3.7 Hz), 128.12 (CF$_3$, q, J =261.3 Hz), 130.13 (phenyl C$_1$, q, J = 22.6 Hz), 130.67 (2CH), 135.33 (C), 136.23 (C), 143.09 (C), 152.48 (C). HRMS (m/z): [M + H]$^+$ calcd for $C_{21}H_{22}F_3N_3$: 374.1839; found: 374.1833.

3.2.10. 2-(4-Methoxyphenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2g)

IR (KBr, cm^{-1}): υ_{max} 3070 (aromatic C-H stretching), 2935 (aliphatic C-H stretching), 1612-1450 (C = N and C = C stretching), 1307-1029 (C-N and C-O stretching) 837 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d$_6$, ppm) δ: 1.33 (6H, br s, piperidine -CH$_2$-), 2.21 (4H, br s, piperidine -CH$_2$-), 2.56 (2H, t, J = 6.5 Hz, -CH$_2$-), 3.84 (3H, s, OCH$_3$), 4.35 (2H, t, J =6.6 Hz, -CH$_2$-), 7.11 (2H, d, J = 8.9 Hz, phenyl H$_3$, H$_5$), 7.19–7.28 (2H, m, benzimidazole H$_5$, H$_6$), 7.59–7.65 (2H, m, benzimidazole H$_4$, H$_7$), 7.78 (2H, d, J = 8.8 Hz, phenyl H$_2$, H$_6$). ^{13}C NMR (75 MHz, DMSO-d$_6$, ppm) δ: 24.24 (CH$_2$), 25.89 (2CH$_2$), 42.65 (CH$_2$), 54.69 (2CH$_2$), 55.78 (OCH$_3$), 57.75 (CH$_2$), 111.25 (CH), 114.49 (2CH), 119.35 (CH), 122.21 (CH), 122.51 (CH), 123.32 (C), 131.21 (2CH), 136.17 (C), 143.13 (C), 153.83 (C), 160.09 (C). HRMS (m/z): [M + H]$^+$ calcd for $C_{21}H_{25}N_3O$: 336.2070; found: 336.2061.

3.2.11. 2-(4-Ethoxyphenyl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-benzimidazole (2h)

IR (KBr, cm^{-1}): υ_{max} 3053 (aromatic C-H stretching), 2970 (aliphatic C-H stretching), 1612–1452 (C = N and C = C stretching), 1246–1041 (C-N and C-O stretching) 850 (parasubstituted benzene). ^{1}H NMR (300 MHz, DMSO-d$_6$, ppm) δ: 1.31-1.39 (9H, m, piperidine -CH$_2$-, -OCH$_2$C$\underline{\text{H}}_3$), 2.21 (4H, br s, piperidine -CH$_2$-), 2.57 (2H, t, J = 6.5 Hz, -CH$_2$-), 4.12 (2H, q, J = 7 Hz, -OC$\underline{\text{H}}_2$CH$_3$), 4.35 (2H, t, J = 6.5 Hz, -CH$_2$-), 7.09 (2H, d, J = 8.8 Hz, phenyl H$_2$, H$_6$), 7.19–7.28 (2H, m, benzimidazole H$_5$, H$_6$), 7.59–7.65 (2H, m, benzimidazole H$_4$, H$_7$), 7.76 (2H, d, J = 8.8 Hz phenyl H$_3$, H$_5$). ^{13}C NMR (75 MHz, DMSO-d$_6$, ppm) δ: 15.06 (OCH$_2$$\underline{\text{C}}H_3$), 24.24 ($CH_2$), 25.89 ($2CH_2$), 42.62 ($CH_2$), 54.67 ($2CH_2$), 57.75 ($CH_2$), 63.73 ($O\underline{C}H_2CH_3$), 111.24 (CH), 114.90 (2CH), 119.35 (CH), 122.20 (CH), 122.50 (CH), 123.18 (C), 131.21 (2CH), 136.16 (C), 143.13 (C), 153.86 (C), 159.96 (C). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{27}N_3O$: 350.2227; found: 350.2215.

3.3. Pharmacology

3.3.1. Animals

Experiments were carried out with adult Wistar rats (body weight: 250–350 g) that were housed in well-ventilated and thermoregulated rooms in a cycle of dark (12 h) and light (12 h) at a temperature of 24 ± 1 °C. Twelve hours before the experimental session began, animals received only drinking water in order to prevent food interference with the absorption of the compounds. Animals were brought to the laboratory at least 48 h before the experiments to ensure that they acclimatized to the environment. The experimental protocol of this study was approved by the Local Ethics Committee on Animal Experimentation of Anadolu University, Turkey.

3.3.2. Administration of drugs and chemical compounds

Animals were randomly divided into ten groups: control group, reference group (morphine sulfate), and test groups **2a**–**2h**. Each of the experimental groups consisted of seven rats.

Test compounds were dissolved in sunflower oil and administered orally (p.o.) at a dose of 10 mg/kg.[28] The control solution was sunflower oil since test compounds were dissolved in it. The reference drug (morphine sulfate, 5 mg/kg) was intraperitoneally injected at a volume of 0.1 mL.[55]

Experiments were performed 30 min after the administration of morphine and 60 min after the administration of the control solution and the test compounds.

3.3.3. Nociceptive tests

3.3.3.1. Hot-plate test

The hot-plate test was performed using a hot/cold plate device (Ugo-Basile, 37100, Verase, Italy) that consisted of an aluminum plate and a Plexiglas compartment (20 × 25 cm) settled on it. Reactions of animals against thermal stimuli were evaluated by recording the time between placement of rats with all four paws on the plate maintained at a constant temperature of 55 ± 1 °C and their first hind paw licking and/or jumping behavior. After each session, the plate was cleaned with ethanol to eliminate the odor of the previous rat. A cut-off time of 40 s was set as the maximum stimuli period in order to avoid possible tissue damage.[56,57]

3.3.3.2. Paw-pressure test

The paw-pressure test was performed using a Randall–Selitto analgesy-meter (Ugo-Basile, 37215), as previously described by Bujalska-Zadrożny et al.[58] This device is used to apply incremental pressure at a constant rate (32 g/s) to the dorsal surface of the rat's paw. The nociceptive threshold was defined as the force (g) at the time the rat attempted to withdraw its hind paw. A cut-off limit of 480 g was determined to avoid tissue damage.

The data obtained from the hot plate and Randall–Selitto tests were expressed as a percentage of the maximum possible effect (MPE) using the following equation:[59]

$$\text{MPE\%} = (\text{postdrug value} - \text{predrug value}) / (\text{cut-off value} - \text{predrug value}) \times 100.$$

3.3.3.3. Formalin test

The formalin test was performed by subcutaneous administration of 5% formalin solution (in a volume of 100 μL) into the plantar region of the right hind paw of the animal. The duration of time spent licking or biting the injected paw was measured every 5 min. Following the formalin injection, the first 0–10 min was accepted as the "early phase" or "acute phase" and the next 10–45 min was accepted as the "late phase" or "prolonged tonic response".[60]

Inhibition of nociceptive response was calculated by the following equation:

Inhibition% = [(control group − treated group) / control group] × 100.

3.3.4. Motor coordination tests

Motor deficits of the animals were evaluated with a rotarod test device (Ugo Basile, 7560) having five disks forming four equal sections between them. The rotating mill was adjusted at 16 rpm.

Rats were subjected to pretraining for 3 consecutive days to be acclimatized. On the day of the experiment, animals were placed on a rotating mill 60 min after the drug administrations and falling latencies from the mill were recorded automatically by the device.[61,62] Endurance time on the treadmill was accepted as a parameter for motor coordination. Task time-out was chosen as 300 s.

3.4. Statistical evaluation

GraphPad Prism for Windows version 6.01 (GraphPad Software, San Diego, CA, USA) was used for the statistical evaluation. Analysis of the experimental data was performed using one-way ANOVA with Tukey's post hoc test. The results were presented as mean ± standard error of the mean (SEM). $P < 0.05$ was considered as statistically significant.

3.5. Theoretical calculation of ADME parameters

In order to evaluate ADME profiles of the synthesized compounds, some physicochemical parameters were calculated using the Molinspiration property calculation program (http://www.molinspiration.com/services/properties.html).

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