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Tezin Yabancı Dilde Adı:	Designing New Benzimidazole Derivatives, Docking Studies, Synthesis and Investigation of Anti-Inflammatory, Antioxidant Activities
Anahtar Kelimeler:	Benzimidazol, Sentez, Anti-enflamatuvar, Antioksidan
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Introduction

Inflammation is a protective response of the nonspecific immune system that serves to localize, neutralize, or destroy an injurious agent to prepare for the healing process. It is a local reaction of the vascular and supporting elements of a tissue to injury that results in the formation of protein-rich exudates. Rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functio laesa (loss of function) are the primary indicators of inflammation. Physical, chemical, immunological, and pathogenic organism infections are all potential causes of inflammation.[1] There are two types of inflammation: acute and chronic. The exudation of fluid and plasma proteins (oedema) and the emigration of leukocytes, primarily neutrophils, are hallmarks of acute inflammation. Chronic inflammation is seen as inflammation that lasts for a long time (weeks or months), during which time there is concurrent active inflammation, tissue destruction, and attempts at healing. Chronic inflammation includes some of the most prevalent and debilitating human diseases, including rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases.[2]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used as the first line of treatment for a variety of inflammatory illnesses like arthritis and rheumatisms as well as to alleviate ordinary aches and pains.[3] Classical NSAIDs exhibit their action by restricting the biosynthesis of prostaglandin, some of which are pro-inflammatory. This is essentially brought about by inhibiting the rate-limiting cyclooxygenase (COX) enzyme involved in the inflammatory cascade.[4] Among different types of NSAIDs, imidazole and fused imidazole with six-membered rings,[5] occupy a central position among those compounds that are used as analgesic and anti-inflammatory agents. Reactive oxygen species (ROS), including hydroxyl radicals, hydrogen peroxide, and superoxide anions, are created by cytochrome P450s and other common biological processes (CYPs).[6]

Under stress, the body produces more ROS, resulting in oxidative stress, which can damage cell membranes, membrane lipids, and nucleic acids. This process has been observed in aging pathogens and diseases such as atherosclerosis, cancer, and Alzheimer's disease.[7,8]

A superfamily of enzymes known as CYPs is responsible for oxidizing a variety of xenobiotics. CYP1A1/2 can convert aromatic amines and polycyclic aromatic hydrocarbons into their final, mutagenic, or carcinogenic metabolites. Additionally, the EROD activity-catalysing enzyme CYP1A1/2 can produce ROS.[6] Lipid peroxidation is a reaction that takes place when free radicals such as oxyl, peroxyl, and hydroxyl radicals strip electrons from lipids, producing reactive intermediates that can be employed in subsequent reactions.[9] Since antioxidants are essential for shielding living organisms from excessive ROS and there is a significant scientific interest in

producing various forms of antioxidants for medical treatment, removing free radicals and related species has attracted a lot of attention in recent years.

Benzimidazole and its derivatives are an important class of bioactive molecules in the field of drugs and pharmaceuticals. They work against a wide range of diseases [10,11], such as antitumor [12,13], antidiabetic [14], anti-inflammatory [15], antiviral [16], anticoagulant [15], antifungal [16,17], antimicrobial [15-18] anticonvulsant, antidepressant, antileishmanial, antioxidant, antihypertensive [19,20].

The structural optimization of benzimidazole derivatives has resulted in several potent drugs that are now being used in the market, including Albendazole (inhibitor of Encephalitozoon Intestinalis infection in AIDS patients), Omeprazole (proton pump inhibitor), Pimobendan (ionodilator), and Mebendazole (anthelmintic). As a result, the preparation of benzimidazole has received a lot of attention in recent years.

Materials and methods

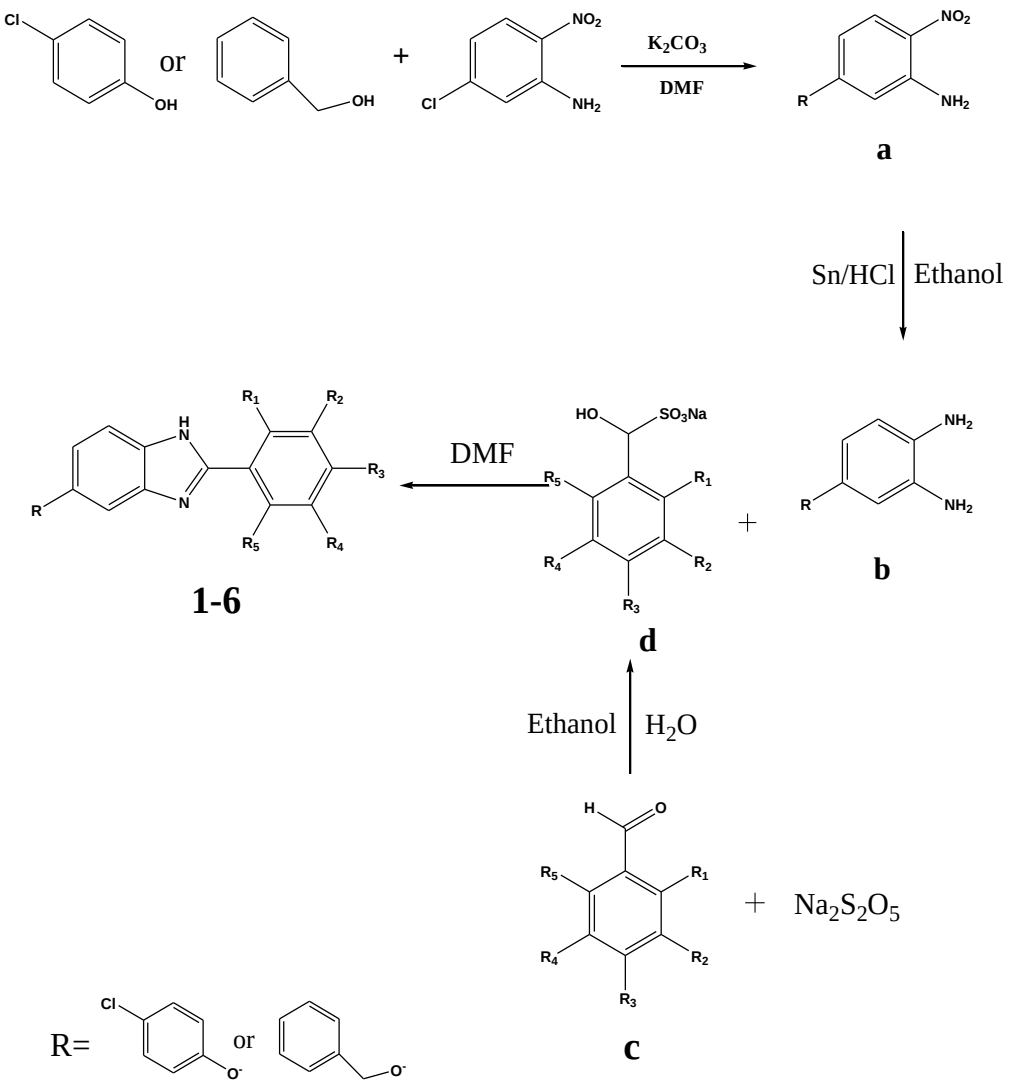
1. Synthesis of compounds 1-6

4-Chloro-phenol or benzyl alcohol was reacted with 2-nitro-5-chloroaniline in DMF in the presence of K_2CO_3 and 5-(benzyloxy) or 5-(4-chlorophenoxy)-2-nitroaniline **a** was obtained.[21-23]

5-(Benzyloxy) or 5-(4-chlorophenoxy)-2-nitroaniline **a** was dissolved in ethanol and reduced in the medium with Sn/HCl and stirred at room temperature for 30 minutes to obtain 4-(benzyloxy) or 4-(4-chlorophenoxy)-ortho-phenylenediamine derivatives **b**.

For the synthesis of 5-substituted-2-substituted-1H-benzimidazole derivatives 1-6, different benzaldehydes were converted to Na-bisulfite salt. To prepare these salts, first of all, aldehydes **c** was dissolved in 50 mL EtOH, and sodium bisulfite (1.6 g) was dissolved in water (10 mL) and then they were added together to a cooling solution in portions, stirred vigorously, and a little bit EtOH was added and cooled. The precipitate was filtered off and dried.

The sodium metabisulfite salts of the respective substituted benzaldehydes **d** were prepared as described above, and these salts were heated in DMF with 4-benzyloxy-ortho-phenylenediamine or 4-(4-chlorophenyloxy)-ortho-phenylenediamine **b** at 135 ^{0}C for 4 hours, the reaction medium was cooled and poured into water. The precipitate was filtered and dried, the solid was collected and purified by column chromatography.[24]



Scheme 1: Synthesis of compounds **1-6**.

Compounds	R	R_1	R_2	R_3	R_4	R_5
1	benzyloxy	H	H	$-OCH_3$	H	H
2	benzyloxy	$-OCH_3$	H	H	H	H
3	benzyloxy	F	H	H	F	H
4	benzyloxy	H	H	CN	H	H
5	4-chlorophenoxy	H	$-OCH_3$	$-OCH_3$	H	H
6	benzyloxy	Cl	H	H	H	Cl

6-(Benzyloxy)-2-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazole (1)

Light brown powder; Yield: 80%, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.88 (3H, s, -OCH_3), 5.23 (2H, s, -OCH_2-), 7.21-7.34 (5H, m, ArH), 7.37-7.41 (2H, m, ArH), 7.47 (1H, s, ArH), 7.49 (1H, d, J_m=1.6, ArH), 7.70 (1H, d, J_o=8.8, ArH), 8.13 (2H, dd, J_o=9.2, J_m=2.0, ArH), 12.50 (1H, bs, NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.78, 70.12, 97.63, 110.88, 113.76, 114.58, 115.27, 116.06, 125.82, 127.69, 128.49, 129.69, 132.37, 136.60, 148.38, 157.02, 163.21. ESI-MS m/z: 331 ($[M+1]^+$, 100%). $C_{21}H_{18}N_2O_2$.

6-(Benzyloxy)-2-(2-methoxyphenyl)-1*H*-benzo[*d*]imidazole (2)

Beige powder; Yield: 90 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.02 (3H, s, -OCH_3), 5.16 (2H, s, -OCH_2-), 6.92 (1H, dd, J_o=8.90, J_m=1.92, ArH), 7.10 (1H, t, ArH), 7.22 (2H, d, J_o=6.64, ArH), 7.36 (1H, t, ArH), 7.41-7.53 (6H, m, ArH), 8.30 (1H, dd, J_o=5.16, Jm=1.00, ArH), 11.99 (1H, bs, NH). ESI-MS m/z: 331 ($[M+1]^+$, 100%). $C_{21}H_{18}N_2O_2$.

6-(Benzyloxy)-2-(2,5-difluorophenyl)-1*H*-benzo[*d*]imidazole (3)

Off-white powder; Yield: 75 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm, 5.14 (2H, s, -OCH_2-), 7.34-7.55 (5H, m, ArH), 7.53 (1H, s, ArH), 7.54 (1H, d, J_m=1.2, ArH), 7.68-7.71 (2H, m, ArH), 7.84 (1H, d, J_o=9.2, ArH), 8.03-8.07 (1H, m, ArH), 12.49 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 70.42, 97.74, 111.02, 113.87, 115.61, 116.73, 117.85, 119.58, 122.37, 126.26, 128.01, 128.82, 132.77, 136.83, 142.17, 154.87, 157.32, 157.92, 159.68. ESI-MS m/z: 337 ($[M]^+$, 100%). $C_{20}H_{14}F_2N_2O$.

4-(6-(Benzyloxy)-1H-benzo[d]imidazol-2-yl)benzonitrile (4)

Yellow powder; Yield: 95 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.27 (2H, s, -OCH_2-), 7.29-7.34 (3H, m, ArH), 7.35-7.41 (2H, t, ArH), 7.42-7.51 (2H, d, ArH), 7.80 (1H, d, J_o=7.2, ArH), 8.19 (2H, dd, J_o=5.4, J_m=1.6, ArH), 8.32 (2H, dd, J_o=5.4, J_m=1.60, ArH), 14.70 (1H, s, NH).

^{13}C NMR (100MHz, DMSO-d_6): δ ppm 70.51, 97.91, 112.01, 114.30, 115.30, 115.72, 116.59, 117.70, 118.27, 118.88, 127.01, 127.88, 128.40, 128.72, 128.91, 133.43, 136.92, 146.87, 157.90. ESI-MS m/z: 326 ($[M+1]^+$, 100%). $C_{21}H_{15}N_3O$.

6-(4-Chlorophenoxy)-2-(3,4-dimethoxyphenyl)-1*H*-benzo[*d*]imidazole (5)

Brown powder; Yield: 75 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.86 (6H, d, J=15.56, -OCH_3), 6.93-7.00 (2H, ArH), 7.13 (2H, d, J_o=6.72, ArH), 7.32 (1H, s, ArH), 7.40 (2H, d, J_m=1.96, ArH), 7.54-7.75 (3H, m, ArH), 12.83 (1H, d, NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 56.17, 103.85, 110.85, 112.60, 114.80, 115.52, 118.55, 120.53, 122.27, 128.22, 130.32, 132.72, 149.76, 149.98, 153.70, 154.95, 156.16, 172.32. ESI-MS m/z: 381 ($[M+1]^+$, 100%). $C_{21}H_{17}ClN_2O_3$.

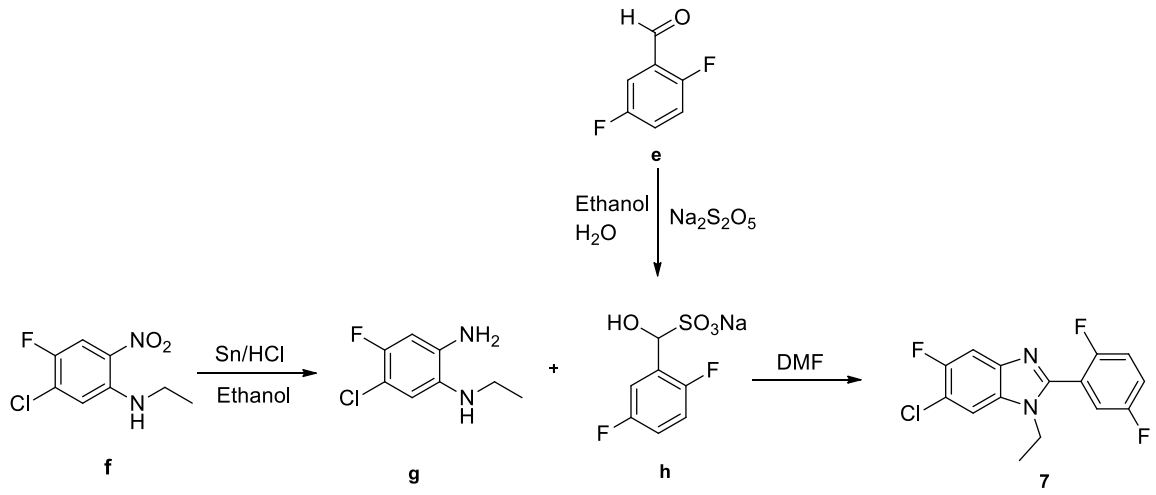
6-(Benzyloxy)-2-(2,6-dichlorophenyl)-1*H*-benzo[*d*]imidazole (6)

Light yellow powder; Yield: 85 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.18 (2H, s, -O<u>CH$_2$-</u>), 6.98 (1H, dd, J_o=7, J_m=1.92, ArH), 7.20 (1H, d, J_m=1.6, ArH), 7.34 (1H, t, J=5.88, ArH), 7.41 (2H, t, J_o=6.16, ArH), 7.50 (2H, d, J_o=5.64, ArH), 7.54 (1H, d, J_o=7.04, ArH), 7.60 (1H, t, J_o=5.56, ArH), 7.66 (2H, dd, J_o=6.88, J_m=1.2, ArH).

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 60.70, 69.99, 70.23, 70.47, 70.82, 113.18, 128.03, 128.20, 128.80, 128.91, 130.95, 132.80, 135.62, 137.90, 146.63, 155.37. ESI-MS m/z: 369 ([M]$^+$, 100%). $C_{20}H_{14}Cl_2N_2O$.

2. Synthesis of compound 7

The sodium metabisulfite salt of the 2,5-difluorobenzaldehyde was prepared from 2,5-Difluorobenzaldehyde as method mentioned above, then heated in DMF with 5-chloro-N^1-ethyl-4-fluorobenzene-1,2-diamine at 135 ^{0}C for 4 hours, the reaction medium was cooled and poured into water. The precipitate was filtered and dried, the solid was collected and purified by column chromatography.



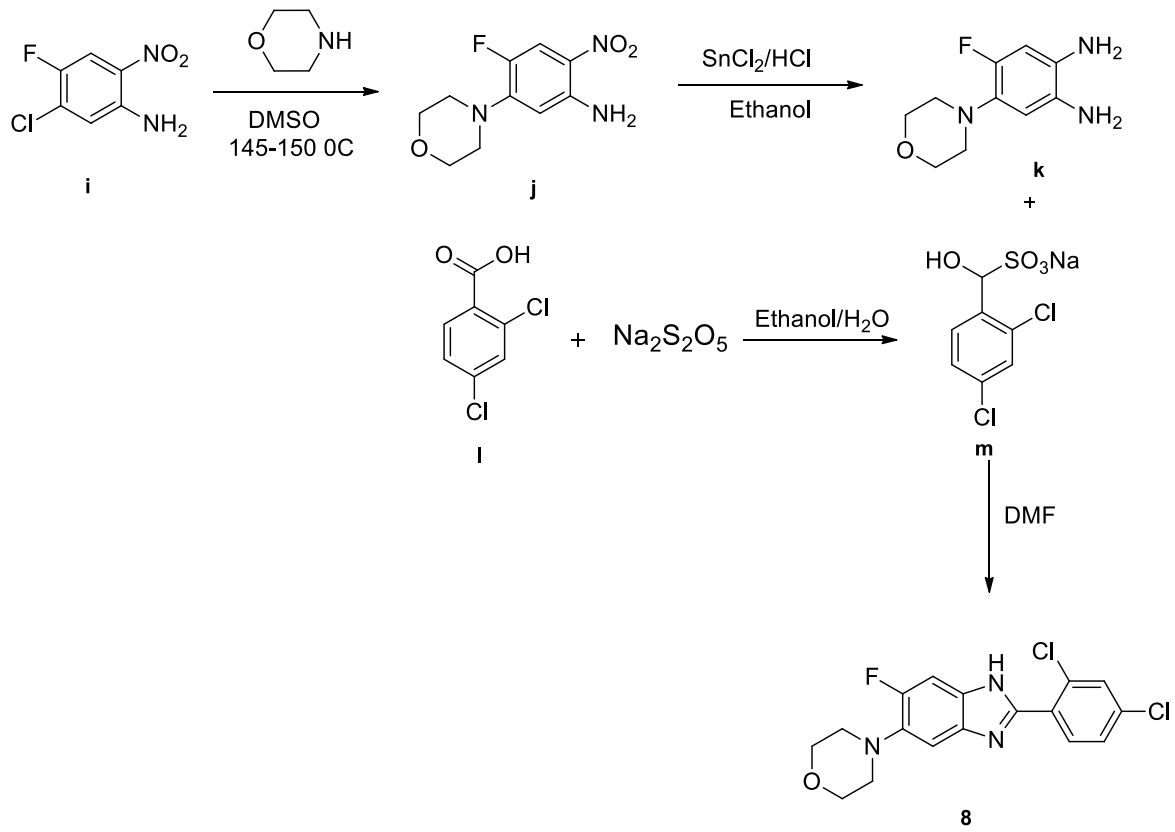
Scheme 2: Synthesis of compounds **7**.

6-Chloro-2-(2,5-difluorophenyl)-1-ethyl-5-fluoro-1H-benzo[d]imidazole (7)

Beige powder; Yield: 80 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.22 (3H, t, J_o=5.7, CH_3), 4.18 (2H, q, J_o=5.7, CH_2), 7.55 (2H, m, ArH), 7.60 (1H, m, ArH), 7.78 (1H, d, J_o=7.84, ArH), 8.09 (1H, d, J_o=5.36, ArH). ESI-MS m/z: 311 ($[M+1]^+$, 100%). $C_{15}H_{10}ClF_3N_2$.

3. Synthesis of compound 8

5-Chloro-4-fluoro-2-nitroaniline **i** (1 mmol) dissolved in 10ml DMSO and 1mmol from morpholine was added and the mixture was heated at 145-150 ^{0}C. The product **j** was isolated and dissolved in ethanol then reduced in the medium with Sn/HCl at room temperature for 30 minutes to obtain 4-chloro-5-fluorobenzene-1,2-diamine **k.** 4-Chloro-5-fluorobenzene-1,2-diamine **k** was heated in DMF with sodium metabisulfite salt of the 2,4-dichlorobenzaldehyde **m** at 135 ^{0}C for 4 hours, the reaction medium was cooled and poured into water. The precipitate was filtered and dried, the solid was collected and purified by column chromatography.



Scheme 2: Synthesis of compounds **8**.

4-(2-(2,4-dichlorophenyl)-6-fluoro-1H-benzo[d]imidazol-5-yl)morpholine (8)

Light yellow powder; Yield: 50 %, ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.076 (4H, s, -NCH_2-), 3.782 (4H, s, -OCH_2-), 7.38 (1H, d, J_o=7.20, ArH), 7.743 (1H, s, ArH), 7.77 (1H, dd, J_o=8.40, J_m=2.40, ArH), 7.95 (1H, d, J=7.95, ArH), 7.97 (1H, ds, J_m=1.6, ArH), 14.80 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 51.29, 66.38, 102.14, 103.64, 122.29, 126.58, 128.78, 130.70, 133.89, 134.27, 138.88, 140.86, 145.48, 153.91, 156.36. ESI-MS m/z: 366 ($[M]^+$, 100%). $C_{17}H_{14}Cl_2FN_3O$.

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