

Molecular Dynamic Study and Synthesis of 1H-benzo[d]imidazole-5-carboxamide Derivatives as Inhibitors for Yellow Fever and Zika Virus Replication

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ABSTRACT

Flaviviridae family comprises the flavivirus genotype that represents a significant world health problem as it includes the Yellow fever virus (YFV) and Zika virus (ZIKV) which are responsible for large outbreaks and for which novel therapies are in urgent demand. The benzimidazole scaffold has been widely reported for its antiviral activity, and hence a new series of 1H-benzo[d]imidazole-5-carboxamide derivatives (**VIIa-x**, **VIIIa-h** & **IXa, b**) was designed, synthesized, and biologically evaluated for their antiviral activity. Five Compounds (**VIIId**, **VIIe**, **VIIIh**, **VIIIn**, and **VIIIi**) showed antiviral activity against YFV in the low micromolar range using the human hepatoma Huh-7 cells and Vero cells. One compound (**VIIId**) exhibited activity on both YFV ($EC_{50} = 1.7 \pm 0.8 \mu\text{M}$) and ZIKV ($EC_{50} = 4.5 \pm 2.1 \mu\text{M}$). Molecular docking and molecular dynamics simulation studies were conducted to understand the SAR of newly synthesized compounds, to explore the potential target of compound **VIIId**, and to investigate the possible binding mode to its target.

Keywords: 1H-benzo[d]imidazole; Flavivirus; Yellow Fever virus; Zika virus; Molecular dynamics simulation.

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1. INTRODUCTION

The *Flaviviridae* family comprises a large number of genera representing a major cause of public health problems, mainly the Hepacivirus genus, the Flavivirus genus, and the Pestivirus genus [1–3]. The Hepacivirus genus includes, amongst others, the hepatitis C virus (HCV), and the pestivirus genus includes the bovine viral diarrhea virus (BVDV) [2]. The Flavivirus genus comprises more than 53 members including those

causing large outbreaks in the human population: Dengue virus (DENV), Yellow Fever virus (YFV), and Zika virus (ZIKV).

Flaviviruses began to spread about 75,000 years ago due to human migration from Africa [4]. According to the world health organization (WHO), 200,000 new YFV cases occur annually with outbreaks recorded in 2016-2017 in several countries in Africa and the Americas [5–7]. As for ZIKV, an estimated 440,000–1,300,000 cases

were recorded in late 2015 during the Brazilian outbreak, and as a result of an increase in ZIKV-associated microcephaly cases in 2016, the WHO declared ZIKV a Public Health Emergency of International Concern [8, 9].

Despite the availability of a highly efficacious YFV vaccine (17D vaccine) the recent large YFV outbreaks clearly show that antivirals against YFV and all flaviviruses are desperately needed. Aiming to decrease the viremia during early infection and block the viral replication and growth, various viral proteins are targeted [10]. The flaviviral proteins are classified into structural and non-structural proteins. The structural proteins include the capsid protein (C), the envelope protein (E), and the membrane protein (M) [11–13]. The non-structural proteins include: NS1, NS2A, NS2B, NS3 (serine protease/ helicase enzyme), NS4A, NS4B, NS5

(methyltransferase/ polymerase enzyme) [14–19].

The benzimidazole nucleus has been reported to be involved as a structural unit in many bioactive molecules, such as anticancer agents^[20], antioxidants [21], antihypertensives [22], immunomodulatory [23], anti-inflammatory agents [24], CNS stimulants as well as anti-depressants, [25] antiparasitic agents, [26] antidiabetics, [27] antimicrobials [28] and antivirals [29]. It is worth noting that most of the reported biologically active compounds comprising a benzimidazole nucleus to have their functional groups at positions 1,2 and/or 5 (or 6) [25, 30, 31].

Various benzimidazole-based compounds were reported to be active leads in the search for new antiviral agents acting against *Flaviviridae* family members especially yellow fever virus and HCV (Fig. 1) [32–35].

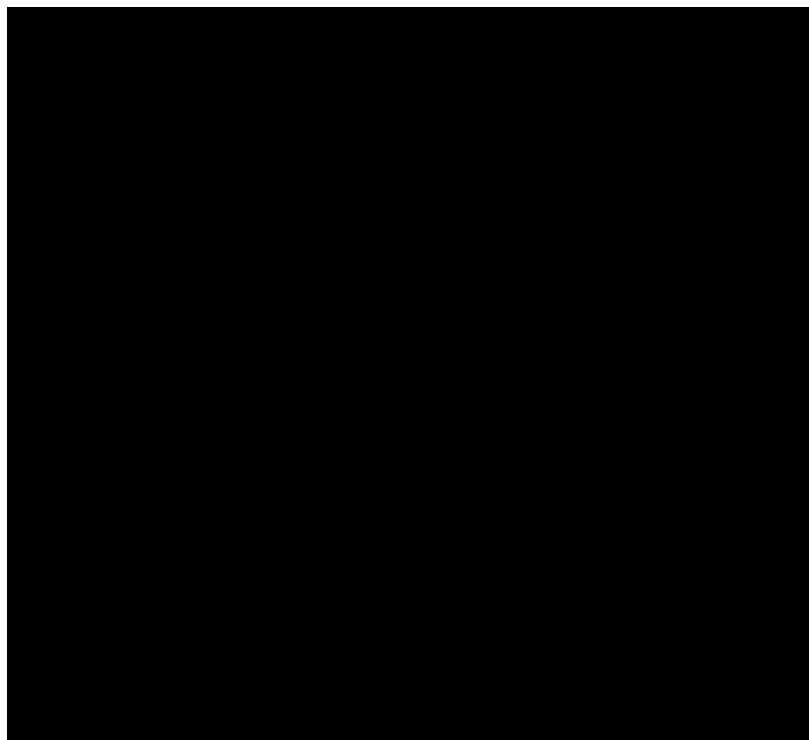


Fig.1. Benzimidazole-based reported anti-*Flaviviridae* compounds

Herein, a variety of 1H-benzo[d]imidazole-5-carboxamide derivatives (**VIIa-x**, **VIIIa-h** & **IXa, b**) were synthesized and biologically evaluated for their anti-YFV activity. Besides, molecular modeling studies (docking and molecular dynamics) of compound **VIIId** which exhibited antiviral activity against both YFV (on Huh-7 and VeroA cells) and ZIKV were conducted to gain insight into its antiviral mechanism. These studies suggested that **VIIId** may be a ZIKV polymerase inhibitor.

1.1. Rationale and Design

G. Vitale has reported -as a part of the anti-Flaviviridae Project- several derivatives of benzimidazole-based structures which were divided into four series: arylbenzimidazoles, naphthyl benzimidazoles, **[36, 37]** styrylbenzimidazoles **[38]** and 5-acetyl-2-arylbenzimidazoles **[35]**. These compounds were assayed against different members of the Flaviviridae family, YFV (*Flaviviruses*), BVDV (*Pestiviruses*), and HCV (*Hepacivirus*). Many of the compounds showed very good anti-YFV with EC_{50} values ranging from 0.5-27 μM (**Fig. 2**).

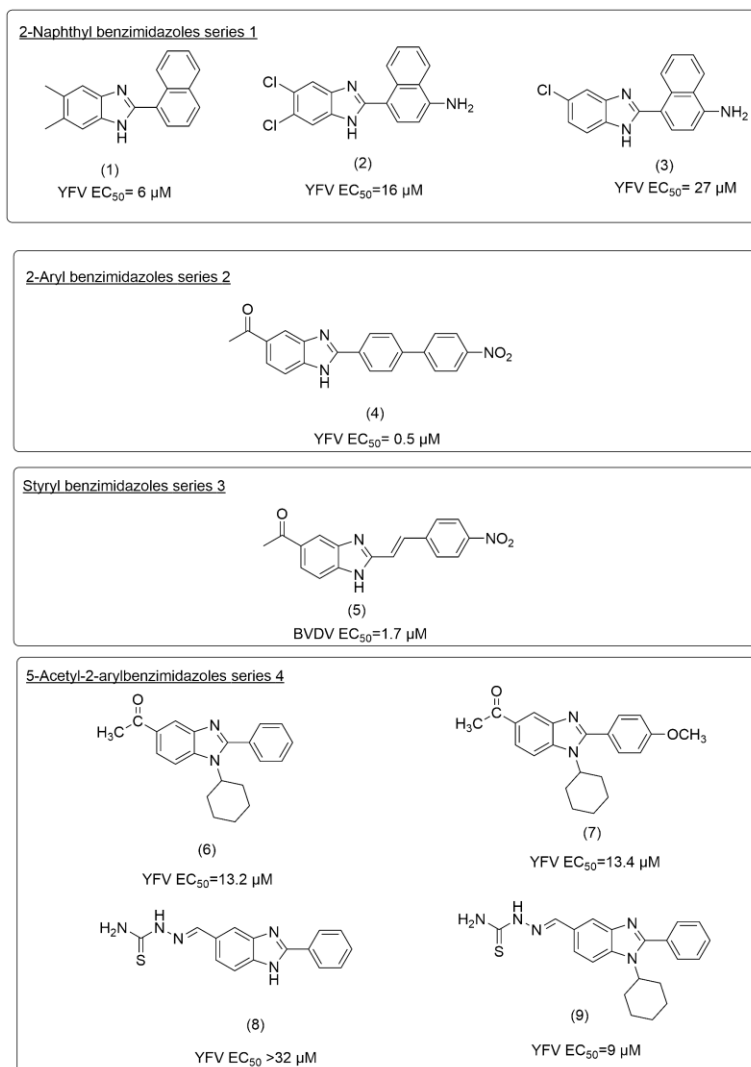


Fig. 2. Benzimidazole-based compounds showing activity against Flaviviridae family

The first series (2-naphthyl benzimidazoles) evaluated the effect of different substituents at positions 5 and 6 of the benzimidazole ring, nearly 3 compounds (**1-3**) showed activity on YFV.

The second series, (2-aryl benzimidazoles), replacing the 2-naphthalene moiety in series 1 with a 2-[4-R-biphenyl] moiety. Only compound (**4**) showed anti-YFV activity with scarce chance to conclude a SAR.

As for the third series, (styrylbenzimidazoles), the phenyl ring was separated from the benzimidazole nucleus by an ethylene spacer, and compounds with various substituents at position 5, 6 of the benzimidazole nucleus and the phenyl ring were tested. All compounds from this series showed no activity against YFV. Compound **5** displayed the highest antiviral activity against BVDV among all active derivatives.

The fourth series (5-acetyl-2-arylbenzimidazoles) showed the best anti-Flaviviridae activity with about 9 compounds showing antiviral activity ranging between 0.8-8 μM on YFV, BVDV, and HCV representing the three genera of *Flaviviridae* family (Flavivirus, Pestivirus, and Hepacivirus) respectively.

SAR studies within the reported compounds demonstrated that:

- 1) 5-Acetyl group improved the anti-BVDV and anti-YFV activity due to its electronegativity.
- 2) 5-Acetyl group derivatizations to ketoximes, semicarbazones, and thiosemicarbazones improved the potency.
- 3) Cyclohexyl substituent introduction at position 1 improved the anti-YFV activity better than the unsubstituted derivative and that was obvious in compounds **8** and **9**.

Based on the SAR study of the previously

reported compounds, in the present study, we were able to design, synthesize and biologically evaluate novel series of benzimidazole derivatives targeting the *Flaviviridae* family (especially YFV and ZIKV).

Our structure-based design strategy was based on:

- 1) Keeping the benzimidazole nucleus which is beneficial for anti-viral activity.
- 2) Keeping the cyclohexyl ring in the N1 position.
- 3) Exploration of various substituents on the 2-phenyl ring compared to the unsubstituted derivatives.
- 4) Exploration of various substituents on 5 positions of benzimidazole ring.

Accordingly, three series of compounds were designed based on the modification of the lead compound **4**, which possesses high potency to be synthesized and biologically evaluated against YFV and ZIKV as shown in (**Fig. 3**).

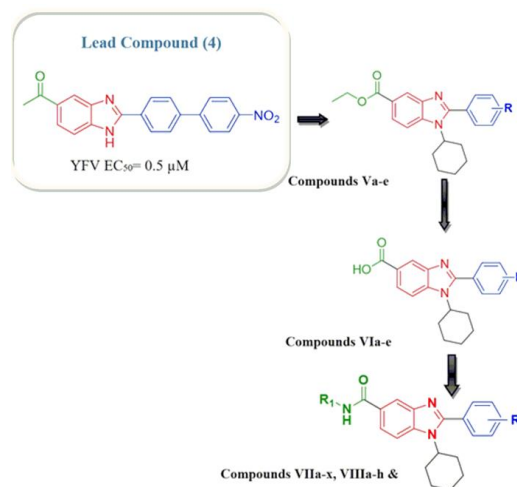


Fig. 3. Design of new anti-Flavivirus agents by bioisosteric modifications of lead compound 4

2. MATERIALS AND METHODS

2.1. Chemistry

Starting materials and reagents were purchased from Sigma-Aldrich (USA), Loba Chemie (India), and Alfa-Aesar organics and used without further purification. Solvents were purchased from Fisher Scientific and Sigma-Aldrich and utilized after drying. Reactions were monitored by analytical TLC, performed on silica gel 60 F₂₅₄ packed on Aluminum sheets, purchased from Merck, visualized under U.V. light (254 nm). For flash chromatography, Merck silica gel 60 was used with a particle size (230-400 mesh). Melting points were measured on the Stuart Scientific apparatus. ¹HNMR spectra were recorded in δ scale given in ppm on a Bruker 400 MHz and referred to TMS as an internal reference and ¹³CNMR spectra were recorded on a Bruker 101 MHz at the Center of Drug Discovery and Research Development, Ain Shams University. EI-MS spectra and Elemental analyses were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

2.1.1. General procedure to prepare Intermediates I-III

The starting compound (4-chlorobenzoic acid) was purchased from Sigma-Aldrich (USA) and used as it is. 4-Chlorobenzoic acid (6.24 g, 40 mmol) was dissolved in 20 mL concentrated sulfuric acid portion-wise in an ice bath at 0 °C, then 20 mL of concentrated nitric acid was added dropwise for 30 minutes at 0 °C. The reaction mixture was left to stir at room temperature for 3 h, then poured dropwise on ice to give a white precipitate. The precipitate was filtered and washed with excess water to get rid of excess acids and left to dry to give the titled compound **I** as a white powder. To a solution of compound **1** (6 g, 29.8 mmol) in 48 mL ethanol, 6 mL of concentrated sulfuric acid was added dropwise while in an ice bath at 0 °C. Then the reaction mixture was stirred at 70 °C for 24 h. After the reaction was completed, it was poured on

ice/water to give an off-white precipitate. The precipitate was neutralized with sodium carbonate then filtered and washed with excess water and left to dry. The product was crystallized from ethanol to give the titled compound **II** as an off-white powder. For the preparation of compound **III**, cyclohexylamine (9.35 mL, 93.3 mmol, 3 equivalents) and triethylamine (9.3 g) were added to a solution of compound **II** (7.14 g, 31.1 mmol, 1 equivalent) in DMSO (23 mL). The reaction mixture was then stirred at 90 °C for 4 h. After completion of the reaction, the reaction mixture was poured dropwise on ice with vigorous stirring to give a yellow precipitate. The precipitate was filtered and washed with water and diethyl ether, then left to dry and then crystallized from n-hexane to yield the titled compound **III** as yellow solid. ⁽³⁹⁻⁴¹⁾

2.1.2. General procedure to prepare Intermediate IV

A solution of Ethyl 4-(cyclohexyl amino)-3-nitrobenzoate **III** (2 g, 6.8 mmol) and 10% Pd/C (0.2 g) in ethyl acetate (100 mL) were placed in a parr bottle and the bottle was placed in the parr hydrogenator apparatus, flushed 3 times with hydrogen and filled with hydrogen (40 psi). The mixture was agitated at room temperature for 30 minutes during which the pressure dropped to approximately 20 psi. The bottle was again filled with hydrogen (40 psi), and agitation was continued for 4 h until no pressure drop is observed. The catalyst was removed by filtration using filter aid (celite) and the filtrate was evaporated under vacuum till complete dryness then crystallized from the diethyl ether to give the titled compound **IV** as brown crystals [42].

2.1.3. General procedure to prepare Intermediate compounds Vad

To a solution of compound **III** (3 g, 10.27 mmol, 1 equivalent) in 30 mL DMSO,

benzaldehyde derivative (1.1 equivalent) and sodium dithionite (5.3 g, 30.81 mmol, 3 equivalents) was added. The reaction mixture was stirred at 90 °C for 7 h. After completion of the reaction, the reaction was poured onto ice-cold water with vigorous stirring where precipitate was formed. The precipitate was then filtered and crystallized from the appropriate solvent to yield target compounds **Va-d**.

2.1.3.1. Ethyl 1-cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxylate (Va)

The product was crystallized from ethanol to yield the titled compound **Va** as white powder (2.7 g, 72.78%); m.p. 128-132 °C as reported [43]; ¹HNMR (400 MHz, DMSO-*d*₆) δ 8.29 (d, *J* = 1.5 Hz, 1H, benzimidazole H), 8.05 (m, 2H, ArH), 7.93 (dd, *J* = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.66 (d, *J* = 8 Hz, 1H, benzimidazole H), 7.64 (m, 3H, ArH), 4.32 (q, *J* = 7.1 Hz, 2H, ester), 4.04 (q, 1H, cyclohexyl), 2.39–2.13 (m, 2H, cyclohexyl), 2.00–1.79 (m, 4H, cyclohexyl), 1.69–1.53 (m, 1H, cyclohexyl), 1.36 (t, *J* = 7.1 Hz, 3H, ester), 1.27 (m, 3H, cyclohexyl); FT-IR(U max, cm⁻¹): 3124 (CH aromatic), 2937 (CH aliphatic), 1698(C=O ester); MS (Mwt = 348.44), *m/z* (% rel. int.): 348.19 (M⁺, 91%), 349.2 (M⁺, 25.3%); Anal. Calcd for C₂₂H₂₄N₂O₂: C, 75.83; H, 6.94; N, 8.04; Found: C, 76.14; H, 7.03; N, 8.22.

2.1.3.2. Ethyl 1-cyclohexyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (Vb)

The product was crystallized from hexane to yield the titled compound **Vb** as an off-white powder (2.8 g, 72.11%); m.p. 114-118 °C; ¹HNMR (400 MHz, DMSO-*d*₆) δ 8.24 (d, *J* = 1.5 Hz, 1H, benzimidazole H), 7.90 (dd, *J* = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.82 (dd, *J* = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.60 (d, *J* = 8 Hz, 1H, benzimidazole H), 7.14 (dd, *J* = 8.6, 1.7, 2H, 4-OMe ArH) 4.32 (q, *J* = 7.1 Hz, 2H, ester), 4.04

(q, 1H, cyclohexyl), 3.86 (s, 3H, methoxy), 2.39–2.13 (m, 2H, cyclohexyl), 2.00–1.79 (m, 4H, cyclohexyl), 1.69–1.53 (m, 1H, cyclohexyl), 1.36 (t, *J* = 7.1 Hz, 3H, ester), 1.27 (m, 3H, cyclohexyl); MS (Mwt= 378.46), *m/z* (% rel. int.): 378.19 (M⁺, 91%), 379.2 (M⁺, 25.3%); Anal. Calcd for C₂₃H₂₆N₂O₃: C, 72.99; H, 6.92; N, 7.40; Found: C, 73.11; H, 7.03; N, 7.51.

2.1.3.3. Ethyl 1-cyclohexyl-2-(2-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (Vc)

The product was crystallized from hexane to yield the titled compound **Vc** as an off-white powder (2.7 g, 67%); m.p. 108-110 °C; ¹HNMR (400 MHz, DMSO-*d*₆) δ 8.23 (d, *J* = 1.5 Hz 1H, benzimidazole H), 7.92 (dd, *J* = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.86 (d, *J* = 8.9 Hz, 1H, 2-O Me ArH), 7.63–7.55 (d, *J* = 8 Hz 1H, benzimidazole H), 7.46 (d, *J* = 7.3 Hz, 1H, 2-OMe ArH), 7.24 (d, *J* = 8.4 Hz, 1H, 2-OMe ArH), 7.14 (t, *J* = 7.6 Hz, 1H, 2-OMe ArH), 4.32 (q, *J* = 7.1 Hz, 2H, ester), 4.04 (q, 1H, cyclohexyl), 3.81 (s, 3H, methoxy), 2.39–2.13 (m, 2H, cyclohexyl), 2.00–1.79 (m, 4H, cyclohexyl), 1.69–1.53 (m, 1H, cyclohexyl), 1.36 (t, *J* = 7.1 Hz, 3H, ester), 1.27 (m, 3H, cyclohexyl); MS (Mwt= 378.46), *m/z* (% rel. int.): 378.22 (M⁺, 82 %), 379.2 (M⁺, 25.7%); Anal. Calcd for C₂₃H₂₆N₂O₃: C, 72.87; H, 7.11; N, 7.45; Found: C, 73.11; H, 7.03; N, 7.51.

2.1.3.4. Ethyl 1-cyclohexyl-2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (Vd)

The product was crystallized from hexane to yield the titled compound **Vd** as an off-white powder (2.5 g, 64.43%); m.p. 164-165 °C; ¹HNMR (400 MHz, DMSO-*d*₆) δ 10.00 (s, 1H, OH D₂O exchangeable), 8.2 (d, *J* = 1.5 Hz 1H, benzimidazole H), 8.11 (dd, *J* = 8.7, 2.8 Hz, 2H, 4-OH ArH), 7.93 (dd, *J* = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.85 (dd, *J* = 8.8, 2.8 Hz, 2H,

4-OH ArH), 7.56 – 7.51 (d, $J = 8$ Hz 1H, benzimidazole H), 4.32 (q, $J = 7.1$ Hz, 2H, ester), 4.2 (q, 1H, cyclohexyl) 2.39–2.13 (m, 2H, cyclohexyl), 2.00 – 1.79 (m, 4H, cyclohexyl), 1.69 – 1.53 (d, $J = 12.5$ Hz, 1H, cyclohexyl), 1.36 (t, $J = 7.1$ Hz, 3H, ester), 1.27 (m, 3H, cyclohexyl); **MS** (Mwt = 364.44), m/z (% rel. int.): 364.22 (M^+ , 82 %), 365.2 (M^+ , 24.7%); **Anal.** Calcd for $C_{22}H_{24}N_2O_3$: C, 72.50; H, 6.64; N, 7.69; Found: C, 73.08; H, 6.79; N, 7.91.

2.1.3.5. Ethyl 1-cyclohexyl-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylate (Ve)

A solution of Ethyl 3-amino-4-(cyclohexylamino) benzoate **IV** (0.5 g, 1.8 mmol, 1 equivalent), 4-nitrobenzaldehyde (0.2 g, 1.8 mmol, 1 equivalent) and Na acetate (0.15 g, 1.8 mmol, 1 equivalent) in absolute ethanol was stirred at 70 °C for 4 h, the precipitate was filtered, washed with diethyl ether and left to dry to give the titled compound **Ve** as an orange powder. The product was crystallized from hexane to yield the titled compound **Ve** as orange powder. yield: 93.35%; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 8.36 (dd, $J = 8.7, 1.7$ Hz, 2H, 4-NO₂ArH), 8.30 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.02 (dd, $J = 8.6, 1.7$ Hz, 2H, 4-NO₂ArH), 7.80–7.77 (dd, $J = 8.5, 1.5$ Hz, 1H, benzimidazole H), 7.74 (d, $J = 8$ Hz, 1H, benzimidazole H), 4.96 (p, 1H, cyclohexyl), 4.26 (q, $J = 7.1$ Hz, 2H, ester), 2.23 (m, 2H, cyclohexyl), 1.96 (d, $J = 10.0$ Hz, 2H, cyclohexyl), 1.73 (m, 2H, cyclohexyl), 1.62 (d, 1H, cyclohexyl), 1.38 (d, $J = 10.0$ Hz, 3H, cyclohexyl), 1.31 (t, $J = 7.1$ Hz, 3H, ester); **MS** (Mwt= 393.44), m/z (% rel. int.): 393.19 (M^+ , 82%), 394.20 (M^+ , 24.9%); **Anal.** Calcd for $C_{22}H_{23}N_3O_4$: C, 67.16; H, 5.89; N, 10.68; Found: C, 67.43; H, 6.08; N, 11.03.

2.1.4. General procedure to prepare Intermediates VIa-d

To a solution of LiOH.H₂O (1.17 g, 28.1 mmol, 2 equivalents) in ethanol (50%, 100 mL),

the respective ethyl ester **Va-d** (14.08 mmol, 1 equivalent) was added. The mixture was stirred under reflux for 2 h. The resulting solution was cooled to 0 °C in an ice bath and 10% HCl was added dropwise with stirring till precipitate was formed. The precipitate was filtered, washed with water, and recrystallized from diethyl ether to yield the titled compounds **VIa-d** as white powders.

2.1.4.1. 1-Cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxylic acid (VIa)

The titled compound **VIa** was separated as white crystals (3.2 g, 71%); m.p. 270 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 13-12.5 (s, 1H, COOH D₂O exchangeable) 8.26 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 7.97 (dd, $J = 8.7, 1.5$ Hz, 1H, benzimidazole H), 7.88 (dd, $J = 8.5, 1.7$ Hz, 2H, ArH), 7.67 (d, $J = 8$ Hz, 1H, benzimidazole H), 7.61 (d, $J = 3.7$ Hz, 3H, ArH), 4.27 (q, $J = 8.5$ Hz, 1H, cyclohexyl), 2.35–2.20 (m, 2H, cyclohexyl), 1.95–1.88 (m, 2H, cyclohexyl), 1.84 (m, 2H, cyclohexyl), 1.64 (d, $J = 12.5$ Hz, 1H, cyclohexyl), 1.32–1.11 (m, 3H, cyclohexyl); **FT-IR (U max, cm⁻¹):** 3449-2980 (OH carboxylic), 3010 (CH aromatic), 2936 (CH aliphatic), 1686 (C=O acid); **MS** (Mwt= 320.38), m/z (% rel. int.): 320.15 (M^+ , 92 %), 321.16 (M^+ , 25.3%); **Anal.** Calcd for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74; Found: C, 75.11; H, 6.42; N, 9.02.

2.1.4.2. 1-Cyclohexyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (VIb)

The titled compound **VIb** was separated as white crystals (3.3 g, 72.74%); m.p. 298 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 12.76 (s, 1H, COOH D₂O exchangeable), 8.24 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 7.90 (dd, $J = 8.7, 1.5$ Hz, 1H, benzimidazole H), 7.82 (dd, $J = 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 7.60 (d, $J = 8$ Hz, 1H, benzimidazole H), 7.14 (dd, $J = 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 4.29 (q, $J = 8.5$ Hz, 1H, cyclohexyl), 3.86 (s, 3H, methoxy) 2.27 (m, 2H,

cyclohexyl), 1.97–1.78 (m, 4H, cyclohexyl), 1.63–1.58 (d, 1H, cyclohexyl), 1.29 (d, 3H, cyclohexyl); **MS** (Mwt= 350.41), *m/z* (% rel. int.): 350.22 (M^+ , 87 %), 351.19 (M^+ , 23.7%); **Anal.** Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99; Found: C, 72.16; H, 6.52; N, 8.22.

2.1.4.3. 1-Cyclohexyl-2-(2-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (VIc)

The titled compound **VIc** was separated as white crystals (3.1 g, 68.33%); m.p. 292–293 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.84 (s, 1H, COOH D₂O exchangeable), 8.23 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 7.92 (dd, *J*= 8.8, 1.5 Hz, 1H, benzimidazole H), 7.86 (d, *J*= 8.9 Hz, 1H, 2-OMe ArH), 7.63 – 7.55 (d, *J*= 8 Hz, 1H, benzimidazole H), 7.46 (d, *J*= 7.3 Hz, 1H, 2-OMe ArH), 7.24 (d, *J*= 8.4 Hz, 1H, 2-OMe ArH), 7.14 (t, *J*= 7.6 Hz, 1H, 2-OMe ArH), 4.37 (q, 1H, cyclohexyl), 3.81 (s, 3H, methoxy), 2.29 – 2.06 (m, 2H, cyclohexyl), 1.82 (m, 4H, cyclohexyl), 1.62 (d, *J*= 12.4 Hz, 1H, cyclohexyl), 1.21 (m, 3H, cyclohexyl); **MS** (Mwt= 350.41), *m/z* (% rel. int.): 350.16 (M^+ , 84%), 351.17 (M^+ , 23.1%); **Anal.** Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99; Found: C, 72.11; H, 6.41; N, 8.09.

2.1.4.4. 1-Cyclohexyl-2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (VIId)

The titled compound **VIId** was separated as white crystals (3 g, 66.3%); m.p. 340 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H, COOH D₂O exchangeable), 10.00 (s, 1H, OH D₂O exchangeable), 8.21 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 8.11 (dd, *J*= 8.7, 2.8 Hz, 2H, 4-OH ArH), 7.91 (dd, *J*= 8.6, 1.5 Hz, 1H, benzimidazole H), 7.85 (dd, *J*= 8.8, 2.8 Hz, 2H, 4-OH ArH), 7.56–7.51 (d, *J*= 8 Hz, 1H, benzimidazole H), 4.27 (q, 1H, cyclohexyl), 2.39 – 2.21 (m, 2H, cyclohexyl), 1.9–1.79 (m, 4H, cyclohexyl), 1.69 – 1.62 (d, *J*= 12.5 Hz, 1H, cyclohexyl), 1.25 (m, 3H, cyclohexyl); **MS** (Mwt= 336.38), *m/z* (% rel. int.): 336.16 (M^+ , 88

%), 337.17 (M^+ , 22.9%); **Anal.** Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33; Found: C, 71.91; H, 6.22; N, 8.59.

2.1.4.5. 1-Cyclohexyl-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid (VIe)

Yield: 47.38% To a solution of LiOH.H₂O (0.1 g, 2.53 mmol, 2 equivalents) in methanol (66%, 100 mL), the respective ethyl ester **Ve** (0.5 g, 1.26 mmol, 1 equivalent) was added. The mixture was heated under reflux for 24 h. The resulting solution was cooled to 0 °C in ice bath and anhydrous glacial acetic acid was added drop wise with stirring then poured on ice with vigorous stirring till precipitate was formed. The precipitate was filtered, washed with water and crystallized from diethyl ether to yield the titled compound **VIe** as red powder. 1H NMR (400 MHz, DMSO- d_6) δ 12.8 (s, 1H, COOH D₂O exchangeable), 8.36 (dd, *J*= 8.7, 1.7 Hz, 2H, 4-NO₂ArH), 8.30 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 8.02 (d, *J*= 8.7, 1.7 Hz, 2H, 4-NO₂ArH), 7.80 – 7.77 (dd, *J*= 8.5, 1.5 Hz, 1H, benzimidazole H), 7.74 (d, *J*= 8 Hz, 1H, benzimidazole H), 4.96 (p, 1H, cyclohexyl), 2.23 (m, 2H, cyclohexyl), 1.96 (d, *J*= 10.0 Hz, 2H, cyclohexyl), 1.73 (m, 2H, cyclohexyl), 1.62 (d, 1H, cyclohexyl), 1.38 (d, *J*= 10.0 Hz, 3H, cyclohexyl); **MS** (Mwt = 365.38), *m/z* (% rel. int.): 365.16 (M^+ , 71 %), 366.17 (M^+ , 14.1%); **Anal.** Calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50; Found: C, 65.86; H, 5.39; N, 11.62.

2.1.5. General procedure to prepare compounds (VIIa-x)

A suspension of the appropriate acid derivative **VIa-e** (3.14 mmol, 1 equivalent) in dry DCM (15 mL) was cooled to 0 °C in an ice bath and thionyl chloride (5 mL, 4 equivalent) was added dropwise with stirring, the reaction mixture was stirred under reflux for 2–4 h. The solvent was then evaporated under vacuum giving brownish solid of the respective acid

chloride that was used directly without further purification.

To a stirring solution of the appropriate acid chloride (1 mmol, 1 equivalent) in dry DCM (20 mL) at 0 °C, a solution of appropriate amine (4.39 mmol, 1.4 equivalent) (cyclohexylamine, aniline, benzylamine, 2-methyl-4-nitro aniline, 3,4-dichloro aniline, 2-phenylethylamine, isobutyl amine, 4-fluoro aniline, 3-chloro aniline, and butylamine), respectively, for compounds (**VIIa-j**) and cyclohexylamine, aniline, benzylamine, 2-methyl-4-nitro aniline, isobutyl amine, and cyclopropyl amine, respectively, for compounds (**VIIk-p**) and aniline, benzylamine, 2-methyl-4-nitro aniline, 3,4-dichloro aniline, 2-phenylethylamine), respectively, for compounds (**VIIq-u**) and cyclohexylamine, aniline, and benzylamine, for compounds (**VIIv-x**) in dry DCM (2 mL) and 4 drops of TEA was added dropwise. Then the ice bath was removed, and the reaction is left to stir at room temperature for 48-72 h. The reaction mixture was poured to ice/H₂O/10% HCl with vigorous stirring where precipitate was formed. The precipitate was filtered and purified using flash chromatography (the system used: hexane: ethyl acetate= 6:1 then changed to 4:1 and finally 2:1) to give the titled compounds (**VIIa-u**). As for compounds (**VIIv-x**), the flash chromatography system used was hexane: ethyl acetate= 9:1 then changed to 8:1 and finally 6:1.

2.1.5.1. N,1-Dicyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (**VIIa**)

The titled compound **VIIa** is white powder (0.3 g, 76.71%); m.p. 222-224 °C (charring); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.25 (s, 1H, NH D₂O exchangeable), 8.20 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 7.89 (dd, *J*= 8.6, 1.5 Hz, 1H, benzimidazole H), 7.80 (dd, *J*= 8.7, 1.7 Hz, 1H, ArH), 7.70-7.63 (d, *J*= 8 Hz, 1H, benzimidazole H), 7.61-7.58 (m, 4H, ArH), 4.27 (p, 1H, cyclohexyl), 3.81 (q, *J*= 7.6 Hz, 1H, cyclohexyl),

2.35-2.20 (m, 2H, cyclohexyl), 1.95 - 1.80 (m, 6H, cyclohexyl), 1.75 (dd, *J*= 9.5, 3.8 Hz, 2H, cyclohexyl), 1.65-1.58 (m, 2H, cyclohexyl), 1.42 - 1.13 (m, 8H, cyclohexyl); **MS** (Mwt= 401.5), *m/z* (% rel. int.): 401.25 (M⁺, 79%), 402.25 (M⁺, 28.51%); **Anal.** Calcd for C₂₆H₃₁N₃O: C, 77.77; H, 7.78; N, 10.46; Found: C, 77.91; H, 7.89; N, 10.58.

2.1.5.2. 1-Cyclohexyl-N,2-diphenyl-1H-benzo[d]imidazole-5-carboxamide (**VIIb**)

The titled compound **VIIb** is white powder (0.25 g, 64.9%); m.p. 100-102°C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.25 (s, 1H, NH D₂O exchangeable), 8.41 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 8.00 (dd, *J*= 8.6, 1.5 Hz, 1H, benzimidazole H), 7.91 (d, *J*= 8 Hz, 1H, benzimidazole H), 7.84 (d, *J*= 8.0 Hz, 2H, ArH), 7.69 (dd, *J*= 6.6, 3.1 Hz, 2H, NH-ArH), 7.62 (q, *J*= 2.9 Hz, 3H, ArH), 7.37 (t, *J*= 7.7 Hz, 2H, NH-ArH), 7.11 (t, *J*=7.5 Hz, 1H, NH-ArH), 4.37-4.23 (m, 1H, cyclohexyl), 2.37-2.23 (m, 2H, cyclohexyl), 2.02-1.79 (m, 4H, cyclohexyl), 1.65 (d, *J*= 12.3 Hz, 1H, cyclohexyl), 1.33 - 1.19 (m, 3H, cyclohexyl); **MS** (Mwt= 395.5), *m/z* (% rel. int.): 395.2 (M⁺, 81%), 396.2 (M⁺, 29.3%); **Anal.** Calcd for C₂₆H₂₅N₃O: C, 78.96; H, 6.37; N, 10.62; Found: C, 79.12; H, 6.45; N, 10.87.

2.1.5.3. N-Benzyl-1-cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (**VIIc**)

The titled compound **VIIc** is white powder (0.29 g, 72.71%); m.p. 184-186 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.08 (s, 1H, NH D₂O exchangeable), 8.29 (d, *J*= 1.5 Hz, 1H, benzimidazole H), 7.94 (dd, *J*= 8.7, 1.5 Hz, 1H, benzimidazole H), 7.85 (d, *J*= 8 Hz, 1H, benzimidazole H), 7.71-7.55 (m, 5H, ArH), 7.35 (d, *J*= 4.6 Hz, 4H, ArH), 7.26 (d, *J*= 7.2 Hz, 1H, ArH), 4.53 (d, *J*= 5.6 Hz, 2H, benzyl), 4.37 - 4.15 (m, 1H, cyclohexyl), 2.29 (q, *J*= 12.3 Hz, 2H, cyclohexyl), 1.98-1.75 (m, 4H, cyclohexyl), 1.63 (d, *J*= 12.0 Hz, 1H, cyclohexyl), 1.32 (dd,

$J = 52.6, 11.6$ Hz, 3H, cyclohexyl); **MS** (Mwt= 409.52), m/z (% rel. int.): 409.22 (M^+ , 94%), 410.22 (M^{+1} , 29.6%); **Anal.** Calcd for $C_{27}H_{27}N_3O$: C, 79.19; H, 6.65; N, 10.26; Found: C, 79.05; H, 6.81; N, 10.49.

2.1.5.4. 1-Cyclohexyl-N-(2-methyl-4-nitrophenyl)-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIId)

The titled compound **VIId** is white powder (0.3 g, 67.77%); m.p. 124-126 °C; 1H NMR (400 MHz, DMSO- d_6) δ 10.09 (s, 1H, NH D₂O exchangeable), 8.43 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.21 (s, 1H, 2-Me-4-NO₂ArH), 8.14 (d, $J = 8.9$ Hz, 1H, 2-Me-4-NO₂ArH), 8.03 (d, $J = 8.7$ Hz, 1H, 2-Me-4-NO₂ArH), 7.92 (dd, $J = 8.6, 1.8$ Hz, 1H, benzimidazole H), 7.87 (d, $J = 8$ Hz, 1H, benzimidazole H), 7.72–7.67 (m, 2H, ArH), 7.64–7.60 (m, 3H, ArH), 4.31 (p, $J = 5.8$ Hz, 1H, cyclohexyl), 2.45 (s, 3H, methyl), 2.31 (dt, $J = 16.8, 7.3$ Hz, 2H, cyclohexyl), 2.00 – 1.91 (m, 2H, cyclohexyl), 1.87 (d, $J = 13.1$ Hz, 2H, cyclohexyl), 1.66 (d, $J = 12.3$ Hz, 1H, cyclohexyl), 1.34–1.22 (m, 2H, cyclohexyl), 0.86 (td, $J = 8.4, 7.7, 2.7$ Hz, 1H, cyclohexyl). ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.3, 155.5, 144.5, 143.3, 140.4, 137.2, 134.3, 130.8, 130.5, 129.9, 129.3, 127.9, 126.1, 125.9, 122.7, 122.0, 120.0, 113.5, 57.3, 31.0, 26.0, 24.8, 18.4; **MS** (Mwt= 454.5), m/z (%rel. int.): 455.2 (M^+ , 36.8%), 454.21 (100%); **Anal.** Calcd for $C_{27}H_{26}N_4O_3$: C, 71.35; H, 5.77; N, 12.33; Found: C, 71.18; H, 5.89; N, 12.6.

2.1.5.5. 1-Cyclohexyl-N-(3,4-dichlorophenyl)-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIe)

The titled compound **VIIe** is white powder (0.4 g, 88.44%); m.p. 136-138 °C (charring); 1H NMR (400 MHz, DMSO- d_6) δ 10.51 (s, 1H, NH D₂O exchangeable), 8.42 (d, $J = 1.6$ Hz, 1H, ArH), 8.23 (d, $J = 1.5$ Hz, 1H, benzimidazole H),

8.03 (d, $J = 8.6$ Hz, 1H, ArH), 7.90 (dd, $J = 8.5, 1.5$ Hz, 1H, benzimidazole H), 7.83 (dd, $J = 9.0, 2.5$ Hz, 1H, ArH), 7.71–7.68 (d, $J = 8$ Hz, 1H, benzimidazole H), 7.64 (s, 1H, ArH), 7.62 (d, $J = 4.0$ Hz, 4H, ArH), 4.46–4.12 (m, 1H, cyclohexyl), 2.31 (td, $J = 13.7, 10.0$ Hz, 2H, cyclohexyl), 1.98 – 1.91 (m, 2H, cyclohexyl), 1.9 – 1.81 (m, 2H, cyclohexyl), 1.74–1.54 (m, 1H, cyclohexyl), 1.53–1.19 (m, 3H, cyclohexyl); ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.5, 155.5, 143.2, 140.1, 136.6, 131.3, 130.9, 130.8, 130.5, 129.9, 129.3, 128.2, 125.3, 122.6, 121.8, 120.7, 119.8, 113.4, 57.3, 31.0, 26.0, 24.8; **MS** (Mwt= 464.39), m/z (% rel. int.): 465.08 (M^+ , 30.51%), 303.15 (100%); **Anal.** Calcd for $C_{26}H_{23}Cl_2N_3O$: C, 67.25; H, 4.99; N, 9.05; Found: C, 67.49; H, 5.12; N, 9.21.

2.1.5.6. 1-Cyclohexyl-N-phenethyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIf)

The titled compound **VIIIf** is white powder (0.28 g, 67.88%); m.p. 176-178 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.60 (s, 1H, NH D₂O exchangeable), 8.22 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 7.92 (dd, $J = 8.6, 1.5$ Hz, 1H, benzimidazole H), 7.79 (d, $J = 8.5$ Hz, 1H, ArH), 7.68 – 7.65 (d, $J = 8$ Hz, benzimidazole H), 7.62 – 7.59 (m, 4H, ArH) 7.39–7.23 (m, 4H, ArH), 7.21 (t, $J = 7.1$ Hz, 1H, ArH), 4.27 (p, $J = 6.5$ Hz, 1H, cyclohexyl), 3.53 (q, $J = 6.8$ Hz, 2H, NHCH₂CH₂), 2.89 (t, $J = 7.4$ Hz, 2H, NHCH₂CH₂), 2.37 – 2.21 (m, 2H, cyclohexyl), 1.97 – 1.80 (m, 4H, cyclohexyl), 1.63 (d, $J = 12.4$ Hz, 1H, cyclohexyl), 1.25 (d, $J = 13.5$ Hz, 3H, cyclohexyl); **MS** (Mwt= 423.55), m/z (% rel. int.): 423.23 (M^+ , 76%), 424.23 (M^{+1} , 31.4%); **Anal.** Calcd for $C_{28}H_{29}N_3O$: C, 79.4; H, 6.9; N, 9.92; Found: C, 79.63; H, 7.04; N, 10.13.

2.1.5.7. 1-Cyclohexyl-N-isobutyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIg)

The titled compound **VIIIf** is white powder (0.27 g, 73.83%); m.p. 118-120 °C; 1H NMR

(400 MHz, DMSO- d_6) δ (ppm) 8.48 (t, J = 5.8 Hz, 1H, NH D₂O exchangeable), 8.24 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.91 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.80 (dd, J = 8.6, 1.7 Hz, 1H, ArH), 7.69–7.65 (d, J = 8 Hz, 1H, benzimidazole H), 7.62 – 7.55 (m, 4H, ArH), 4.27 (p, J = 6.8 Hz, 1H, cyclohexyl), 3.13 (d, J = 7.0 Hz, 2H, isobutyl), 2.29 (q, 2H, cyclohexyl) 2.27 (m, 1H, isobutyl), 1.96–1.81 (m, 4H, cyclohexyl), 1.64 (d, J = 12.4 Hz, 1H, cyclohexyl), 1.46–1.32 (m, 1H, cyclohexyl), 1.27 (ddd, J = 16.8, 8.5, 3.5 Hz, 2H, cyclohexyl), 0.91 (d, J = 6.7 Hz, 6H, isobutyl); **MS** (Mwt = 375.51), m/z (% rel. int.): 375.23 (M^+ , 79%), 376.23 (M^{+1} , 27.1%); **Anal.** Calcd for C₂₄H₂₉N₃O: C, 76.76; H, 7.78; N, 11.19; Found: C, 76.95; H, 7.90; N, 11.34.

2.1.5.8. 1-Cyclohexyl-N-(4-fluorophenyl)-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIIh)

The titled compound **VIIIh** is white powder (0.3 g, 74.5%); m.p. 112-114 °C; **¹H NMR** (400 MHz, DMSO- d_6) δ (ppm) 9.06 (s, 1H, NH D₂O exchangeable), 8.26 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.91 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.82 (d, J = 8 Hz, 1H, benzimidazole H), 7.61 (d, J = 8.7 Hz, 2H, 4-F ArH), 7.37–7.33 (m, 4H, ArH), 7.28–7.23 (m, 1H, ArH), 7.15 (d, J = 8.7 Hz, 2H, 4-F ArH), 4.33 – 4.24 (m, 1H, cyclohexyl), 2.37 – 2.23 (m, 2H, cyclohexyl), 1.88 (t, J = 15.7 Hz, 4H, cyclohexyl), 1.65 (d, J = 12.3 Hz, 1H, cyclohexyl), 1.46–1.19 (m, 3H, cyclohexyl); **¹³C NMR** (101 MHz, DMSO- d_6) δ 165.3, 156.8, 143.3, 136.4, 130.9, 130.4, 129.9, 129.3, 128.7, 126.8, 122.6, 122.6, 119.6, 115.7, 115.5, 113.3, 57.3, 31.1, 26.0, 24.9; **MS** (Mwt= 413.49), m/z (%rel. int.): 413.09 (M^+ , 10%), 303.00 (100%); **Anal.** Calcd for C₂₆H₂₄FN₃O: C, 75.52; H, 5.85; N, 10.16; Found: C, 75.68; H, 5.94; N, 10.37.

2.1.5.9. N-(3-Chlorophenyl)-1-cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide

(VIIIi)

The titled compound **VIIIi** is white powder (0.32 g, 76.42%); m.p. 114-116 °C; **¹H NMR** (400 MHz, DMSO- d_6) δ (ppm) 10.42 (s, 1H, NH D₂O exchangeable), 8.41 (d, J = 1.5 Hz, 1H, benzimidazole H), 8.04 (m, 1H, ArH), 8.01 (s, 1H, 3-Cl ArH), 7.90 (dd, J = 8.6, 1.6 Hz, 1H, benzimidazole H), 7.78 (d, J = 8.3 Hz, 1H, 3-Cl ArH), 7.72 – 7.67 (d, J = 8 Hz 1H, benzimidazole H), 7.64 – 7.60 (m, 4H, ArH), 7.40 (t, J = 8.1 Hz, 1H, 3-Cl ArH), 7.16 (d, J = 8.2 Hz, 1H, 3-Cl ArH), 4.30 (p, J = 6.8 Hz, 1H, cyclohexyl), 2.41 – 2.18 (m, 2H, cyclohexyl), 1.94 (d, J = 11.9 Hz, 2H, cyclohexyl), 1.86 (d, J = 13.1 Hz, 2H, cyclohexyl), 1.65 (d, J = 12.5 Hz, 1H, cyclohexyl), 1.35–1.20 (m, 3H, cyclohexyl); **MS** (Mwt= 429.94), m/z (% rel. int.): 429.16 (M^+ , 54%), 430.16 (M^{+1} , 29.2%), 431.16 (M^{+2} , 32.3%); **Anal.** Calcd for C₂₆H₂₄ClN₃O: C, 72.63; H, 5.63; N, 9.77; Found: C, 72.80; H, 5.75; N, 9.89.

2.1.5.10. N-Butyl-1-cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIj)

The titled compound **VIIj** is white powder (0.26 g, 71.09%); m.p. 88-90 °C; **¹H NMR** (400 MHz, DMSO- d_6) δ 8.46 (s, 1H, NH D₂O exchangeable), 8.22 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.91 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.79 (d, J = 8.5 Hz, 1H, ArH), 7.68–7.64 (d, J = 8 Hz, 1H, benzimidazole H), 7.63–7.58 (m, 4H, ArH), 4.26 (p, J = 6.8 Hz, 1H, cyclohexyl), 3.30 (d, J = 6.5 Hz, 2H, butyl), 2.36–2.22 (m, 2H, cyclohexyl), 1.95–1.80 (m, 4H, cyclohexyl), 1.68–1.60 (m, 2H, butyl), 1.54 (t, J = 7.4 Hz, 1H, cyclohexyl), 1.35 (q, J = 7.1 Hz, 2H, butyl), 1.25 (d, J = 15.3 Hz, 3H, cyclohexyl), 0.92 (t, J = 7.2 Hz, 3H, butyl); **MS** (Mwt= 375.51), m/z

(%rel. int.): 375.23 (M^+ , 72%), 376.23 (M^{+1} , 27.1%); **Anal.** Calcd for $C_{24}H_{29}N_3O$: C, 76.76; H, 7.78; N, 11.19; Found: C, 76.59; H, 7.94; N, 11.32.

2.1.5.11. N,1-Dicyclohexyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIk)

The titled compound **VIIk** is white powder (0.27 g, 69.93%); m.p. 120-122 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.21 (s, 1H, NH D₂O exchangeable), 8.17 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.86 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.76 (dd, J = 8.5, 1.7 Hz, 1H, benzimidazole H), 7.60 (d, J = 8 Hz, 1H, benzimidazole H), 7.15 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 4.27 (p, J = 6.6 Hz, 1H, cyclohexyl), 3.87 (s, 3H, methoxy), 3.80 (m, 1H, cyclohexyl), 2.29 (m, 2H, cyclohexyl), 1.94 – 1.82 (m, 6H, cyclohexyl), 1.77 (d, J = 9.5 Hz, 2H, cyclohexyl), 1.64 (m, 2H, cyclohexyl), 1.32 (dt, J = 25.0, 12.3 Hz, 6H, cyclohexyl), 1.13 (d, J = 25.3 Hz, 2H, cyclohexyl); ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.1, 160.8, 154.9, 143.3, 135.9, 131.3, 128.9, 123.1, 122.0, 118.9, 114.7, 112.8, 57.1, 55.8, 48.89, 33.0, 31.0, 26.0, 25.8, 25.5, 24.9; **MS** (Mwt= 431.57), m/z (%rel. int.): 431.22 (M^+ , 61%), 432.22 (M^{+1} , 30.33%); **Anal.** Calcd for $C_{27}H_{33}N_3O_2$: C, 75.14; H, 7.71; N, 9.74; Found: C, 75.02; H, 7.8; N, 9.87

2.1.5.12. 1-Cyclohexyl-2-(4-methoxyphenyl)-N-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIII)

The titled compound **VIII** is white powder (0.3 g, 78.8 %); m.p. 154-156 °C; 1H NMR (400 MHz, DMSO- d_6) δ 10.24 (s, 1H, NH D₂O exchangeable), 8.37 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.97 (dd, J = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.88 (d, J = 8 Hz, 1H, benzimidazole H), 7.82 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.66 – 7.59 (m, 2H, ArH), 7.36 (t,

J = 7.9 Hz, 2H, ArH), 7.19–7.15 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.10 (t, J = 7.4 Hz, 1H, ArH), 4.31 (m, 1H, cyclohexyl), 3.87 (s, 3H, methoxy), 2.31 (q, J = 12.3 Hz, 2H, cyclohexyl), 1.98 – 1.81 (m, 4H, cyclohexyl), 1.66 (d, J = 12.2 Hz, 1H, cyclohexyl), 1.49–1.21 (m, 3H, cyclohexyl); **MS** (Mwt= 425.52), m/z (% rel. int.): 425.22 (M^+ , 78%), 426.21 (M^{+1} , 30.4%); **Anal.** Calcd for $C_{27}H_{27}N_3O_2$: C, 76.21; H, 6.40; N, 9.87; Found: C, 76.33; H, 6.51; N, 9.91.

2.1.5.13. N-Benzyl-1-cyclohexyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIm)

The titled compound **VIIm** is white powder (0.29 g, 73.75%); m.p. 184-186 °C; 1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.06 (t, J = 6.0 Hz, 1H, NH D₂O exchangeable), 8.26 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.91 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.82 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.67–7.53 (d, J = 8 Hz, 1H, benzimidazole H), 7.35-7.34 (m, 4H, ArH), 7.25 (dq, J = 7.8, 2.9 Hz, 1H, ArH), 7.21–7.06 (dd, J = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 4.53 (d, J = 5.9 Hz, 2H, benzyl), 4.28 (p, J = 6.6 Hz, 1H, cyclohexyl), 3.87 (s, 3H, methoxy), 2.38 – 2.22 (m, 2H, cyclohexyl), 1.88 (t, J = 15.7 Hz, 4H, cyclohexyl), 1.65 (d, J = 12.3 Hz, 1H, cyclohexyl), 1.46–1.17 (m, 3H, cyclohexyl); **MS** (Mwt= 439.55), m/z (% rel. int.): 439.23 (M^+ , 77%), 440.23 (M^{+1} , 30.7%); **Anal.** Calcd for $C_{28}H_{29}N_3O_2$: C, 76.51; H, 6.65; N, 9.56; Found: C, 76.49; H, 6.78; N, 9.78.

2.1.5.14. 1-Cyclohexyl-2-(4-methoxyphenyl)-N-(2-methyl-4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIn)

The titled compound **VIIn** is white powder (0.32 g, 73.82%); m.p. 142-144 °C; 1H NMR (400 MHz, DMSO- d_6) δ 10.07 (s, 1H, NH D₂O exchangeable), 8.40 (d, J = 1.5 Hz, 1H, benzimidazole H), 8.21 (s, 1H, 2-Me-4-NO₂ArH), 8.13 (dd, J = 8.9 Hz, 2H, 2-Me-4-

NO₂ArH), 8.02 – 7.98 (dd, $J=8.5, 1.5$ Hz, 1H, benzimidazole H), 7.89 (dd, $J= 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 7.64 (d, $J= 8.0$ Hz, 1H, benzimidazole H), 7.17 (dd, $J= 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 4.31 (p, $J= 6.7$ Hz, 1H, cyclohexyl), 3.88 (s, 3H, methoxy), 2.45 (s, 3H, methyl), 2.32 (m, 2H, cyclohexyl), 1.99 – 1.81 (m, 4H, cyclohexyl), 1.70–1.62 (d, 1H, cyclohexyl), 1.36 – 1.22 (m, 3H, cyclohexyl); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.8, 161.2, 154.9, 140.4, 138.6, 136.0, 137.2, 133.3, 131.4, 129.1, 127.7, 126.9, 125.8, 124.9, 121.0, 119.8, 114.7, 111.8, 62.8, 55.8, 31.0, 26.0, 24.8, 18.4; MS (Mwt= 484.55), m/z (%rel. int.): 484.19 (M⁺, 75.97%); Anal. Calcd for C₂₈H₂₈N₄O₄: C, 69.41; H, 5.82; N, 11.56; Found: C, 69.52; H, 5.97; N, 11.75.

2.1.5.15. 1-Cyclohexyl-N-isobutyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIo)

The titled compound VIIo is white powder (0.25 g, 68.91%); m.p. 116-118 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.89 (t, $J= 5.8$ Hz, 1H, NH D₂O exchangeable), 8.42 (d, $J= 1.5$ Hz, 1H, benzimidazole H), 8.25 (dd, $J= 8.5, 1.5$ Hz, 1H, benzimidazole H), 8.08 – 7.86 (dd, $J= 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 7.68 (d, $J= 8$ Hz, 1H, benzimidazole H), 7.23 (dd, $J= 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 4.29 (p, $J= 6.6$ Hz, 1H, cyclohexyl), 4.14 (q, $J= 7.0$ Hz, 2H, isobutyl), 3.40 (s, 3H, methoxy), 2.34(m, 1H, isobutyl) 2.27- 2.22 (m, 2H, cyclohexyl), 1.95 – 1.81 (m, 4H, cyclohexyl), 1.64 (d, $J= 12.2$ Hz, 1H, cyclohexyl), 1.21-1.19 (m, 3H, cyclohexyl), 0.85 (d, $J= 6.7$ Hz, 6H, isobutyl); MS (Mwt= 405.53), m/z (% rel. int.): 405.21 (M⁺, 81%), 406.24 (M⁺, 28.1%); Anal. Calcd for C₂₅H₃₁N₃O₂: C, 74.04; H, 7.70; N, 10.36; Found: C, 74.21; H, 7.89; N, 10.19.

2.1.5.16. 1-Cyclohexyl-N-cyclopropyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIp)

The titled compound VIIp is white powder (0.24 g, 68.87%); m.p. 120-122 °C (charring); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.43 (d, $J= 4.2$ Hz, 1H, NH D₂O exchangeable), 8.17 (d, $J= 1.5$ Hz, 1H, benzimidazole H), 7.87 (dd, $J= 8.7, 1.5$ Hz, 1H, benzimidazole H), 7.75 (dd, $J= 8.5, 1.6$ Hz, 2H, 4-OMe ArH), 7.60 (d, $J= 8$ Hz, 1H, benzimidazole H), 7.15 (dd, $J= 8.6, 1.7$ Hz, 2H, 4-OMe ArH), 4.32 – 4.23 (m, 1H, cyclohexyl), 3.86 (s, 3H, methoxy), 2.88 (dq, $J= 7.3, 3.7$ Hz, 1H, cyclopropyl), 2.28 (q, $J= 11.9, 11.2$ Hz, 2H, cyclohexyl), 1.92–1.81 (m, 4H, cyclohexyl), 1.64 (d, $J= 12.3$ Hz, 1H, cyclohexyl), 1.27 (d, $J= 13.6$ Hz, 3H, cyclohexyl), 0.70 (dt, $J= 6.9, 3.1$ Hz, 2H, cyclopropyl), 0.63–0.58 (m, 2H, cyclopropyl); MS (Mwt= 389.49), m/z (% rel. int.): 389.21 (M⁺, 69%), 390.21 (M⁺, 27.1%); Anal. Calcd for C₂₄H₂₇N₃O₂: C, 74.01; H, 6.99; N, 10.79; Found: C, 74.04; H, 7.12; N, 10.95.

2.1.5.17. 1-Cyclohexyl-2-(2-methoxyphenyl)-N-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIq)

The titled compound VIIq is white powder (0.3 g, 78.8%); m.p. 122-124 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.23 (s, 1H, NH D₂O exchangeable), 8.39 (d, $J= 1.5$ Hz, 1H, benzimidazole H), 7.96 (d, $J= 7.9$ Hz, 1H, 2-OMe ArH), 7.92–7.87 (dd, $J= 8.5, 1.5$ Hz, 1H, benzimidazole H), 7.84 (t, $J= 7.9$ Hz, 1H, 2-OMe ArH), 7.60 (td, $J= 8.0, 1.9$ Hz, 2H, ArH), 7.47 (d, $J= 8$ Hz, 1H, benzimidazole H), 7.37 (t, $J= 7.9$ Hz, 1H, 2-OMe ArH), 7.25 (d, $J= 7.8$ Hz, 1H, 2-OMe ArH), 7.15 (t, $J= 7.5$ Hz, 2H, ArH), 7.10 (t, $J= 7.5$ Hz, 1H, ArH), 3.89 (p, $J= 6.8$ Hz, 1H, cyclohexyl), 3.83 (s, 3H, methoxy), 2.23 (m, 2H, cyclohexyl), 1.9-1.84 (m, 4H, cyclohexyl), 1.63 (d, $J= 12.5$ Hz, 1H, cyclohexyl), 1.25 (m, 3H, cyclohexyl); MS (Mwt= 425.52), m/z (% rel. int.): 425.21 (M⁺, 100%), 426.21 (M⁺, 30.4%); Anal. Calcd for C₂₇H₂₇N₃O₂: C, 76.21; H, 6.40; N, 9.87; Found: C, 76.40; H, 6.52; N, 9.98.

2.1.5.18. N-Benzyl-1-cyclohexyl-2-(2-

methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIr)

The titled compound **VIIr** is white powder (0.28 g, 71.2%); m.p. 136-138 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 9.06 (t, J = 6.1 Hz, 1H, NH D₂O exchangeable), 8.27 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.90 (d, J = 7.8 Hz, 1H, 2-OMe ArH), 7.84 (dd, J = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.59 (t, J = 7.8 Hz, 1H, 2-OMe ArH), 7.45 (d, J = 8Hz, 1H, benzimidazole H), 7.38 – 7.31 (m, 5H, ArH), 7.25 (d, J = 7.8 Hz, 1H, 2-OMe ArH), 7.14 (t, J = 7.8 Hz, 1H, 2-OMe ArH), 4.53 (d, J = 5.9 Hz, 2H, NHCH_2), 3.86 (p, J = 6.8 Hz, 1H, cyclohexyl), 3.81 (s, 3H, methoxy), 2.20 (m, 2H, cyclohexyl), 1.83 (m, 4H, cyclohexyl), 1.62 (d, J = 12.4 Hz, 1H, cyclohexyl), 1.25 (m, 3H, cyclohexyl); **MS** (Mwt= 439.55), m/z (% rel. int.): 439.23 (M^+ , 88%), 440.23 (M^+ , 30.7%); **Anal.** Calcd for C₂₈H₂₉N₃O₂: C, 76.51; H, 6.65; N, 9.56; Found: C, 76.64; H, 6.71; N, 9.70.

2.1.5.19. 1-Cyclohexyl-2-(2-methoxyphenyl)-N-(2-methyl-4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIs)

The titled compound **VIIIs** is yellow powder (0.3 g, 69.2 %); m.p. 120-122 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 10.25 (s, 1H, NH D₂O exchangeable), 8.38 (d, J = 1.5 Hz, 1H, benzimidazole H), 8.26 (s, 1H, 2-Me-4-NO₂ArH), 8.11 (d, J = 8.9 Hz, 1H, 2-Me-4-NO₂ArH), 7.97 (d, J = 8.7 Hz, 1H, 2-Me-4-NO₂ArH), 7.89 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.84 (t, J = 7.8 Hz, 1H, 2-OMe ArH), 7.61-7.58 (d, J = 8 Hz, 1H, benzimidazole H), 7.47 (d, J = 7.4 Hz, 1H, 2-OMe ArH), 7.25 (d, J = 8.4 Hz, 1H, 2-OMe ArH), 7.14 (t, J = 7.5 Hz, 1H, 2-OMe ArH), 3.89 (p, J = 6.8 Hz, 1H, cyclohexyl), 3.82 (s, 3H, methoxy), 2.45 (s, 3H, methyl), 2.27 (m, 2H, cyclohexyl), 1.91 – 1.8 (m, 4H, cyclohexyl), 1.63 (d, J = 12.5 Hz, 1H, cyclohexyl), 1.23 (d, 3H, cyclohexyl); **MS** (Mwt= 484.55), m/z (%rel. int.): 484.21 (M^+ ,

31.8%); **Anal.** Calcd for C₂₈H₂₈N₄O₄: C, 69.41; H, 5.82; N, 11.56; Found: C, 69.70; H, 5.98; N, 11.49.

2.1.5.20. 1-Cyclohexyl-N-(3,4-dichlorophenyl)-2-(2-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIt)

The titled compound **VIIt** is off-white powder (0.31 g, 70%); m.p. 198-200 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 10.49 (s, 1H, NH D₂O exchangeable), 8.40 (s, 1H, 3,4-diCl ArH), 8.24 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.97 (d, J = 8.6 Hz, 1H, 3,4-diCl ArH), 7.89 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.84 (t, J = 7.8 Hz, 1H, 2-OMe ArH), 7.63–7.62 (d, 1H, 3,4-diCl ArH), 7.61-7.58 (d, J = 8 Hz, 1H, benzimidazole H), 7.47 (d, J = 7.4 Hz, 1H, 2-OMe ArH), 7.25 (d, J = 8.4 Hz, 1H, 2-OMe ArH), 7.15 (t, J = 7.5 Hz, 1H, 2-OMe ArH), 3.89 (q, J = 6.8 Hz, 1H, cyclohexyl), 3.82 (s, 3H, methoxy), 2.22 (m, 2H, cyclohexyl), 1.91–1.72 (m, 4H, cyclohexyl), 1.63 (d, J = 12.5 Hz, 1H, cyclohexyl), 1.23 (d, 3H, cyclohexyl); $^{13}\text{C NMR}$ (101 MHz, DMSO- d_6) δ 166.5, 157.6, 153.4, 143.5, 140.1, 139.4, 136.3, 132.4, 131.7, 130.9, 127.8, 125.2, 123.7, 122.4, 121.8, 121.2, 120.7, 119.6, 112.9, 111.9, 57.5, 55.8, 31.0, 26.0, 24.8.; **MS** (Mwt= 494.4), m/z (%rel. int.): 494.79 (M^+ , 14.1%), 495.13 (M^+ , 64.3%), 493.13 (100%); **Anal.** Calcd for C₂₇H₂₅Cl₂N₃O₂: C, 65.59; H, 5.10; N, 8.5; Found: C, 65.70; H, 5.23; N, 8.6.

2.1.5.21. 1-Cyclohexyl-2-(2-methoxyphenyl)-N-phenethyl-1H-benzo[d]imidazole-5-carboxamide (VIIu)

The titled compound **VIIu** is white powder (0.32 g, 78.86 %); m.p. 90-92 °C; $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 8.55 (t, J = 5.6 Hz, 1H, NH D₂O exchangeable), 8.17 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.87 (dd, J = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.78 – 7.75 (d, J = 7.8 Hz, 1H, 2-OMe ArH), 7.59 (t, J = 7.9 Hz, 1H, 2-OMe ArH), 7.44 (d, J = 8 Hz, 1H, benzimidazole H),

7.34–7.24 (m, 5H, ArH), 7.22 (d, $J = 7.8$ Hz, 1H, 2-OMe ArH) 7.14 (t, $J = 7.9$ Hz, 1H, 2-OMe ArH), 3.84 (p, $J = 6.8$ Hz, 1H, cyclohexyl), 3.81 (s, 3H, methoxy), 3.52 (q, $J = 6.8$ Hz, 2H, NHCH₂CH₂), 2.89 (t, $J = 7.4$ Hz, 2H, NHCH₂CH₂), 2.20 (m, 2H, cyclohexyl), 1.83 (m, 4H, cyclohexyl), 1.60 (d, $J = 12.5$ Hz, 1H, cyclohexyl), 1.21 (m, 3H, cyclohexyl); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.9, 157.6, 152.9, 143.5, 140.2, 135.6, 132.4, 132.3, 129.2, 128.8, 128.4, 126.5, 121.9, 121.2, 120.0, 118.9, 112.6, 111.8, 100.0, 57.4, 55.8, 41.5, 35.7, 31.0, 26.0, 25.0; **MS** (Mwt= 453.58), m/z (%rel. int.): 453.35 (M⁺, 100%), 454.39 (M⁺¹, 32.30%); **Anal.** Calcd for C₂₉H₃₁N₃O₂: C, 76.79; H, 6.89; N, 9.26; Found: C, 76.90; H, 6.94; N, 9.45.

2.1.5.22. N,1-Dicyclohexyl-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIv)

The titled compound VIIv is buff powder (0.28 g, 63.34%); m.p. 112–114 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 8.44 (dd, $J = 8.5$, 1.7 Hz, 2H, 4-NO₂ArH), 8.29 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.24 (dd, $J = 8.5$, 1.5 Hz, 1H, benzimidazole H), 7.98 (dd, $J = 8.7$, 1.8 Hz, 2H, 4-NO₂ArH), 7.94 (s, 1H, NH D₂O exchangeable), 7.83 (d, $J = 8$ Hz, 1H, benzimidazole H), 4.27 (m, 1H, cyclohexyl), 3.80 (q, $J = 7.5$ Hz, 1H, cyclohexyl), 2.34–2.24 (m, 2H, cyclohexyl), 1.96 (m, 3H, cyclohexyl), 1.85 (m, 3H, cyclohexyl), 1.80–1.71 (m, 2H, cyclohexyl), 1.63 (d, $J = 11.4$ Hz, 2H, cyclohexyl), 1.34 (q, $J = 11.2$, 9.6 Hz, 5H, cyclohexyl), 1.15 (d, $J = 12.5$ Hz, 1H, cyclohexyl), 0.83 (td, $J = 9.8$, 8.9, 5.1 Hz, 2H, cyclohexyl); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 157.4, 156.1, 152.9, 148.6, 136.8, 136.1, 131.4, 129.5, 124.4, 119.5, 115.4, 113.3, 57.5, 54.9, 48.9, 33.0, 31.1, 25.9, 25.8, 25.5; **MS** (Mwt= 446.5), m/z (% rel. int.): 446.11 (M⁺, 49%), 447.22 (M⁺¹, 59.76%); **Anal.** Calcd for C₂₆H₃₀N₄O₃: C, 69.93; H, 6.77; N, 12.55; Found: C, 70.1; H, 6.89; N, 12.78.

2.1.5.23. 1-Cyclohexyl-2-(4-nitrophenyl)-N-

phenyl-1H benzo[d] imidazole-5-carboxamide (VIIw)

The titled compound VIIw is buff powder (0.32 g, 73.38 %); m.p. 130–132 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.28 (s, 1H, NH D₂O exchangeable), 8.46 (dd, $J = 8.6$, 1.7 Hz, 2H, 4-NO₂ArH), 8.44 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.06 (dd, $J = 8.7$, 1.5 Hz, 1H, benzimidazole H), 8.02–7.99 (m, 2H, ArH), 7.94 (dd, $J = 8.7$, 1.8 Hz, 2H, 4-NO₂ArH), 7.84 (d, $J = 8.0$ Hz, 1H, benzimidazole H), 7.37 (t, $J = 7.8$ Hz, 2H, ArH), 7.11 (t, $J = 7.5$ Hz, 1H, ArH), 4.30 (m, 1H, cyclohexyl), 2.37–2.26 (m, 2H, cyclohexyl), 1.99 (d, $J = 12.3$ Hz, 2H, cyclohexyl), 1.86 (d, $J = 12.3$ Hz, 2H, cyclohexyl), 1.66 (d, $J = 11.6$ Hz, 1H, cyclohexyl), 1.47–1.23 (m, 3H, cyclohexyl); **MS** (Mwt= 440.49), m/z (% rel. int.): 440.18 (M⁺, 66.21%), 441.19 (M⁺¹, 29.76%); **Anal.** Calcd for C₂₆H₂₄N₄O₃: C, 70.89; H, 5.49; N, 12.72; Found: C, 71.08; H, 5.67; N, 12.64.

2.1.5.24. N-benzyl-1-cyclohexyl-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIx)

The titled compound VIIx is brown powder (0.25 g, 55.5 %); m.p. 126–128 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.04 (t, $J = 5.9$ Hz, 1H, NH D₂O exchangeable), 8.4 (dd, $J = 8.7$, 1.9 Hz, 2H, 4-NO₂ArH), 8.27 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.14 (dd, $J = 8.5$, 1.5 Hz, 1H, benzimidazole H), 7.98 (dd, $J = 8.7$, 1.8 Hz, 2H, 4-NO₂ArH), 7.83 (d, $J = 8$ Hz, 1H, benzimidazole H), 7.71–7.55 (m, 5H, ArH), 4.51 (d, $J = 5.6$ Hz, 2H, benzyl), 4.27 (m, 1H, cyclohexyl), 2.27 – 2.24 (m, 2H, cyclohexyl), 1.98–1.8 (m, 4H, cyclohexyl), 1.63 (d, $J = 12.5$ Hz, 1H, cyclohexyl), 1.32 (m, 3H, cyclohexyl); **MS** (Mwt= 454.52), m/z (%rel. int.): 454.20 (M⁺, 82.27%), 455.20 (M⁺, 30.8%); **Anal.** Calcd for C₂₇H₂₆N₄O₃: C, 71.35; H, 5.77; N, 12.33; Found: C, 71.49; H, 5.89; N, 12.50.

2.1.6. General procedure to prepare

compounds (VIIIa-h)

A mixture of 1-cyclohexyl-2-substituted-1H-benzo[d]imidazole-5-carboxylic acid (**VIa-d**) (0.33 mmol, 1 equivalent), the amine (0.33 mmol, 1 equivalent), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluranium tetrafluoroborate (TBTU) (0.214 g, 0.66 mmol, 2 equivalent) and 4-(Dimethylamino)pyridine (DMAP) (0.07 g, 0.66 mmol, 2 equivalent) was stirred in dry DMF under N₂ atmosphere for 24 h. The mixture was poured on ice/H₂O with continuous stirring, the resulting solid was filtered and washed with H₂O several times and allowed to dry. The solid was purified using flash chromatography (twice) (the system used: hexane: ethyl acetate = 6:1 then changed to 4:1 and finally 2:1) to give the titled compounds (**VIIIa-h**).

2.1.6.1 (S)-Ethyl 2-(1-cyclohexyl-2-(2-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamido)-3-phenylpropanoate (VIIIa)

The titled compound **VIIIa** is white powder (0.16 g, 84.65 %); m.p. 88-90 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.80 (d, *J* = 7.7 Hz, 1H, NH D₂O exchangeable), 8.20 (d, *J* = 1.5 Hz, 1H, benzimidazole H), 7.88 (dd, *J* = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.73 (d, *J* = 8 Hz, 1H, benzimidazole H), 7.60 (td, *J* = 8.0, 7.3, 1.8 Hz, 1H, 2-OMe ArH), 7.45 (dd, *J* = 7.4, 1.7 Hz, 1H, 2-OMe ArH), 7.34 (d, *J* = 7.2 Hz, 1H, 2-OMe ArH), 7.29 (d, *J* = 7.4 Hz, 1H, 2-OMe ArH), 7.26 (d, *J* = 4.9 Hz, 2H, ArH), 7.24–7.19 (m, 1H, ArH), 7.14 (t, *J* = 7.5 Hz, 2H, ArH), 4.67 (q, *J* = 7.5 Hz, 1H, -CHCH₂), 4.11 (q, *J* = 7.1 Hz, 2H, -OCH₂CH₃), 3.85-3.82 (p, *J* = 6.2 Hz, 1H, cyclohexyl), 3.81 (s, 3H, -OCH₃), 3.19 – 3.13 (d, *J* = 10 Hz, 2H, -CHCH₂), 2.20 (m, 2H, cyclohexyl), 1.83 (m, 4H, cyclohexyl), 1.62 (d, *J* = 12.5 Hz, 1H, cyclohexyl), 1.37 (d, *J* = 13.3 Hz, 3H, cyclohexyl), 1.15 (t, *J* = 7.1 Hz, 3H, -OCH₂CH₃); **MS** (Mwt = 525.64), *m/z* (% rel. int.): 525.26 (M⁺, 78.2%), 526.27 (M⁺, 35.2%); **Anal.** Calcd for C₃₂H₃₅N₃O₄: C, 73.12; H, 6.71; N, 7.99;

Found: C, 73.18; H, 6.74; N, 8.12.

2.1.6.2 (S)-Ethyl 2-(1-cyclohexyl-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxamido)-3-phenylpropanoate (VIIIb)

The titled compound **VIIIb** is white powder (0.15 g, 79.36 %); m.p. 96-98 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.81 (d, *J* = 7.6 Hz, 1H, NH D₂O exchangeable), 8.21 (d, *J* = 1.5 Hz, 1H, benzimidazole H), 8.19 (dd, *J* = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.89 (dd, *J* = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 7.73 (d, *J* = 8 Hz, 1H, benzimidazole H), 7.61 (t, *J* = 9.4 Hz, 1H, ArH), 7.30 (dt, *J* = 16.8, 9.5 Hz, 4H, ArH), 7.16 (dd, *J* = 8.6, 1.7 Hz, 2H, 4-OMe ArH), 4.67 (q, *J* = 7.5 Hz, 1H, -CHCH₂), 4.29 (p, *J* = 6.2 Hz, 1H, cyclohexyl), 4.16 – 4.05 (q, 2H, -OCH₂CH₃), 3.35 (s, 3H, methoxy), 3.16 (d, *J* = 10.0 Hz, 2H, -CHCH₂), 2.27 (m, 2H, cyclohexyl), 1.97 – 1.76 (m, 4H, cyclohexyl), 1.65 (d, *J* = 12.5 Hz, 1H, cyclohexyl), 1.32–1.21 (m, 3H, cyclohexyl), 1.16 (t, *J* = 7 Hz, 3H, -OCH₂CH₃); **MS** (Mwt = 525.64), *m/z* (% rel. int.): 525.27 (M⁺, 32.7%); **Anal.** Calcd for C₃₂H₃₅N₃O₄: C, 73.12; H, 6.71; N, 7.99; Found: C, 73.40; H, 6.89; N, 8.06.

2.1.6.3 N-(6-Chlorobenzothiazol-2-yl)-1-cyclohexyl-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIIIc)

The titled compound **VIIIc** is white powder (0.15 g, 78.32 %); m.p. 178-180 °C (charring); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.58 (s, 1H, NH D₂O exchangeable), 8.18 (d, *J* = 1.5 Hz, 1H, benzimidazole H), 8.06 (s, 1H, benzothiazole H), 7.7 (d, 1H, benzothiazole H), 7.79 (dd, *J* = 8.7, 1.5 Hz, 1H, benzimidazole H), 7.69 (d, *J* = 8 Hz, 1H, benzimidazole H), 7.65 – 7.60 (m, 5H, ArH), 7.50 (dd, *J* = 8.6, 2.2 Hz, 1H, benzothiazole H), 4.29 (p, *J* = 6.2 Hz, 1H, cyclohexyl), 2.37 – 2.24 (m, 2H, cyclohexyl), 1.99 – 1.91 (m, 2H, cyclohexyl), 1.86 (d, *J* = 12.8 Hz, 2H, cyclohexyl), 1.65 (d, *J* = 12.3 Hz, 1H, cyclohexyl), 1.33–1.18 (m, 3H, cyclohexyl); **MS**

(Mwt= 487.02), m/z (%rel. int.): 486.3 (M^+ , 100%), 488.13 (M^+ , 36.9%), 489.13 (M^{+2} , 10.9%); **Anal.** Calcd for $C_{27}H_{23}ClN_4OS$: C, 66.59; H, 4.76; N, 11.50; Found: C, 66.75; H, 4.89; N, 11.64.

2.1.6.4 1-Cyclohexyl-N-(6-nitrobenzo[d]thiazol-2-yl)-2-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIII d)

The titled compound **VIII d** is yellow powder (0.15 g, 76.65 %); m.p. 182-184 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.64 (d, J = 2.6 Hz, 1H, NH D_2O exchangeable), 8.25 (s, 1H, benzothiazole H), 8.10 (dd, J = 8.9, 2.5 Hz, 1H, benzothiazole H), 7.97 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.88 (dd, J = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.67 (d, J = 8 Hz, 1H, benzimidazole H), 7.64–7.58 (m, 5H, ArH), 7.49 (d, J = 8.9 Hz, 1H, benzothiazole H), 4.27 (p, J = 6.3 Hz, 1H, cyclohexyl), 2.34 – 2.21 (m, 2H, cyclohexyl), 1.89 (m, 4H, cyclohexyl), 1.64 (d, J = 12.2 Hz, 1H, cyclohexyl), 1.26 (m, 3H, cyclohexyl); **MS** (Mwt= 497.57), m/z (% rel. int.): 497.15 (M^+ , 88%), 498.16 (M^{+1} , 29.6%); **Anal.** Calcd for $C_{27}H_{23}N_5O_3S$: C, 65.17; H, 4.66; N, 14.08; Found: C, 65.41; H, 4.88; N, 14.21.

2.1.6.5 N,1-Dicyclohexyl-2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIII e)

The titled compound **VIII e** is white powder (0.12 g, 76.73 %); m.p. 240-242 °C; 1H NMR (400 MHz, DMSO- d_6) δ 9.99 (s, 1H, OH D_2O exchangeable), 8.19 (s, 1H, NH D_2O exchangeable), 8.16 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.84 (dd, J = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.75 (dd, J = 8.7, 2.8 Hz, 2H, 4-OH ArH), 7.48 (d, J = 8 Hz, 1H, benzimidazole H), 6.96 (dd, J = 8.7, 2.8 Hz, 2H, 4-OH ArH), 4.29 (p, J = 6.6 Hz, 1H, cyclohexyl), 3.79 (m, 1H, cyclohexyl), 2.28 (m, 2H, cyclohexyl), 1.87 (m, 6H, cyclohexyl), 1.76 (d, J = 9.5 Hz, 2H, cyclohexyl), 1.64 (t, J = 11.8 Hz, 2H, cyclohexyl), 1.32 (dt, J = 23.0, 12.0 Hz, 7H, cyclohexyl), 1.17

(d, 1H, cyclohexyl); ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.1, 159.3, 155.4, 143.3, 135.9, 131.3, 128.9, 121.9, 121.4, 118.8, 116.0, 112.7, 57.1, 48.8, 33.0, 31.0, 26.0, 25.8, 25.5, 24.9; **MS** (Mwt= 417.54), m/z (%rel. int.): 417.23 (M^+ , 29.14%), 418.23 (M^{+1} , 100%); **Anal.** Calcd for $C_{26}H_{31}N_3O_2$: C, 74.79; H, 7.48; N, 10.06; Found: C, 74.95; H, 7.53; N, 10.27.

2.1.6.6 1-Cyclohexyl-2-(4-hydroxyphenyl)-N-phenyl-1H-benzo[d]imidazole-5-carboxamide (VIII f)

The titled compound **VIII f** is white powder (0.11 g, 71.37%); m.p. 204-206 °C; 1H NMR (400 MHz, DMSO- d_6) δ 10.22 (s, 1H, NH D_2O exchangeable), 10.01 (s, 1H, OH D_2O exchangeable), 8.36 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.95 (dd, J = 8.5, 1.5 Hz, 1H, benzimidazole H), 7.87 (d, J = 8 Hz, 1H, benzimidazole H), 7.83 (dd, J = 8.4, 2.7 Hz, 2H, 4-OH ArH), 7.51 (d, J = 8.5 Hz, 2H, ArH), 7.36 (t, J = 7.8 Hz, 3H, ArH), 6.97 (dd, J = 8.5, 2.7 Hz, 2H, 4-OH ArH), 4.27 (m, 1H, cyclohexyl), 2.37–2.26 (m, 2H, cyclohexyl), 1.89 (t, J = 14.6 Hz, 4H, cyclohexyl), 1.66 (d, J = 12.5 Hz, 1H, cyclohexyl), 1.36–1.26 (m, 3H, cyclohexyl); ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.2, 159.4, 155.7, 143.3, 139.4, 136.4, 131.4, 129.0, 128.7, 123.9, 122.2, 121.3, 120.8, 119.3, 116.0, 113.1, 57.2, 31.0, 26.0, 24.9; **MS** (Mwt= 411.5), m/z (% rel. int.): 411.13 (M^+ , 52%), 412.15 (M^{+1} , 33.72%); **Anal.** Calcd for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21; Found: C, 75.96; H, 6.27; N, 10.3.

2.1.6.7 N-Benzyl-1-cyclohexyl-2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxamide (VIII g)

The titled compound **VIII g** is white powder (0.13 g, 81.56%); m.p. 264-266 °C; 1H NMR (400 MHz, DMSO- d_6) δ 10.01 (s, 1H, OH D_2O exchangeable), 9.05 (t, J = 5.9 Hz, 1H, NH D_2O exchangeable), 8.24 (d, J = 1.5 Hz, 1H,

benzimidazole H), 7.89 (dd, $J = 8.6, 1.5$ Hz, 1H, benzimidazole H), 7.81 (dd, $J = 8.7, 1.7$ Hz, 2H, 4-OH ArH), 7.51–7.46 (m, 1H, ArH), 7.36–7.33 (m, 4H, ArH), 7.25 (d, $J = 8$ Hz, 1H, benzimidazole H), 6.99–6.93 (dd, $J = 8.7, 2.8$ Hz, 2H, 4-OH ArH), 4.52 (d, $J = 6.0$ Hz, 2H, -NHCH₂), 4.35–4.24 (m, 1H, cyclohexyl), 2.29 (qd, $J = 11.7, 11.2, 5.3$ Hz, 2H, cyclohexyl), 1.92–1.80 (m, 4H, cyclohexyl), 1.65 (d, $J = 12.0$ Hz, 1H, cyclohexyl), 1.37 (m, 3H, cyclohexyl); **MS** (Mwt = 425.52), m/z (%rel. int.): 425.21 (M⁺, 100%), 426.21 (M⁺, 30.14%); **Anal.** Calcd for C₂₇H₂₇N₃O₂: C, 76.21; H, 6.40; N, 9.87; Found: C, 76.34; H, 6.49; N, 9.98.

2.1.6.8 1-Cyclohexyl-2-(4-hydroxyphenyl)-N-(2-methyl-4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (VIIIh)

The titled compound **VIIIh** is buff powder (0.15 g, 85.11%); m.p. 252-254 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.06 (s, 1H, OH D₂O exchangeable), 8.38 (s, 1H, NH D₂O exchangeable), 8.21 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.13 (dd, $J = 8.7, 2.8$ Hz, 2H, 4-OH ArH), 7.98 (dd, $J = 8.6, 1.5$ Hz, 1H, benzimidazole H), 7.88 (d, $J = 8.2$ Hz, 1H, ArH), 7.51 (d, $J = 8.2$ Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.29 (d, $J = 8$ Hz, 1H, benzimidazole H), 6.98 (dd, $J = 8.8, 3.1$ Hz, 2H, 4-OH ArH), 4.33 (m, 1H, cyclohexyl), 2.44 (s, 3H, methyl), 2.38 – 2.26 (m, 2H, cyclohexyl), 1.89 (t, $J = 14.6$ Hz, 4H, cyclohexyl), 1.66 (d, $J = 11.7$ Hz, 1H, cyclohexyl), 1.36–1.26 (m, 3H, cyclohexyl); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.3, 159.4, 155.9, 144.4, 143.9, 143.3, 136.7, 134.19, 131.3, 127.8, 126.0, 125.8, 122.3, 122.0, 121.2, 119.6, 116.1, 113.3, 57.2, 31.0, 26.0, 24.9, 18.4; **MS** (Mwt = 470.52), m/z (% rel. int.): 470.22 (M⁺, 50.52%), 319.43 (100%); **Anal.** Calcd for C₂₇H₂₆N₄O₄: C, 68.92; H, 5.57; N, 11.91; Found: C, 69.08; H, 5.73; N, 12.08.

2.1.7. General procedure to prepare compounds (IXa, b)

Na metal (5 mmol, 10 equivalent) was added to methanol (20 mL) at 0 °C to produce sodium methoxide which was added dropwise on a mixture of ester **Ve** (0.2 g, 0.5 mmol, 1 equivalent) and appropriate amine (2-methyl-4-nitro aniline and 3,4-dichloro aniline), respectively, (2.54 mmol, 5 equivalent) while in an ice bath, then the ice bath was removed and the reaction was refluxed for 24 h. The solvent was then evaporated under vacuum and the concentrate was poured on ice/H₂O/10% HCl with vigorous stirring where precipitate was formed, The precipitate was filtered and purified using flash chromatography (the system used: hexane: ethyl acetate= 9:1 then changed to 8:1 and finally 6:1) to give the titled compounds (**IXa, b**).

2.1.7.1 1-Cyclohexyl-N-(2-methyl-4-nitrophenyl)-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (IXa)

The titled compound **IXa** is buff powder (0.18 g, 70.89 %); m.p. 126-128 °C (charring); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.49 – 8.44 (dd, $J = 8.7, 1.7$ Hz, 2H, 4-NO₂ArH), 8.39 (s, 1H, NH D₂O exchangeable), 8.22 (d, $J = 1.5$ Hz, 1H, benzimidazole H), 8.14 (s, 1H, 2-Me-4-NO₂ArH), 8.07 (d, $J = 8.7$ Hz, 1H, 2-Me-4-NO₂ArH), 8.04 – 7.93 (dd, $J = 8.7, 1.8$ Hz, 2H, 4-NO₂ArH), 7.87 (dd, $J = 8.5, 1.5$ Hz, 1H, benzimidazole H), 7.36 (d, $J = 8.7$ Hz, 1H, 2-Me-4-NO₂ArH), 7.07 (d, $J = 8$ Hz, 1H, benzimidazole H), 3.77 (p, $J = 6.3$ Hz, 1H, cyclohexyl), 2.45 (s, 3H, methyl), 2.34 (m, 2H, cyclohexyl), 1.99-1.86 (m, 4H, cyclohexyl), 1.66 (d, $J = 12.3$ Hz, 1H, cyclohexyl), 1.25 (d, 3H, cyclohexyl); **MS**

(Mwt= 499.52), m/z (% rel. int.): 499.19 (M^+ , 43%), 500.19 (M^{+1} , 29.8%); **Anal.** Calcd for $C_{27}H_{25}N_5O_5$: C, 64.92; H, 5.04; N, 14.02; Found: C, 65.13; H, 5.21; N, 13.97.

2.1.7.2 1-Cyclohexyl-N-(3,4-dichlorophenyl)-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide (IXb)

The titled compound **IXb** is buff powder (0.15 g, 57.93%); m.p. 156-158 °C; 1H NMR (400 MHz, DMSO- d_6) δ 9.05 (m, 1H, NH D₂O exchangeable), 8.33 (dd, J = 8.5, 1.6 Hz, 2H, 4-NO₂ArH), 8.13 (d, J = 1.5 Hz, 1H, benzimidazole H), 7.90 (dd, J = 8.6, 1.5 Hz, 1H, benzimidazole H), 7.88 (d, J = 8 Hz, 1H, benzimidazole H), 7.86 (d, J = 2.2 Hz, 1H, 3,4 diClArH), 7.85 (s, 1H, 3,4 diClArH), 7.78 (dd, J = 8.8, 1.7 Hz, 2H, 4-NO₂ArH), 7.05 (d, J = 9.1 Hz, 1H, 3,4 diClArH), 4.14 (p, J = 6.8 Hz, 1H, cyclohexyl), 1.98 (d, J = 6.6 Hz, 2H, cyclohexyl), 1.71 (d, J = 11.7 Hz, 4H, cyclohexyl), 1.62 (d, J = 12.8 Hz, 1H, cyclohexyl), 1.46 (m, 3H, cyclohexyl); **MS** (Mwt= 509.38), m/z (% rel. int.): 510.19 (M^+ , 63.9%), 511.11 (M^{+2} , 18.5%), 508.11 (100%); **Anal.** Calcd for $C_{26}H_{22}Cl_2N_4O_3$: C, 61.31; H, 4.35; N, 11.00; Found: C, 61.49; H, 4.52; N, 10.89.

2.2. Biological Evaluation

The antiviral activity against both YFV and ZIKV was determined using a CPE-based assay. For YFV assay using Huh-7 cells, cells were seeded in 96-well plates at a density 5.5×10^3 cells/well in 100 μ L culture medium: Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids (NEAA) and 2% HEPES. Cells could adhere overnight in a CO₂ incubator at 37 °C. The next day, compounds were serially diluted in the culture medium. 100 μ L/well of YFV diluted in assay medium (DMEM supplemented with 2% FCS,

1% NEAA, and 2% HEPES) was added to the 96-well plates, after which plates were incubated for 4 days at 37 °C. For YFV assays using Vero cells, cells were seeded at a density of 2×10^4 cells/well in 100 μ L culture media (MEM Rega3, 10% FBS, 2 mM L-glutamine, and 0.075% sodium bicarbonate) in 96-well plates. The next day, the culture medium was removed and 100 μ L/well of assay medium (same as culture medium except that 10% FBS was replaced by 2% FBS) containing serial dilution of the compounds was added to the 96-well plates followed by an incubation period of 7 days. A potential toxic effect on the host cells was tested in parallel assays using the same protocol except that virus infection was omitted. In both antiviral and metabolic assays, the colorimetric readout was performed by using the MTS/PMS method, as described previously.^[44] CPE-based assays for ZIKV were performed as described previously [45]. The 50% effective concentration (EC₅₀; the compound concentration that is required to protect 50% of the cells from virus-induced CPE) and the 50% cytotoxic concentration (CC₅₀; the concentration that reduces the number of viable cells by 50%) were determined using logarithmic interpolation, as described previously [44].

2.3. Molecular Modelling

2.3.1. Molecular Docking

Molecular docking was performed using AutoDock Vina version 1.1.2 [46]. First, the protein (PDB ID: 5WZ3) was prepared using Discovery Studio 2.5 protein preparation protocol, and missing loops were added. Both protein and ligand files were converted to AutoDock's PDBQT format. The XYZ coordinates were chosen to be 24.1, 72.6, and 130.1, respectively. An equal length of X, Y, and Z axes was chosen to be 15 angstroms. A random seed number and standard exhaustiveness were used. Nine poses were generated where the best pose showed a binding affinity of -5.6

kcal/mol.

2.3.2. Molecular Dynamics Simulations

Molecular dynamics simulations were performed using Sybyl-X molecular modeling software. The simulation length was adjusted to 500 ps. The time step was set to 2 fs. An NVT ensemble was used with a temperature adjusted to 310 K. Boltzmann distribution was used to calculate the initial velocities and a random seed was used. At the end of the simulation, the total energy vs. time plot was generated to visualize energy changes. Molecular graphics of the docked poses and molecular dynamics were generated using Maestro visualizer.

3. Results and Discussion

3.1. Chemistry

The designed compounds were synthesized according to the chemical pathways outlined in schemes 1 and 2.

The 2-substituted-1H-benzo[*d*]imidazole nucleus was constructed in 4 steps starting by nitration of 4-chloro benzoic acid to give the 4-chloro-3-nitrobenzoic acid (**I**) [39] followed by Fischer esterification of the carboxylic acid moiety using ethanol to give ethyl 4-chloro-3-nitrobenzoate (**II**) [40]. Subsequently, the nucleophilic substitution of chloride with cyclohexylamine in the presence of DMSO and TEA was carried out to yield ethyl 4-(cyclohexylamine)-3-nitrobenzoate (**III**) [41]. Finally, the reductive cyclization method was used in the presence of sodium dithionite as a reducing agent for the nitro group followed by

cyclization using the appropriate aldehyde in a one-pot reaction^[42] to afford the ethyl 1-cyclohexyl-2-substituted-1H-benzo[*d*]imidazole-5-carboxylate derivatives (**Va-d**). Compound **Ve** was prepared by a different method, first by reduction of the nitro group in compound (**III**) via catalytic hydrogenation using palladium on activated charcoal (Pd/C) and hydrogenator^[43] to give Ethyl 3-amino-4-(cyclohexylamine) benzoate (**IV**) followed by its condensation with 4-nitro benzaldehyde in the presence of sodium acetate in ethanol^[47] to give the ethyl 1-cyclohexyl-2-(4-nitrophenyl)-1H-benzo[*d*]imidazole-5-carboxylate derivative **Ve**.

The preparation of the targeted 1-cyclohexyl-*N*-substituted-2-substituted phenyl-1H-benzo[*d*]imidazole-5-carboxamide derivatives was carried out firstly by hydrolysis of the 5-carboxylate group using LiOH.H₂O in ethanol/H₂O or methanol/H₂O to yield the carboxylic acid derivative (**VIa-e**) [48] followed by the preparation of carboxamide derivatives by two methods.

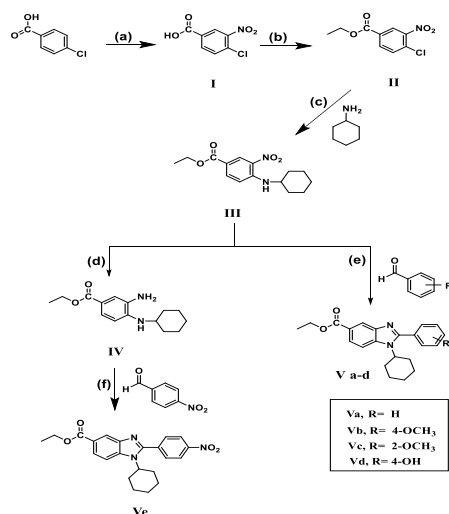
1- Activation of carboxylic acids (**VIa-e**) to their corresponding acid chlorides using thionyl chloride in dry DCM solvent followed by immediate addition of the corresponding amine in presence of TEA base to yield the compounds (**VIIa-x**) [49].

2- Direct coupling of the carboxylic acid derivatives (**VIa-d**) with the appropriate amine in presence of 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluranyl tetrafluoroborate (TBTU), 4-(Dimethylamino) pyridine (DMAP) in DMF, under N₂ atmosphere to yield compounds **VIIIa-h** [50].

Eventually, synthesis of amides directly from esters and amines has been utilized for the preparation of the final two derivatives (**IX a, b**)

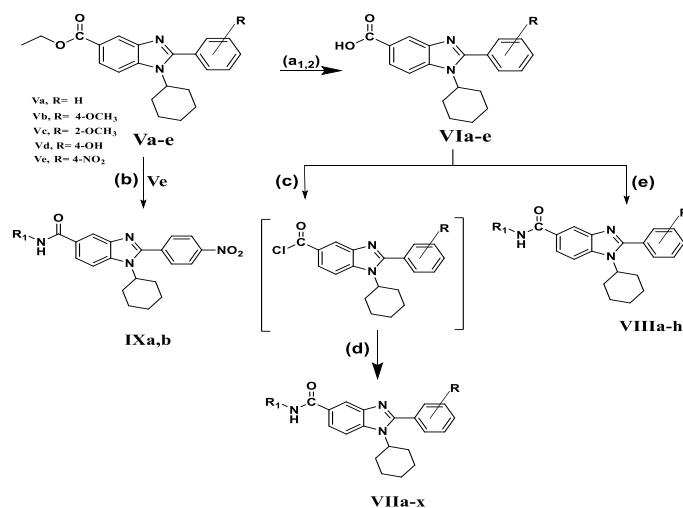
using Na metal in absolute ethanol to prepare sodium ethoxide which was added immediately to compound **Ve** and the corresponding amines.

Scheme 1: preparation of ethyl 1-cyclohexyl-2-substituted-1H-benzo[d]imidazole-5-carboxylate intermediates (Va-e)



Scheme 1, Reagents & Conditions: (a) H_2SO_4 , HNO_3 , rt, 3 h (b) H_2SO_4 , ethanol, 70°C , 24 h (c) TEA, DMSO, 90°C , 4 h (d) H_2 , 10% Pd/C, ethyl acetate (e) $\text{Na}_2\text{S}_2\text{O}_4$, DMSO, 90°C , 7 h (f) Na acetate, ethanol, 70°C , 4 h.

Scheme 2: preparation of 1-cyclohexyl-N-substituted-2-substituted phenyl-1H-benzo[d]imidazole-5-carboxamides (VII a-x), (VIII a-h) and (IXa, b)



Scheme 2, Reagents & Conditions: (a) 1-LiOH.H₂O, EtOH, H₂O, reflux, 2 h for compounds (VIa-d), 2-LiOH.H₂O, MeOH, H₂O, reflux, 24 h for compound VIe, (b) Na metal, EtOH, amine, reflux, 24 h. (c) Dry DCM, SOCl_2 , reflux, 2-4 h (d) Amine, dry DCM, TEA, rt, 48-72 h (e) Amine, TBTU, DMAP, dry DMF, under N_2 , rt, 24h.

Table 1. R and R₁ substitutions of the designed compounds (VII a-x), (VIII a-h) and (IXa, b)

Compound ID	R	R ₁	Compound ID	R	R ₁
VIIa	H		VIIr	2-OCH ₃	
VIIb	H		VIIs	2-OCH ₃	
VIIc	H		VIIt	2-OCH ₃	
VIIId	H		VIIu	2-OCH ₃	
VIIe	H		VIIv	4-NO ₂	
VIIIf	H		VIIw	4-NO ₂	
VIIg	H		VIIx	4-NO ₂	
VIIIh	H		VIIIa	2-OCH ₃	
VIIIi	H		VIIIb	4-OCH ₃	
VIIIj	H		VIIIc	H	
VIIIk	4-OCH ₃		VIIIId	H	

VIII	4-OCH ₃		VIIIe	4-OH	
VIIIm	4-OCH ₃		VIIIf	4-OH	
VIIIn	4-OCH ₃		VIIIg	4-OH	
VIIo	4-OCH ₃		VIIIh	4-OH	
VIIp	4-OCH ₃		IXa	4-NO ₂	
VIIq	2-OCH ₃		IXb	4-NO ₂	

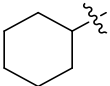
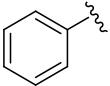
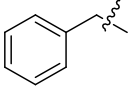
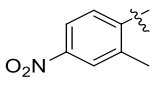
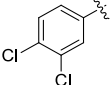
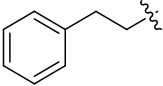
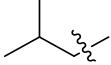
3.2. Biological evaluation

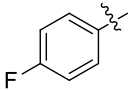
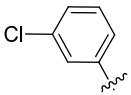



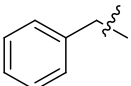
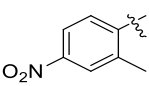



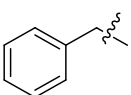
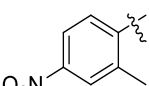
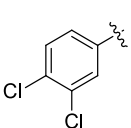
Antiviral activity was first assessed against YFV using the human hepatoma Huh-7 cells, most of the compounds exhibited antiviral activity against YFV in the low micromolar range, as shown in table 2. Next, the activity against YFV was evaluated using a different cell line, i.e. Vero cells (African green monkey kidney). Similar very good antiviral activity was confirmed for 5 compounds, i.e. **VIIId**, **VIIIf**, **VIIIh**, **VIIIn**, and **VIIIt** (Table 2), indicating that antiviral activity of these compounds does not

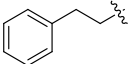
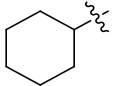
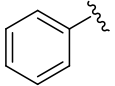

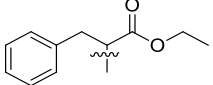
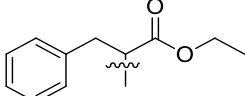
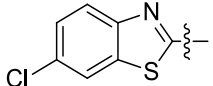
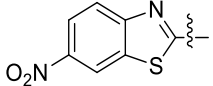
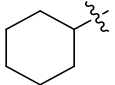
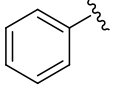
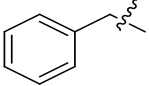
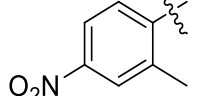
rely on the cell line used in the antiviral assay, but is specific for YFV.

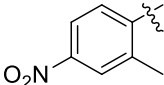
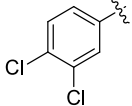
To explore broad-spectrum potential, 14 compounds (**VII d, e, g, h, n, t, v, w, VIII c, e-h, and IXa**) were evaluated against the related ZIKV.10 Compounds out of the 14 ones showed potential activity against YFV with EC₅₀ values <3.5 μM (as determined on Huh-7 cells). None of the tested compounds were active against ZIKV, except for compound **VIIId**, which showed ZIKV EC₅₀= 4.5 ± 2.1 μM (Table 2).

Table 2. Antiviral and anti-metabolic activity of 1H-benzo[d]imidazole-5-carboxamide derivatives. Va, VIa, VIIa-x, VIIIa-h and IXa, b

Compound ID	R	R1	YFV Huh-7 cells		YFV Vero cells		ZIKV VeroE6 cells	
			EC ₅₀ (μM)	CC ₅₀ (μM)	EC ₅₀ (μM)	CC ₅₀ (μM)	EC ₅₀ (μM)	CC ₅₀ (μM)
Va	H	OCH ₂ CH ₃	>100	ND	ND	ND	ND	ND
VIa	H	OH	>211	211.4	ND	>300	ND	ND
VIIa	H		2.5 ± 1.3	11.93	ND	5.4	ND	ND
VIIb	H		9.3 ± 7.7	26.1	ND	6.4	ND	ND
VIIc	H		7.8 ± 3.7	25.7	ND	6.0	ND	ND
VIIId	H		1.7 ± 0.8	>60	1.2 ± 0.02	>12.5	4.5 ± 2.1	>20
VIIe	H		2.4 ± 0.7	>60	<0.8	>50	>20	>20
VIIIf	H		>5.7	5.7	ND	9.9	ND	ND
VIIg	H		12.1 ± 6.4	>20	ND	ND	>7.5	>7.5

VIIIh	H		3.5 ± 1.6	128	2.3 ± 0.1	>3.1	>6.3	>20
VIIIi	H		2.5 ± 0.8	>60	ND	16.3	ND	ND
VIIj	H		11.5 ± 14.3	>60	ND	32.8	ND	ND
VIIIk	4-OCH ₃		3.0 ± 1.4	12.4	ND	6.9	ND	ND
VIII	4-OCH ₃		11.8 ± 0.9	70.3	ND	46.3	ND	ND
VIIIm	4-OCH ₃		5.6 ± 5.6	31.7	ND	20.0	ND	ND
VIIIn	4-OCH ₃		2.1 ± 0.6	>300	1.6 ± 0.01	>6.3	>20	>20
VIIo	4-OCH ₃		>20	ND	ND	ND	ND	ND
VIIp	4-OCH ₃		>100	ND	ND	ND	ND	ND
VIIq	2-OCH ₃		8.2 ± 4.0	53.9	ND	66.0	ND	ND
VIIr	2-OCH ₃		8.6 ± 4.5	51.5	ND	35.1	ND	ND
VIIs	2-OCH ₃		>276	276.5	ND	147	ND	ND
VIIIt	2-OCH ₃		2.2 ± 0.6	>60	0.8 ± 0.04	>100	>20	>20

VIIu	2-OCH ₃		<0.8	6.2 ± 6.3	ND	9.7	ND	ND
VIIv	4-NO ₂		3.3 ± 0.9	>20	ND	ND	>15	>15
VIIw	4-NO ₂		6.9 ± 1.3	>100	ND	ND	>30	>30
VIIx	4-NO ₂		>20	ND	ND	ND	ND	ND
VIIIa	2-OCH ₃		>100	ND	ND	ND	ND	ND
VIIIb	4-OCH ₃		>100	ND	ND	ND	ND	ND
VIIIc	H		8.7 ± 3.7	>20	ND	ND	>30	>30
VIII d	H		>20	ND	ND	ND	ND	ND
VIIIe	4-OH		1.6 ± 0.2	>20	ND	ND	>15	>15
VIII f	4-OH		3.0 ± 1.7	35.2	ND	ND	>7.5	>7.5
VIIIg	4-OH		1.6 ± 0.7	>20	ND	ND	>7.5	>7.5
VIIIh	4-OH		1.3 ± 0.2	>20	ND	ND	>15	>15

IXa	4-NO ₂		22.9	>100	ND	ND	>15	>15
IXb	4-NO ₂		5.5	75.8	ND	7.2	ND	ND
Ribavarin	-	-	>37.5	>100	>50	>100	>37.5	>100

ND, not determined

From the previous results, we can conclude that:

Compounds **Va** and **VIa** bearing the ester and acid moieties respectively showed no YFV inhibition with EC₅₀>100 μm.

Compounds **VIIa**, **VIIId**, **VIIe**, **VIIh**, **VIIi**, **VIIk**, **VIIIn**, **VIIIt**, **VIIv**, **VIIIe**, **VIIIf**, **VIIIg**, and **VIIIh** showed potent antiviral activity against YFV with EC₅₀ below 3.5 μM.

So, modification of the 5-carboxylate ester and 5-carboxylic acid groups into the amide group potentiated the anti-YFV activity.

Compounds **VIIa-j** with **unsubstituted 2-phenyl** showed the best anti-YFV activity with 5 compounds (out of 10 compounds) exhibiting EC₅₀ values below 3.5 μM.

Compounds **VIIIk-p** with **2-(4-methoxyphenyl)** group showed good anti-YFV activity with 2 compounds (out of 6 compounds) exhibiting EC₅₀ values below 3 μM.

Compounds **VIIq-u** with **2-(2-methoxyphenyl)** group showed moderate anti-YFV activity with 1 compound (out of 5 compounds) exhibiting EC₅₀ value below 2.2 μM.

Compounds **VIIIe-h** with the **2-(4-hydroxyphenyl)** group were the most potent compounds with YFV EC₅₀ values of below 3 μM.

Compounds **VIIv-x** and **IXa, b** bearing the **2-(4-nitrophenyl)** group showed moderate anti-YFV activity with one compound (out of 5 compounds) exhibiting EC₅₀ value below 3.3 μM.

Further investigation among the previously obtained results revealed:

In compounds **(VIIa-j)** (**unsubstituted 2-phenyl**) substitutions on the amidic N with phenyl ring bearing electron-withdrawing group as nitro, chloro, and fluoro groups at the para position in compounds **VIIId**, **VIIe** and **VIIh**, respectively, showed very good anti-YFV activity (huh-7 cells) with YFV EC₅₀ values of 1.7±0.8, 2.4 ± 0.7 and 3.5±1.6 μM, respectively. Cyclic aliphatic cyclohexyl substitution in compound **VIIa** also showed the same good activity with YFV (huh-7 cells) EC₅₀ value of 2.5 ±1.3 μM. The other aromatic substitutions as phenyl, benzyl, and phenethyl in compounds **VIIb**, **VIIc**, and **VIIIf**, respectively, and the aliphatic substitution, isobutyl and butyl chains in compounds **VIIg** and **VIIj**, respectively, showed poor anti-YFV activity.

In compounds **(VIIIk-p)** (**2-(4-methoxyphenyl) group**), substitutions on the amidic N with phenyl ring bearing the nitro electron-withdrawing group at the para position in compound **VIIIn** and the aliphatic cyclohexyl substitution in compound **VIIk** showed very good anti-YFV activity with YFV EC₅₀ values of

2.1±0.6 and 3.0±1.4 µM, respectively. The other aromatic substitutions as benzyl and phenyl in compounds **VIIIm** and **VIIIn**, respectively, showed poor anti-YFV activity. While the aliphatic isobutyl and cyclopropyl substituents in compounds **VIIo** and **VIIp**, respectively, showed no anti-YFV activity.

In compounds (**VIIq-u**) (**2-(2-methoxyphenyl) group**), only the N-3,4-dichloro phenyl derivative **VIIIt**, showed very good anti-YFV activity with YFV EC₅₀ value of 2.2±0.6 µM. The other aromatic substitutions as phenyl, benzyl, and phenethyl in compounds **VIIq**, **VIIr**, and **VIIu**, respectively, showed poor anti-YFV activity. Surprisingly the N-2-methyl-4-nitrophenyl group in compound **VIIIs** showed no anti-YFV activity.

In compounds (**VIIv-x**) and (**IXa, b**) (**2-(4-nitrophenyl) group**), only compound **VIIv** bearing N-cyclohexyl group showed very good anti-YFV activity with YFV EC₅₀ value of 3.3±0.9 µM, compound **IXb** featuring N-3,4-dichloro-phenyl substitution showed moderate anti-YFV activity with YFV EC₅₀ value of 5.5 µM. The other aromatic substitutions as phenyl and 2-methyl-4-nitrophenyl in compounds **VIIw** and **IXa**, respectively, showed poor anti-YFV activity and benzyl substitutions in compound **VIIx** showed no anti-YFV activity.

In compounds (**VIIIa-d**), compound **VIIIc** with the 6-Chlorobenzo[*d*]thiazol substitution showed poor anti-YFV activity, and compounds **VIIIa**, **VIIIb** and **VIIId** showed no anti-YFV activity

Compounds (**VIIIe-h**) with the **2-(4-hydroxyphenyl) group** were the most active compounds. Both para-substitution on the N-substituted phenyl ring with the nitro electron-withdrawing group in compound **VIIIh** and the cyclic aliphatic cyclohexyl substitution in compound **VIIIe** showed very good anti-YFV

activity with YFV EC₅₀ values of 1.3±0.2 and 1.6±0.2 µM, aromatic substitutions as benzyl and phenyl in compounds **VIIIg** and **VIIIf**, respectively also showed very good anti-YFV activity with YFV EC₅₀ values of 1.6±0.7 and 3.0±1.7 µM, respectively.

In an attempt to elucidate a SAR study from the previously discussed results, it was clear that the nature of the amidic-N substitution contributes the most to the biological activity, the phenyl ring (substituted with electron-withdrawing groups on its para-position) and the cyclohexyl ring were the most active ones. The variations in the 2-position substituents did not influence the activity much except for the 4-OH phenyl substitution.

3.3. Molecular Modelling

To understand the interesting SAR of our compound series, we conducted different molecular modeling experiments. The goal of our experiments was to explore the potential target of compound **VIIId** which showed very good activity against YFV (Huh-7 and Vero cells) and also against ZIKV VeroE6 cells and to investigate the possible binding mode to its target.

As has been mentioned, the design rationale relied on retaining the benzimidazole scaffold and exploring various substituents on the phenyl ring to improve activity against members of the *Flaviviridae* family. An important observation is that the synthesized compound series has a high structural similarity with CMF, a potent HCV NS5b inhibitor reported by Di Marco *et al.*[51].

The structures show scaffold similarity differing only in one extra nitrogen atom within the benzimidazole nucleus of compound **VIIId**. Both CMF and compound **VIIId** contain phenyl and cyclohexyl on the fused bicyclic nucleus. The major differences between both compounds are an extra morpholine amide found in the CMF

structure, and compound **VIIId** contains an amide substituent on the benzimidazole nucleus instead of the carboxylic acid found in CMF. **Fig. 4.** shows the field alignment of key features of CMF and compound **VIIId**.

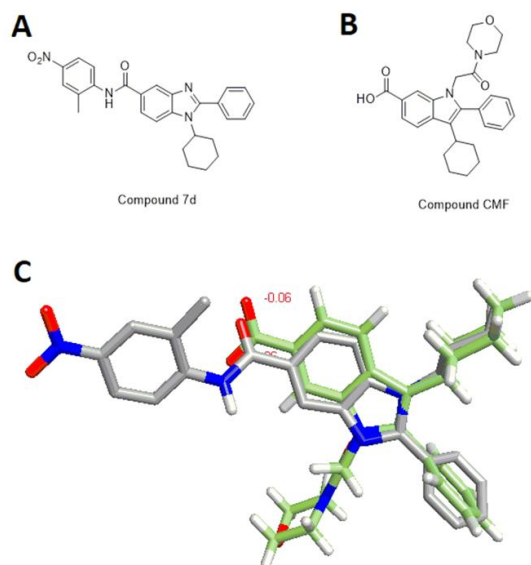


Fig. 4. (A), (B) Structures of compounds **VIIId** and compound CMF respectively. (C) Molecular field alignment of **VIIId** (grey) and CMF (green) with the superposition of key features such as the bicyclic fused nucleus, the cyclohexyl and phenyl moieties

From the previous observations, and due to high structural conservancy among the proteins of the Flaviviridae family, the Zika virus NS5 polymerase was predicted to be a potential target of compound **VIIId** [52, 53]. We conducted several molecular modeling experiments to predict the potential binding mode of compound **VIIId** to Zika virus NS5 polymerase. It is worth mentioning that no co-crystal structure of the Zika virus NS5 polymerase and an inhibitor had been determined yet.

3.3.1. Molecular Docking

Due to the high similarity between CMF and compound **VIIId**, we anticipated the co-crystal structure between CMF and HCV NS5b (PDB ID: 2BRK) as a good starting point to explore the potential target and its binding to compound

VIIId [51] **Fig. 5** shows the 2D interactions between CMF and HCV NS5b.

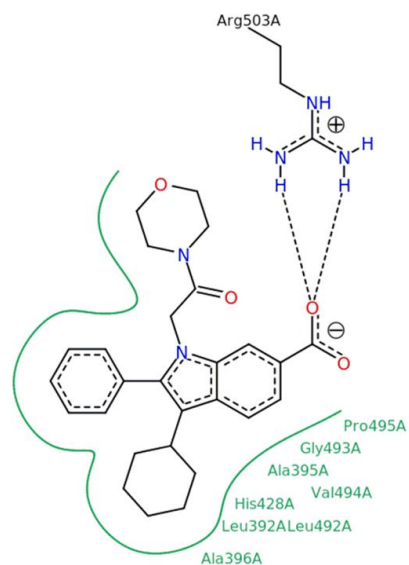


Fig. 5. 2D representation of the interactions between CMF and HCV NS5b (PDB ID: 2BRK)

By examining this crystal structure, it is observed that CMF binds to an allosteric site in the thumb domain of HCV NS5b, this binding relies mainly on Van der Waal's forces with hydrophobic residues. The only charge interaction found is between Arg503 and the carboxylate of CMF. The morpholine amide of CMF does not contribute to binding but protrudes towards the solvent (**Fig. 5**).

molecular docking was utilized to study the possible binding mode of compound **VIIId** to Zika virus NS5 polymerase. Zika virus NS5 crystal structure was prepared (PDB ID: 5WZ3) and docking of compound **VIIId** was performed using AutoDock Vina molecular docking software. The best binding pose showed a binding affinity of -5.6 kcal/mol. Compound **VIIId** occupied a groove within the thumb domain of the protein where the phenyl and cyclohexyl substituents showed hydrophobic contacts with residues at the tip of the priming loop. The nitrolyl moiety showed pi-pi stacking with Tyr885 as shown in (**Fig. 6**).

This binding mode gives insight into key features that improve the binding and activity of the tested compound. Pi-pi stacking with Tyr885 is strengthened with electron-withdrawing groups as the p-nitro group found in compound **VIIId**. Compound binding depends mainly on hydrophobic contacts with the side chains of Val787, Asp810, Met883, Asp884, and Tyr885. This is also observed to be the driving force for the binding of CMF with HCV NS5b [51].

This binding mode also suggests a possible mechanism of action for compound **VIIId** by allosterically influencing the geometry of the

binding site. It was observed that **VIIId** forms hydrophobic contact with Val787 and Asp810. These two residues are at the tip of the priming loop of the protein which extends from Val785 to Asp810 (**Fig. 6**). The dynamics of this loop is crucial for the activity of the NS5 protein as it regulates the allosteric positioning of the 3' terminus of the RNA template at the active site.^[52] Binding of **VIIId** to the tip of the priming loop could lead to a dynamic cascade which leads to disruption of the priming loop and active site deformity. To test this hypothesis, we conducted molecular dynamics simulations.

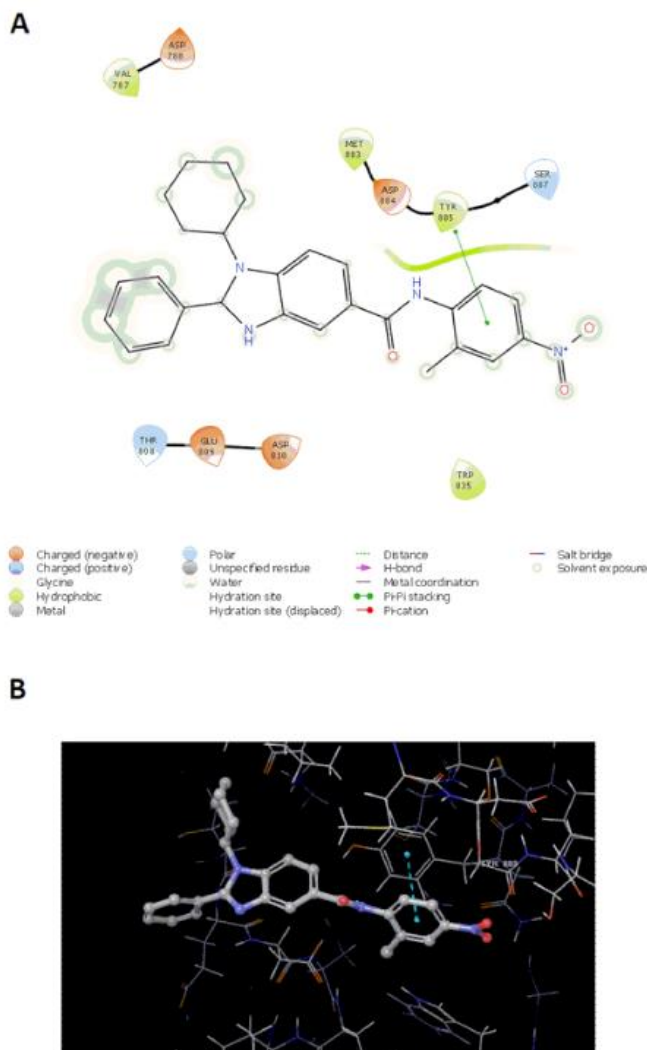


Fig. 6. (A) 2D Representation of the binding mode of compound **VIIId**. (B) 3D Representation of the binding mode of compound **VIIId**. Note that pi-pi stacking with Tyr885 is shown as a cyan dashed line

3.3.2. Molecular Dynamics Simulations

The binding pose generated from our docking experiments suggests a possibility of an allosteric remodeling of the priming loop triggered by hydrophobic contact of **VIIId** with the loop tip residues. To test this hypothesis, we conducted molecular dynamics simulations of the Zika virus NS5 protein (PDB ID: 5WZ3) with compound **VIIId** bound to it using Sybyl-X molecular modeling software. The simulation showed the stabilization of total energy after 500 ps (**Fig. 7**). The priming loop was shown to have been moved significantly throughout the simulation showing an RMSD of 9.59 when compared to its conformation in the protein crystal structure (**Fig. 8**). This significant motility of the priming loop upon binding of **VIIId** could explain the inhibitory mechanism of **VIIId**.

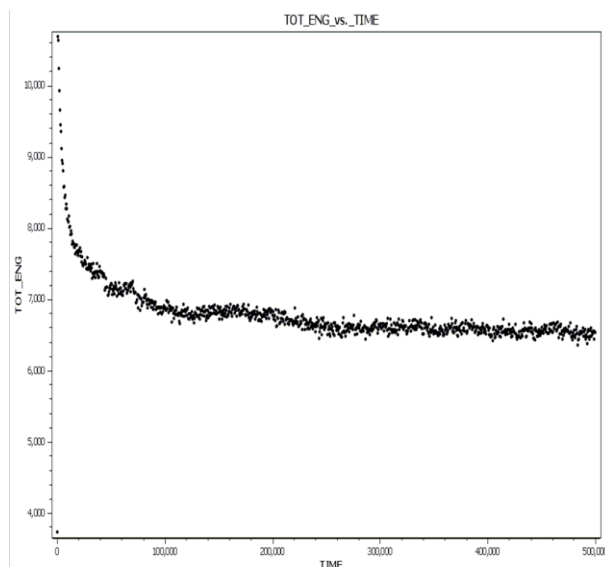


Fig. 7. Plot showing the change of total energy of the system vs. time throughout a 500 ps molecular dynamics simulation

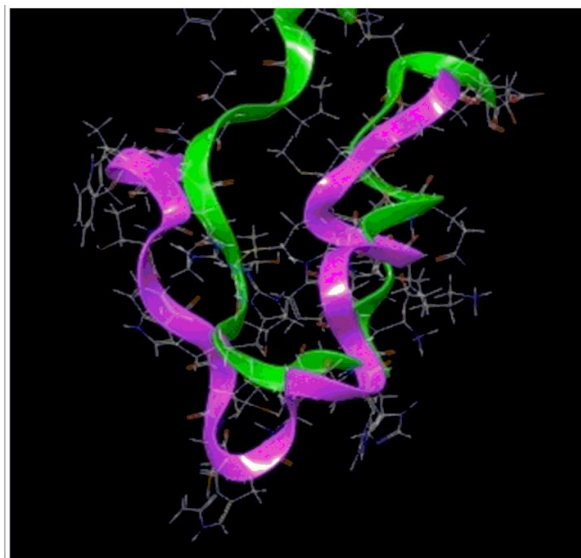


Fig. 8. Conformation Difference of the priming loop in the inhibitor **VIIId**-bound protein (green) and the apo-protein (magenta), the crystal structure (PDB ID: 5WZ3). Residues from both loops are shown as sticks and the rest of the protein structures are hidden for simplicity

Conclusion

34 new compounds of 1H-benzo[d]imidazole-5-carboxamide derivatives were designed, synthesized, and evaluated for their anti-YFV activity. Compounds **VIIId**, **VIIe**, **VIIIh**, **VIIIi**, **VIIIj**, **VIIIk**, **VIIIl**, **VIIIm**, **VIIIn**, **VIIIo**, **VIIIp**, **VIIIq**, **VIIIr**, **VIIIs**, **VIIIt**, **VIIIu**, **VIIIv**, **VIIIw**, **VIIIx**, **VIIIy**, **VIIIz**, **VIIIf**, **VIIIg**, and **VIIIh** which showed good activity on YFV were also evaluated for their anti-ZIKV activity.

SAR study among the compounds showed that modification of the 5-carboxylate ester and 5-carboxylic acid groups into amide group potentiated the anti-YFV activity. Variation of 2-position substituents between phenyl, 4-methoxy phenyl, 2-methoxy phenyl, 4-hydroxy phenyl, and 4-nitro phenyl didn't influence too much in the anti-YFV activity except 4-hydroxy phenyl derivatives (**VIIIe-h**) which demonstrated very good activity. Variation of 5-position carboxamide derivatives had a bigger influence on the anti-YFV activity. The variation of the anti-YFV activity of the carboxamide derivatives depends on the nature of the N-substitution.

Compound **VIIId** was proven to be a hit with YFV $EC_{50}=1.7\pm 0.8$ μ M on Huh-7 cells, YFV $EC_{50}= 1.2\pm 0.02$ μ M on Vero cells, and ZIKV $EC_{50}= 4.5\pm 2.1$ μ M. Molecular docking and molecular dynamics simulations studies for the synthesized compound confirmed the ZIKV NS5 polymerase to be the probable target.

Declarations

Not applicable.

Consent to publish

Not applicable.

Conflict of interest

The authors have declared no conflict of interest.

Funding Statement

No funding source was received.

Authors' contributions

All authors shared in the design of the study, collection, analysis, interpretation of data, and in writing the manuscript.

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