Send Orders for Reprints to reprints@benthamscience.ae

Mini-Reviews in Medicinal Chemistry, 2016, *16*, 67-83

Novel Benzo[d]imidazole-based Heterocycles as Broad Spectrum Anti-viral Agents: Design, Synthesis and Exploration of Molecular Basis of Action

Taha M.A. Eldebss[1,*], Ahmad M. Farag[1], Mohamed M. Abdulla[2] and Reem K. Arafa[3,4]

[1]*Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt;* [2]*Research Unit, Saco Pharm. Co., 6th October City, Egypt;* [3]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, P.O. Box 11562, Cairo, Egypt;* [4]*University of Science and Technology, Zewail City of Science and Technology, Cairo 12588, Egypt*

T.M.A. Eldebss

Abstract: The design and synthesis of a novel series of benzo[d]imidazole-based heterocycles and their biological evaluation as antiviral agents are reported herein. 1-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)-2-thiocyanatoethanone **2** was used as a key intermediate for the synthesis of the thiazolylhydrazine **4**, the thiazolylamine **5** and the methylthiazole **7**. Coupling of compounds **5** or **7** with the appropriate diazotized aromatic amines gave the diazenyl derivatives **6a-c** and **8a-c**, respectively. The quinazoline derivative **12** was also synthesized. On the other hand, the phenylthio **20** and the phenylsulphonyl **22** bioisosteresand their respective diazenyl derivatives **21a-c** and **23a-c** were prepared. The synthesized compounds were evaluated for their HIV-1, HCV, SSPE and H1N1 inhibitory activities and were found to display very promising results. Furthermore, to investigate the underlying possible mechanism of action, *in vitro* and *in silico* screening of this series of benzo[d]imidazoles was performed against the viral enzymes HIV-1 RT, HCV NS3/4A serine protease and H1N1 NA1. Overall findings of the executed investigations highlight these novel compounds as very promising potent, broad spectrum antiviral agents.

Keywords: Antiviral, Benzo[d]imidazole, HCV, H1N1, HIV, thiazolo[2,3-b]quinazolin-5-one.

1. INTRODUCTION

Viral infections pose a serious health hazard to mankind all over the world. The associated infectious diseases can be either relatively simple such as common cold, flu and warts or can lead to severe illnesses such as human immunodeficiency virus-acquired immunodeficiency syndrome (HIV/AIDS) and smallpox. Mortality, morbidity and societal disruption resulting from epidemics and pandemics associated with the three currently most common viral infections caused by influenza, AIDs and hepatitis C virus (HCV, also known as the silent pandemic) call for the widespread use of antiviral agents [1]. On the other hand, the high rates of viral mutations associated with the previously mentioned RNA viruses together with the emerging drug resistance hold the scientific community responsible for discovery of new effective and potent antiviral agents [2]. It is well recognized that pharmaceutically active compounds containing a benzimidazole moiety are of considerable importance because of their multiple biological activities, they have been found to possess antimicrobial, [3] antitubercular, [4] antiviral, [5] antifungal, [6] antiparasitic, [7] anthelmintic, [8, 9] pesticidal, [10] herbicidal [11] and plant-growth regulating [12] properties. Benzimidazoles have also found wide medicinal applications as potent antihypertensive, [13] antihistaminic, [14] anti-cancer [9, 15] and anti-inflammatory [16] agents as well as gastric ulcer inhibitors [17] and for the treatment of cardiovascular diseases [18].

Therefore, the reported potential biological activities of several benzimidazole derivatives have stimulated us to develop the chemistry of this class of compounds as useful precursors for the synthesis of many biologically interesting heterocycles. In continuation of our interest in the synthesis of a variety of heterocyclic systems having biological activities [19-22] containing benzimidazole scaffold from readily obtainable inexpensive starting materials, we report in this study a useful route toward benzimidazole-based heterocycles. The structures of the target compounds were elucidated on the basis of elemental analyses, spectral data and alternative chemical synthesis. All the prepared compounds were subjected to antiviral screening against HIV-1, HCV, SSPE and H1N1 viruses and promising results were obtained from which structure activity relationships (SAR) were drawn. To further explore the molecular basis behind these molecules action, their inhibitory activity against the catalytic activity of the key viral enzymes HIV-1 RT, HCV NS3/4A and H1N1 NA1 was investigated. Finally, a docking study was launched to study the probable binding interaction modes of these molecules to their respective biological targets.

*Address correspondence to this author at the Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt; Tel: +2 37242979-35676602-01001010194; E-mail: taha_eldebss@yahoo.com

1875-5607/16 $58.00+.00

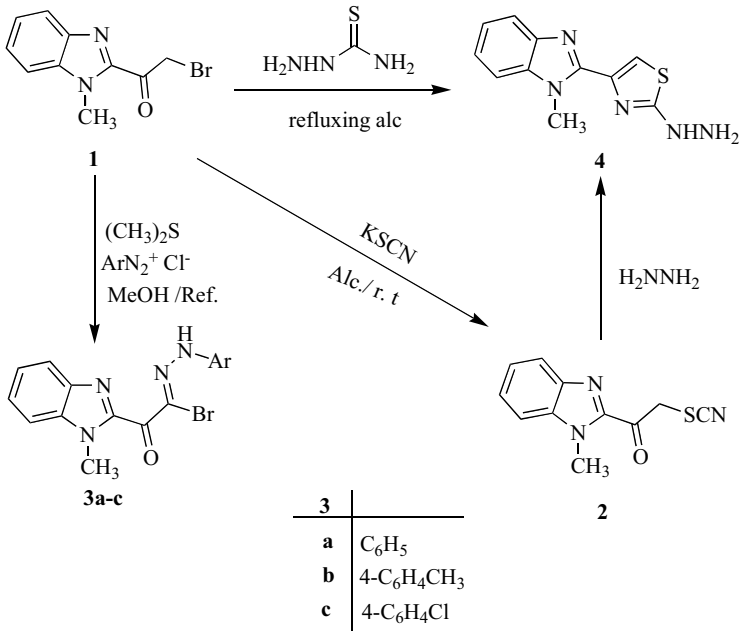
2. CHEMISTRY

Treatment of 2-bromo-1-(1-methyl-1H-benzo[d]imidazole-2-yl)ethanone (**1**) with potassium thiocyanate, in ethanol, at room temperature, afforded the corresponding 1-(1-methyl-1H-benzo[d]imidazole-2-yl)-2-thiocyanatoethanone (**2**) [23] Also, when compound **1** treated with dimethylsulfide in refluxing methanol followed by coupling with the appropriate diazotized aromatic amines in ethanol, buffered with sodium acetate tri hydrate at 0-5°C afforded 2-(2-arylhydrazono)-2-bromo-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethanones (**3a-c**, Scheme **1**). Compound **1** underwent cyclocon-densationreaction with thiosemicarbazide in refluxing ethanol to afford a compound that was identified as 1-(4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)hydrazine (**4**). The structure of compound **4** was inferred from its elemental analysis, IR and ^{1}H-NMR spectral data, in addition to its mass spectrum. Thus, the IR spectrum of compound **4** revealed three bands at 3399, 3208, 3017 cm^{-1} assignable to NH and NH_2 groups in addition to the C=Nabsorption band at 1614 cm^{-1}. Its ^{1}H-NMR spectrum displayed two singlet signals at δ 4.25, 7.25 and a multiplet at 7.55-8.0 ppm corresponding to methyl, thiazole CH and aromatic protons, respectively. Its mass spectrum exhibited a peak at m/z 246 corresponding to its molecular ion. The structure of the isolated product **4** was supported by the alternate chemical synthesis *via* cyclocondensation reaction of compound **2** withhydrazine hydrate in refluxing ethanol in presence of few drops of piperidine (Scheme **1**).

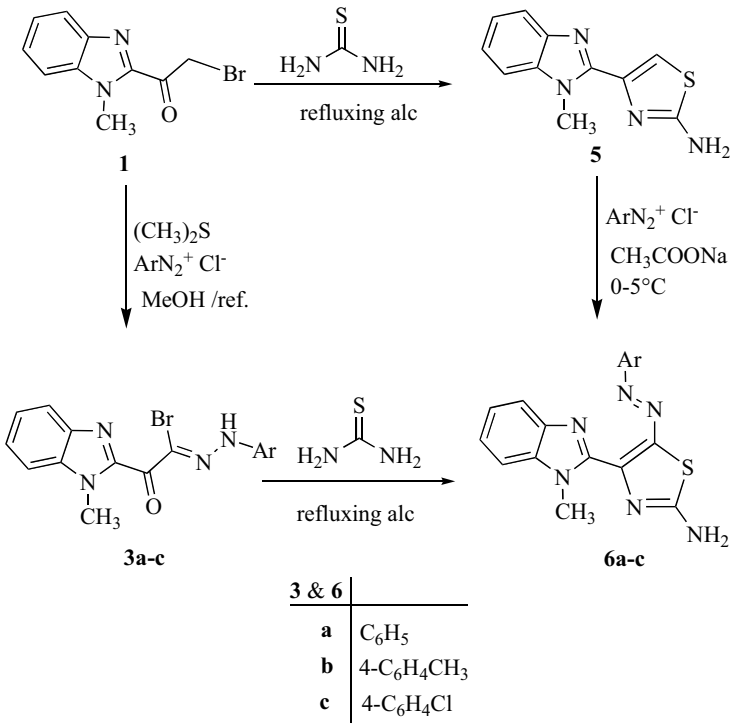
Reaction of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethanone (**1**) with thiourea, in refluxing ethanol yielded 4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2- amine (**5**) on the basis of its elemental analysis and spectral data. For example, the IR spectrum of compound **5** showed two stretching bands at 3296, 3316 cm^{-1} due to two NH groups. Its ^{1}H-NMR spectrum displayed a singlet signal at δ 3.4 ppm due to methyl protons, a broad D_2O-exchangeable signal at δ 3.9 ppm due to NH_2 proton and singlet signal at δ 7.25 due to thiazole CH, in addition to a multiplet at 7.7-9.0 ppm corresponding to aromatic protons. Its mass spectrum exhibited a peak at m/z 252 corresponding to its molecular ion (Scheme **2**).

Coupling of compound **5** with the appropriate diazotized aromatic amines in ethanol, buffered with sodium acetatetrihydrate at 0-5°C gave 5-arylazo4-(1-methyl-1H-benzo[d]imidazole-2-yl)thiazol-2-amine (**6a-c**). The structures of the latter products were established on the basis of their elemental analyses and spectral data (see experimental part). Compound **6** was confirmed by the alternate chemical synthesis from the reaction of hydrazonoyl bromides derivatives **3a-c** with thioureain refluxing ethanol at the same condition. In a similar manner,2-bromo-1-(1-methyl-*1H*-benzo[d]imidazole-2-yl)ethanone (**1**) reacted with thioacetamide in refluxing ethanol to give1-methyl-2-(2-methylthiazol-4-yl)-*1H*-benzo[d]imidazole (**7**) (Scheme **3**).

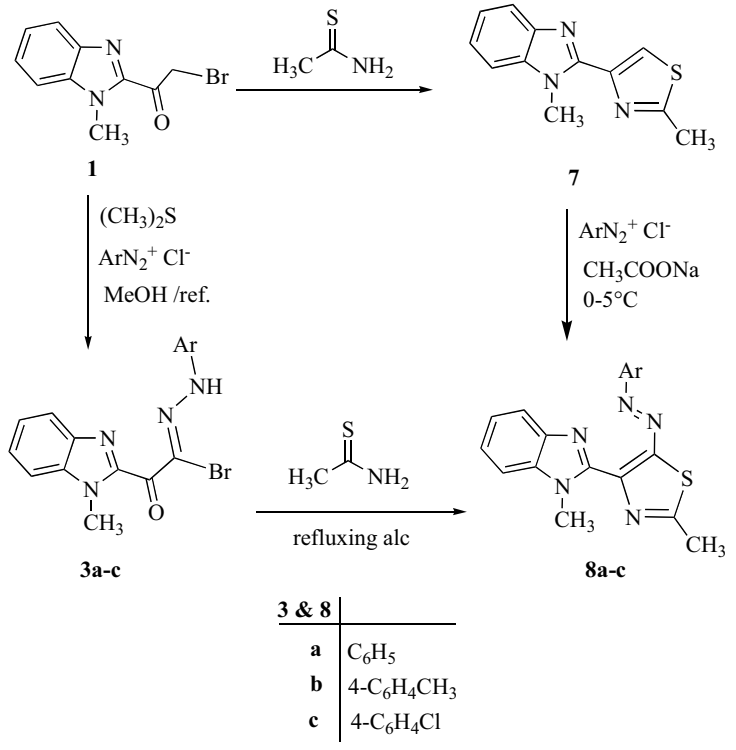
Coupling of compound **7** with the appropriate diazotized aromatic amines in ethanol, buffered with sodium acetate tri hydrate at 0-5°C gave 1-(2-methyl-4-(1-methyl-1*H*-benzo[d]imidazol-2yl-thiazol-5-yl)-2-aryldiazenes **(8a-c)**. The structures of the latter products were established on the basis of their elemental analyses and spectral data. For example, the IR spectra of **8a-c** showed, in each case, the lack of thiazole CH near 7.25 ppm at ^{1}H-NMR spectrum. When compound **2** was treated with hydrobromide salt of ethyl anthranilate in refluxing ethanol, it afforded a compound that was identified as 3-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-5*H*-thiazolo[2,3-b]quinazolin-5-one (**12**) (Scheme **4**). The structure of the latter product was established on the basis of its elemental analysis and spectral data. Its IR spectrum revealed a strong carbonyl absorption bands at 1687 cm^{-1}, whereas its mass spectrum, showed a molecular ion peak at m/z 332.



Scheme (1). Synthesis of derivatives of 2-thiocyanatoethanone 2, 2-arylhydrazono ethanones 3a-c and thiazolylhydrazine 4.

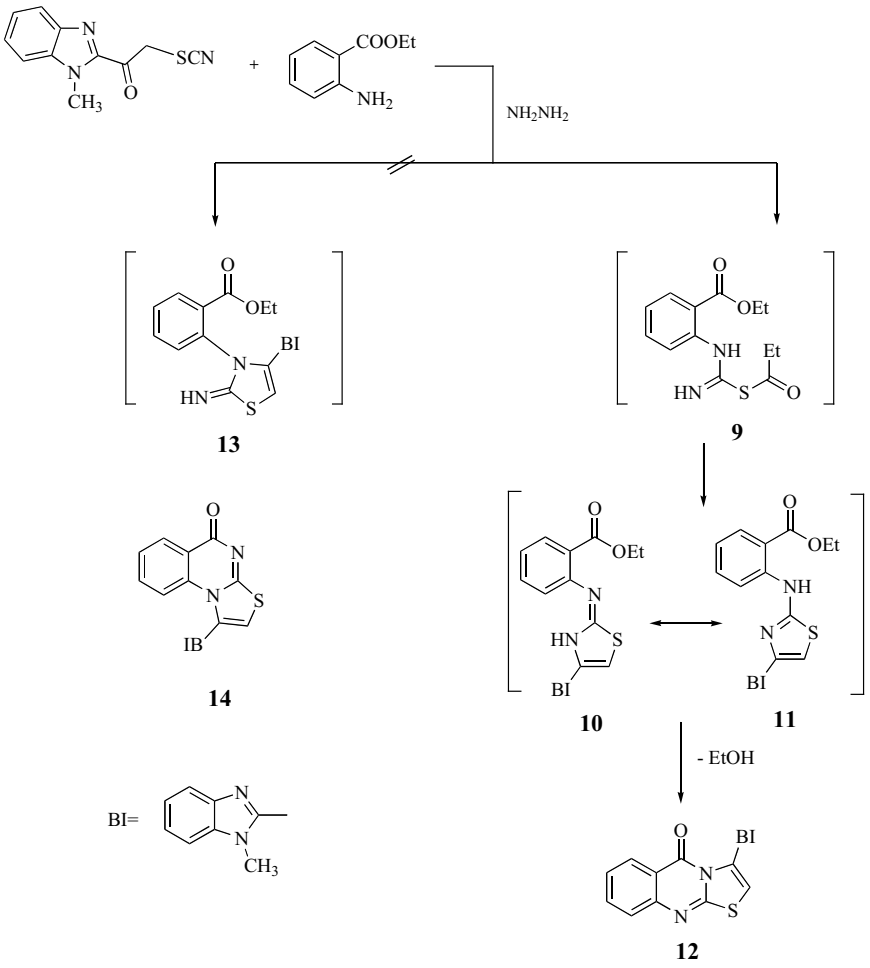


Scheme (2). Synthesis of derivatives of thiazol-2- amine 5 and 5-arylazo thiazol-2- amine 6a-c.

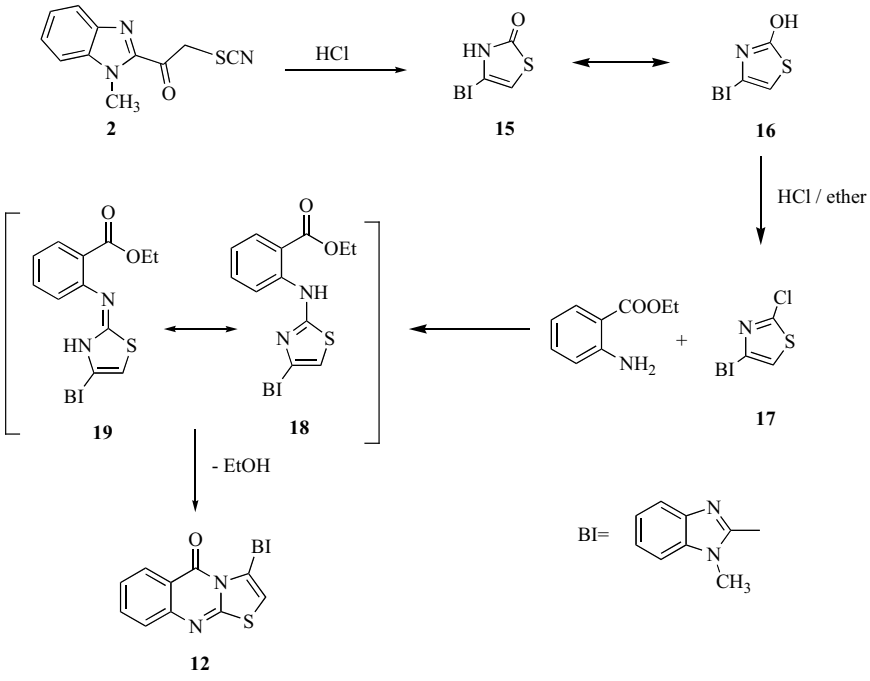


Scheme (3). Synthesis of derivatives of 2-methylthiazol 7 and 5-arylazo 2-methylthiazol 8a-c.

These data provided an additional support for the assigned structure. Also, the structure of the isolated product was proved to be linear **12** rather than the angular isomer **14**, this was confirmed by the alternate chemical synthesis of **12** via the reaction of hydrobromide salt of ethyl anthranilate with 2-(2-chloro)thiazol-4-yl)-1-methyl-1*H*-benzo[d]imidazole (**17**) that afforded a compound identical in all respects (m.p., mixed m.p., spectral data and TLC) with that of compound **12** (Scheme **5**).



Scheme (4). Synthesis of thiazolo[2,3-b]quinazolin-5-one derivative 12.



Scheme (5). Alternative synthesis of thiazolo[2,3-b]quinazolin-5-one derivative 12.

Reaction of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethanone (**1**) with sodium thiophenolate and sodium benzene sulphinate afforded the corresponding 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio)ethanone (**20**) and 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylsulphonyl) ethanone (**22**) [27] (Scheme **5**), respectively on the basis of their elemental analyses and spectral data (see materials and methods).

Compounds **20** and **22** underwent similar coupling with the appropriate diazotized aromatic amines in ethanol, buffered with sodium acetate tri hydrate at 0-5°C to give 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio)-2-aryldiazenyl-ethanone (**21a-c**) and 1-(1-methyl-1*H*-benzo [d]imidazol-2-yl)-2-(phenylsulfonyl)-2-aryldiazenylethanones (**23a-c**) [27], respectively on the basis of their elemental analyses and spectral data that were compatible with the assigned structures of the isolated products in addition to alternate chemical synthesis of both derivatives **21a-c** and **23a-c** *via* the reactions of compounds **20** and **22** with hydrazonoyl bromides **3a-c** in refluxing ethanol (Scheme **6**) (see materials and methods).

3. EXPERIMENTAL

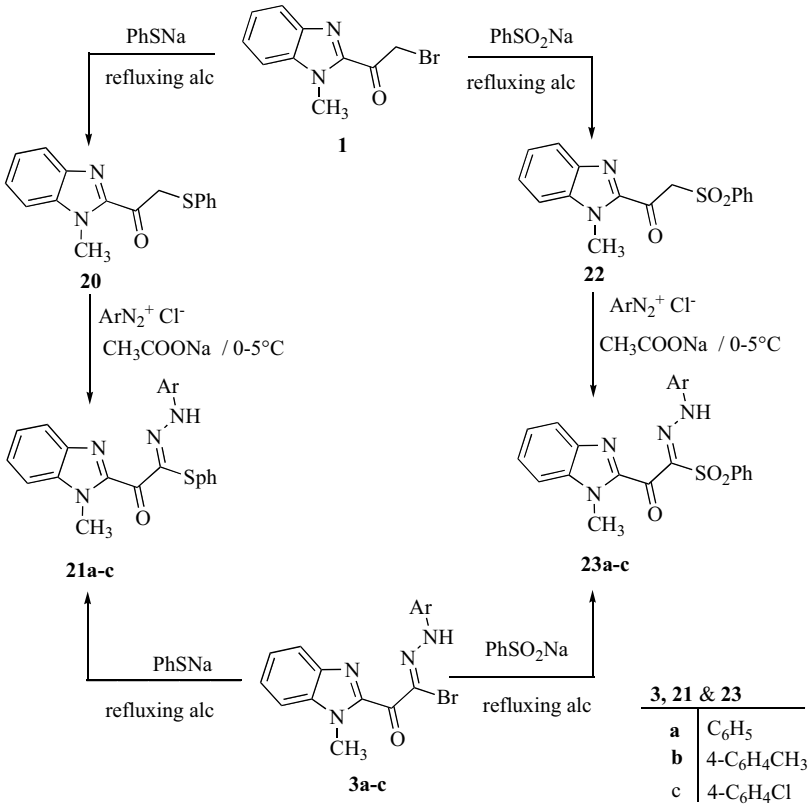
3.1. Chemistry

Melting points were measured with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts are quoted in δ and were related to that of the solvents. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 e.V. Elemental analyses were carried out at the Microanalytical center of Cairo University.

3.2. 1-(4-(1-Methyl -1H-benzo[d]imidazol-2-yl)thiazol-2-yl)hydrazine (4)

Method A

To a solution of 1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-2-thiocyanato-ethanone **(2)** (2.31 g, 10 mmol) in ethanol (20 ml) was added hydrazine hydrate (80%, 10 ml, 10 mmol) and few drops of piperidine The reaction mixture was refluxed for 6-8h then left to cool. The greenish-yellow precipitate so formed was collected by filtration, washed with water and dried. Recrystallization from DMF afforded 1-(4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)hydrazine (**4**) in 73% yield; m.p. 172-3°C; IR (KBr): ν max/cm^{-1} 3399, 3208, 3017 (NH, NH_2) 1614 (C=N); ^{1}H-NMR (DMSO-d_6): δ 4.25 (s, 3H, N-CH_3), 7.25 (s, CH, thiazole), 7.55-8.0 (m, 4H, ArH), 8.10 (s, 2H, NH_2, D_2O exchangeable), 8.30 (s, 1H, NH, D_2O exchangeable). MS: m/z (%), 246 (M^+ + 1, 36), 245 (M^+, 63), 201 (22), 131 (21), 7 (14). For $C_{11}H_{11}N_5S$(245.3): Calcd : C, 53.86; H, 4.52; N, 28.55; S, 13.07%. Found: C, 53.88; H, 4.50; N, 28.52; S, 13.10%.



Scheme (6). Synthesis of derivatives of 2-phenylthio)ethanone 20, 2-phenylthio)ethanone 20, 2-(phenylthio)-2-aryldiazenyl-ethanone 21a-c and 2-(phenylsulfonyl)-2-aryldiazenylethanones 23a-c.

Method B

To a solution of 2-bromo- 1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (**1**) (25.3 g, 100 mmol) in ethanol (20 ml) was added thiosemicarbazide (9.0 g, 100 mmol) and few drops of piperidine The reaction mixture was refluxed for 6-8h then left to cool. The precipitate so formed was collected by filtration, washed with water and dried. Recrystallization from DMF afforded a product identical in all respects (m.p., mixed m.p. and spectra) with that obtained from reaction of compound **2** with hydrazine hydrate as in method A above.

3.3. 4-(1-Methyl-1*H*-benzo[d]imidazole-2-yl)thiazol-2-amine (5)

A mixture of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (**1**) (2.53 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in ethanol (20 mmol) was refluxed for 6 h. The precipitated solid was filtered off, washed with water, dried and recrystallized from dioxane to afford the corresponding to 4-(1-methyl-1*H*-benzo[d]imidazole-2-yl)thiazol-2-amine (**5**) in 75% yield m.p. 175-6°C; IR (KBr): ν max/cm^{-1} 3296, 3164 (2 NH), 1607 (C=N); ^{1}H-NMR (DMSO-d_6): δ 3.4 (s, 3H, N-CH_3), 3.9 (s, 2H, NH_2), 7.40-7.75 (m, 4H, ArH), 7.25 (s, CH-5, thiazole) ppm.; MS: m/z (%), 254 (M^+ + 1, 100), 252 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{11}H_{10}N_4S$ (230.29). Calcd.: C, 57.37; H, 4.38; N, 24.33; S, 13.92%. Found: C, 57.38; H, 4.38; N, 25.28; S, 13.92 %.

3.4. 5-(2-Aryldiazenyl)-4-(1-methyl-1*H*-benzo[d] imidazole-2-yl)thiazol-2-amine (6a-c).

Method A

General Procedure

To a solution of the appropriate hydrazonoyl bromide **3a-c** (10 mmol) in ethanol (50 ml) was added thiourea (0.76 g, 10 mmol) the reaction mixture was refluxed for 1 h., then cooled. The deep colored precipitated solid that formed was filtered off, washed with ammonium hydroxide then with water, dried and recrystallized from dioxane to afford the corresponding 5-(2-aryldiazenyl)-4-(1-methyl-1*H*-benzo[d] imidazole-2-yl)thiazol-2-amine (**6a-c**) in 60-65% yields.

Method B

General Procedure

A cold solution of the appropriate arenediazonium chloride (10 mmol) was added portion wise to a cold solution of compound **5** (2.3 g, 10 mmol) in ethanol (50 ml), in the presence of sodium acetate trihydrate (5 g) with stirring. After the addition was complete, the reaction mixture was stirred at 0-5°C for further 3h., and left to stand in an ice box for 12h., then diluted with water. The solid product was collected, washed with water and dried. Recrystallization from dioxane afforded compounds identical in all respects (m.p., mixed m.p., and spectra) with those obtained by method A above. The compounds synthesized together with their physical data are listed below:

3.4.1. 5-(2-Phenyldiazenyl)-4-(1-methyl-1H-benzo[d] imidazole-2-yl)thiazol-2-amine (6a)

Yield: 65% m.p. 225-6°C; IR (KBr): ν max/cm^{-1} 3290, 3189 (2 NH), 1583 (C=N); ^{1}H-NMR (DMSO-d_6): δ 3.4 (s, 3H, N-CH_3), 3.9 (br. s, 2H, NH_2, D_2O exchangeable), 7.40 - 7.75 (m, 9H, ArH) ppm.; MS: m/z (%), 334 (M^+, 30), 305 (28), 229 (30). For $C_{17}H_{14}N_6$(334.4). Calcd.: C, 61.06; H, 4.22; N, 25.13; S, 9.59%. Found: C, 61.05; H, 4.24; N, 25.14; S, 9.59 %.

3.4.2. 5-(2-p-Tolyldiazenyl)-4-(1-methyl-1H-benzo[d] imidazole-2-yl)thiazol-2-amine (6b)

Yield: 60% m.p.250-2°C; IR (KBr): ν max/cm^{-1} 3569, 33369 (2 NH), 1609 (C=N); ^{1}H-NMR (DMSO-d_6): δ 3.4 (s, 3H, N-CH_3), 3.9 (s,3H,CH_3), 4.3 (br. s, 2H, NH_2, D_2O-exchangeable), 7.73 -7.76 (m, 8H, ArH) ppm.; MS: m/z (%), 349 (M^+ + 1, 90), 252 (M^+, 60), 173 (28), 159 (30). For $C_{18}H_{16}N_6S$ (348.42). Calcd.: C, 62.05; H, 4.63; N, 24.12; S, 9.20%. Found: C, 61.99; H, 4.64; N, 24.13; S, 9.21 %.

3.4.3. 5-(2-(p-Chlorophenyldiazenyl)-4-(1-methyl-1H-benzo[d]imidazole-2-yl)thiazol-2-amine (6c)

Yield: 63% m.p. 256-8°C; IR (KBr): ν max/cm^{-1} 3637, 3101 (2NH), 1583 (C=N); ^{1}H-NMR (DMSO-d_6): δ 3.4 (s, 3H, N-CH_3), 3.9 (s, 2H, NH_2), 7.40 -7.75 (m, 8H, ArH) ppm.; MS: m/z (%), 369 (M^+ + 1, 40), 368 (M+, 40), 367 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{17}H_{13}ClN_6S$ (368.84). Calcd.: C, 55.36; H, 3.55; Cl, 9.61; N, 22.78; S, 8.69%. Found: C, 55.37; H, 3.54; Cl, 9.64; N, 22.67; S, 8.78%.

3.5. 1-Methyl-2-(2- methylthiazol-4-yl)-1*H*-benzo[d] imidazole (7)

A mixture of 2-bromo-1-(1-methyl-1H-benzo[d]imidazole-2-yl)ethanone (**1**) (2.53 g, 10 mmol) and thioacetamide (0.75 g, 10 mmol) in ethanol (20 mmol) was refluxed for 3 h,, then cooled. The precipitated solid was filtered off, washed with water, dried and recrystallized from dioxane to afford the 1-methyl-2-(2-methylthiazol-4-yl)-1*H*-benzo[d]imidazole (**7**) in 85% yield m.p. 214-5°C; IR (KBr): ν max/cm^{-1} 1583 (C=N); ^{1}H-NMR (DMSO-d_6) δ 2.85 (s, 3H, CH_3), 3.5 (s, 3H, N-CH_3), 7.3 (s, 1H, 5-CH thiazole), 7.5-8.3 (m, 4H, ArH) ppm.; MS: m/z (%), 229 (M^+, 30), 228 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{12}H_{11}N_3S$ (229.3). Calcd.: C, 62.86; H, 4.84; N, 18.33; S, 13.98%. Found: C, 62.88; H, 4.82; N, 18.34; S, 13.99%.

3.6. 1-(2-Methyl-4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-thiazol-5-yl)-2-aryldiazene (8a-c)

Method A

General Procedure

A cold solution of the appropriate arenediazonium chloride (10 mmol) was added portion wise to a cold solution of compound **7** (10 mmol) in ethanol (50 ml), in the presence of sodium acetate trihydrate (5 g) with stirring. After the addition was complete, the reaction mixture was stirred at 0-5°C for further 3h., and left to stand in an ice box for 12h., then diluted with water. The solid product was

collected, washed with water and dried. Recrystallization from dioxane afforded the corresponding 1-(2-methyl-4-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-thiazol-5-yl)-2-aryldiazenes **(8a-c**) in 80-85% yields.

Method B

General Procedure

To solution of the appropriate hydrazonyl bromide **3a-c** (10 mmol) in ethanol (50 ml) was added thioacetamide (0.75 g, 10 mmol) the reaction mixture was refluxed for 1 h., where the reactants were dissolved and a red colored precipitate was formed on hot. The mixture was cooled and filtered off, washed with water, dried and recrystallized from dioxane to afforded compounds identical in all respects (m.p., mixed m.p., and spectra) with those obtained by method A above. The compounds synthesized together with their physical data are listed below:

3.6.1. 1-(2-Methyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-thiazol-5-yl)-2-phenyldiazene (8a)

Yield: 80% m.p. 269-70°C; IR (KBr): ν max/cm^{-1} 1607 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.85 (s, 3H, CH_3), 3.5 (s, 3H,N-CH_3), 7.40-7.75 (m, 9H, ArH) ppm.; MS: m/z (%), 334 (M^+ + 1, 20), 332 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{18}H_{15}N_5S$ (333.41). Calcd.: C, 64.84; H, 4.53; N, 21.01; S, 9.62%. Found: C, 64.86; H, 4.54; N, 21.02; S, 9.61 %

3.6.2. 1-(2-Methyl-4-(1-methyl-1H-benzo[d]imidazol-2yl)-thiazol-5-yl)-2-p-toyldiazene (8b)

Yield: 83% m.p. 272-4°C; IR (KBr): ν max/cm^{-1} 1607 (C=N); ^{1}H-NMR (DMSO-d_6) δ 2.65 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 3.5 (s, 3H, N-CH_3), 76.40-7.75 (m, 8H, ArH) ppm.; MS: m/z (%), 348 (M^+ + 1, 30), 346 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{19}H_{17}N_5S$ (347.44). Calcd.: C, 65.68; H, 4.93; N, 20.16; S, 9.23%. Found: C, 65.69; H, 4.92; N, 20.16; S, 9.21 %.

3.6.3. 1-(2-Methyl-4-(1-methyl-1H-benzo[d]imidazol-2yl)-thiazol-5-yl)-2-p-chlorophenyl-diazene (8c)

Yield: 85%; m.p. 277-8°C;IR (KBr):ν max/cm^{-1} 1613 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.85 (s, 3H, CH_3), 3.5 (s, 3H, N-CH_3), 76.40-7.75 (m, 8H, ArH) ppm.; MS: m/z (%), 368 (M^+ + 1, 10), 367 (M^+, 20), 366 (M^+ - 1, 50), 173 (28), 159 (30). For $C_{18}H_{14}ClN_5S$ (367.86). Calcd.: C, 58.77; H, 3.84; N, 19.04; S, 8.72%. Found: C, 58.79; H, 3.82; N, 19.03; S, 8.72 %.

3.7. 3-(1-Methyl-1*H*-benzo[d]imidazole-2-yl)-5*H*-thiazolo [2,3-b]quinazolin-5-one (12).

Method A

To a solution of1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-2-thiocyanato-ethanone (**2**) (2.31 g, 10 mmol) in ethanol (20 ml) was added ethyl antharanilatehydrobromide (3.32 g, 10 mmol) The reaction mixture was refluxed for 6-8h then left to cool. The precipitate so formed was collected by filtration, washed with water and dried. Recrystallization from DMF afforded 3-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-5*H*-thiazolo[2,3-b]quinazolin-5-one (**12**) in 66% yield; m.p.> 300°C; IR (KBr): ν max/cm^{-1} 1687 (C=O), 1602 (C=N); ^{13}C-NMR (DMSO-d_6): δ 165, 150, 145, 141, 138, 134, 132, 127, 126, 122, 121, 120, 117, 115, 112, 110, 47, 32; MS: m/z (%) 333 (M^+ + 1, 43), 332 (M^+, 34), 331 (M^+ - 1, 23), 201 (22), 131 (21), 7 (14). For $C_{18}H_{12}N_4OS$ (332.38) Calcd.: C, 65.04; H, 3.64; N, 16.86; S, 9. 65. Found: C, 65.02; H, 3.56; N, 16.90; S, 9.70 %.

5.8. 2-(2-Chlorothiazol-4-yl)-1-methyl-1*H*-benzo[d] imidazole (17)

Method A

A solution of 1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-2-thiocyanatoethanone (**2**) (2.31 g, 10 mmol) in ether (30 ml), was cooled to 10-15°C and saturated with dry hydrochloric gas during 1.5-2h. After the reaction was complete, the precipitated solid was filtered off, washed with, ether dried and recrystallized from dioxane to afford 2-(2-chlorothiazol-4-yl)-1-methyl-1*H*-benzo[d]imidazole (**17**) in 63% yield; m.p. 223-5°C. IR (KBr): νmax/cm^{-1} 1612 (C=N); MS: m/z (%), 249 (M^+, 52), 248 (M^+ - 1, 44), 173 (20). For $C_{11}H_8ClN_3$ (249.72). Calcd.: C, 52.91; H, 3.23; Cl, 14.20; N, 16.83; S, 12.84 %. Found: C, 52.90; Cl, 14.12; H, 3.0; N, 16.89; S, 12.86 %.

Method B

A mixture of 2-chloro-4-(1-methylbenzimidazol-2-yl) thiazole (**17**) (2.55 g, 10 mmol) and ethyl antharanilatehydrobromide (3.32 g, 10 mmol) and phenol (4ml) was heated on an oil-bath at 150-80°C for 7h., then left to cool. The solid that formed was collected by filtration, washed with water and dried. Recrystallization from acetic acid to afford a product identical in all respects (m.p., mixed m.p. and spectra) with those obtained from reaction of with that obtained from reaction of **2** with methyl antharanilatehydrobromide as in method A above.

3.9. 1-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio)ethanone (20)

Method A

To a stirred solution of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (**1**) (2.53 g, 10 mmol) and thiophenol in ethanol (20 mmol)] was added sodium hydroxide solution (3ml, 20%) dropwise over a period of 1h. while stirring. The resulting mixture was stirred for 3h.then diluted with water The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol to afford the corresponding1-((1-methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylthio)ethanone (**20**). in 75% yields m.p. 119-20°C; IR (KBr): ν max/cm^{-1} 1685 (C=O), 1571 (C=N). For $C_{16}H_{14}N_2OS$ (282.36). Calcd.: C, 68.09; H, 5.00; N, 9.92; S, 11.36%. Found: C, 68.10; H, 4.99; N, 9.92; S, 11.31 %.

Method B

General Procedure

A mixture of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (**1**) (2.53 g, 10 mmol) and sodium thiophenolate (10 mmol) in ethanol (20 mmol)] was refluxed

for 2h. The precipitated solid was filtered off, washed with water, dried and recrystallized from dioxane to afford the corresponding 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio)ethanone (**20**).

3.10. 1-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio))-2-aryldiazenylethanones (21a-c)

Method A

General Procedure

A cold solution of the appropriate arenediazonium chloride (10 mmol) was added portion wise to a cold solution of compound **20** (2.82 gm, 10 mmol) in ethanol (20 ml), in the presence of sodium acetate trihydrate (5 g) with stirring. After the addition was complete, the reaction mixture was stirred at 0-5°C for further 3h., and left to stand in an ice box for 12h., then diluted with water. The solid product was collected, washed with water and dried. Recrystallization from dioxane afforded the corresponding 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylthio)-2-aryldiazenyl-ethanones (**21a-c**).

Method B.

General Procedure

To an ethanolic solution sodium ethoxide solution [prepared from sodium metal (46 mg, 2 mg atom) in ethanol (20 ml)] was added thiophenol (0.2ml, 2mmol) with stirring. To the resulting mixture the appropriate hydrazonoyl bromide **5a-c** (2mmol) was added portion wise while stirring. After the addition was complete, the reaction mixture was stirred for further 6h. The solid product was collected by filteration, washed with water and dried. Recrystallization from acetic acid afforded compounds identical in all respects (m.p., mixed m.p., and spectra) with those obtained by method A above. The compounds synthesized together with their physical data are listed below.

3.10.1. 1-(1-Methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylthio))-2-(phenyldiazenyl)ethanone (21a)

Yield: 75% m.p. 146-8°C; IR (KBr): ν max/cm^{-1} 3144 (NH), 1655 (C=O) 1594 (C=N); ^{1}H-NMR (DMSO-d$_6$): δ 3.5 (s, 3H,N-CH$_3$), 7.4-7.8 (m, 14H, ArH), 11.6, (s, 1H, NH) ppm.; MS: m/z (%), 387 (M$^+$ + 1, 10), 385 (M$^+$ - 1, 60), 173 (28), 159 (30). For $C_{22}H_{18}N_4OS$(386.47). Calcd.: C, 68.37; H, 4.69; N, 14.50; S, 8.30%. Found: C, 68.40; H, 4.66; N, 14. 50; S, 8.28 %.

3.10.2. 1-(1-Methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylthio))-2-(p-tolyldiazenyl)ethanone (21b)

Yield: 78% m.p. 152-3°C; IR (KBr): ν max/cm^{-1}: 3365 (NH), 1641 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d$_6$): δ 2.65 (s, 3H, CH$_3$), 3.5 (s, 3H, N-CH$_3$), 7.4-7.8 (m, 13H, ArH), 11.6 (s, 1H, NH) ppm.; MS: m/z (%), 401 (M$^+$ + 1, 10), 399 (M$^+$ - 1, 60), 173 (28), 159 (30). For $C_{23}H_{20}N_4OS$ (400.5). Calcd.: C, 68.98; H, 5.03; N, 13.99; S, 8.01%. Found: C, 69.03; H, 4.99; N, 13.96; S, 8.01 %.

3.10.3. 1-((1-Methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylthio))-2-(p-chlorophenyldiazenyl)ethanone (21c)

Yield: 85% m.p. 158-9°C; IR (KBr): ν max/cm^{-1} 3380 (NH), 1675 (C=O), 1601 (C=N); ^{1}H-NMR (DMSO-d6): δ 2.65 (s, 3H, CH$_3$), 3.5 (s, 3H, N-CH$_3$), 7.4-7.8 (m, 13H, ArH), 11.6, (s, 1H, NH) ppm.; MS: m/z (%), 421 (M$^+$ + 1, 100), 419 (M$^+$ - 1, 60), 173 (28), 159 (30). For $C_{22}H_{17}ClN_4OS$ (420.91). Calcd.: C, 62.78; H, 4.07; Cl; 5.42 N, 13.31; S, 7.62%. Found: C, 62.77; H, 4.06; N, 13.31; Cl, 5.42; S, 7.62 %.

5.11. 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)ethanone (22)

Method A

General Procedure

A mixture of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (1) (2.53 g, 10 mmol) and sodium benzenesulphinate (1.64 g, 10 mmol) in ethanol (20 mmol) was refluxed for 2h. The precipitated solid was filtered off, washed with water, dried and recrystallized from dioxane to afford the corresponding 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)ethanone (22) [27] in 71% yield: m.p. 161-2°C; IR (KBr): ν max/cm^{-1} 1657 (C=O), 1609 (C=N); ^{1}H-NMR (DMSO-d$_6$): δ 4.1 (s, 3H, CH$_3$), 5.2 (s, 2H, CH$_2$), 7.25-8.05 (m, 9H, ArH) ppm.; ^{13}C-NMR (DMSO-d$_6$): δ 31.77, 60.11, 111.12, 120.46, 123.66, 128.68, 129.66, 133.55, 134.10, 138.29, 138.46, 140.12, 179.43;MS: m/z (%), 315 (M$^+$ + 1, 100), 313 (M$^+$ - 1, 60), 173 (28), 159 (30). For $C_{16}H_{14}N_2O_3S$ (314.36). Calcd.: C, 61.13; H, 4.49; N, 8.91; S, 10.20%. Found: C, 61.13; H, 4.46; N, 8.92; S, 10.21 %.

3.12. 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl))-2-aryldiazenylethanones (23a-c)

Method A

General Procedure

A cold solution of the appropriate arenediazonium chloride (10 mmol) was added portion wise to a cold solution of compound **22** (3.14 g, 10 mmol) in ethanol (50 ml), in the presence of sodium acetate trihydrate (5 g) with stirring. After the addition was complete, the reaction mixture was stirred at 0-5°C for further 3h., and left to stand in an ice box for 12h., then diluted with water. The solid product was collected, washed with water and dried. Recrystallization from dioxane afforded the corresponding 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)-2-aryl-diazenylethanone (**23a-c**).

Method B

General Procedure

To solution of the appropriate hydrazonoyl bromide **3a-c** (10 mmol) in ethanol (20 ml) was added sodium benzenesulphinate (1.64 g, 10 mmol) the reaction mixture was refluxed for 2 h. The mixture was cooled and filtered off, washed with water, dried and recrystallized from dioxane to afforded compounds identical in all respects (m.p., mixed m.p., and spectra) with those obtained by

method A above. The synthesized compounds together with their physical data are listed below:

3.12.1. 1-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)-2-(phenyldiazenyl)-ethanone (23a)

Yield: 76% m.p. 187-8°C; IR (KBr): ν max/cm^{-1} 3218 (NH), 1686 (C=O), 1628 (C=N); ^{1}H-NMR (DMSO-d_6): δ 4.2 (s, 3H, CH_3), 7.4-8.0 (m, 14H, ArH), 8.3 (br. s, 1H, NH, D_2O-exchangeable) ppm.; MS: m/z (%), 419 (M^+ + 1, 100), 416 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{22}H_{18}N_4O_3S$ (418.47). Calcd.: C, 63.14; H, 4.34; N, 13.39; S, 7.66%. Found: C, 63.14; H, 4.32; N, 13.37; S, 7.68%

3.12.2. 1-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)-2-(p-tolyldiazenyl)-ethanone (23b)

Yield: 80% m.p. 192-4°C; IR (KBr): ν max/cm^{-1} 3488 (NH), 1660 (C=O), 1602 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.65 (s, 3H, CH_3), 4.2 (s, 3H, CH_3), 7.4 -8.0 (m, 14H, ArH), 8.3 (br. s, H, NH, D_2O-exchangeable) ppm.; MS: m/z (%), 432 (M^+, 40), 431 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{23}H_{20}N_4O_3S$(432.49). Calcd.: C, 63.87; H, 4.66; N, 12.95; S, 7.41%. Found: C, 63.89; H, 4.68; N, 12.92; S, 7.40%.

3.12.3. 1-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-(phenylsulfonyl)-2-(p-chlorophenyl-diazenyl)ethanone (23c)

Yield: 85% m.p. 203-4°C; IR (KBr): ν max/cm^{-1} 3289 (NH), 1674 (C=O), 1602 (C=N); ^{1}H-NMR (DMSO-d_6): δ 4.3 (s, 3H, CH_3), 7.44-8.20 (m, 14H, ArH), 8.5 (br.s, 1H, NH, D_2O-exchangeable) ppm.; MS: m/z (%), 452 (M^+, 30), 451 (M^+ - 1, 60), 173 (28), 159 (30). For $C_{22}H_{17}ClN_4O_3S$ (452.91). Calcd.: C, 58.34; H, 3.78; Cl, 7.83; N, 12.37; S, 7.08%. Found: C, 58.35; H, 3.79; Cl, 7.85; N, 12.38; S, 7.06%.

3.13. Pharmacological evaluation

3.13.1. In vitro Assay for anti-HIV Activity

The established human cells, laboratory derived virus isolates, and low-passage clinical virus isolates used in these evaluations have previously been described in details [24]. These cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2mM glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). Fresh human cells were obtained from the American Red Cross (Baltimore, Md.).

The inhibitory activities of the compounds against HIV were evaluated by microtiter anti-HIV assays with CEMSS cells or fresh human peripheral blood mononuclear cells (PBMCs); these assays quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication. Quantification was performed by the tetrazolium dye XTT assay (CEM-SS, 174×CEM, MT2 and AA5 cell-based assays), which is metabolized to a colored formazan product by viable cells and/or RT assay (PBMC-based assays). Antiviral and toxicity data are reported as the quantity of drug required for inhibiting virus-induced cell killing or virus production by 50% (CC_{50} and EC_{50}, respectively).

3.13.2. In vitro Assay for HIV RT Inhibitory Activity

Purified RT assays of the newly synthesized compounds were tested for RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL), which utilizes the scintillation proximity assay (SPA) principle, as described in detail [25]. In the assay, a DNA/RNA template is bound to SPA beads via a biotin/streptavidin linkage. The primer DNA is a 16-mer oligo(T), which has been annealed to a poly(rA) template. The primer-template is bound to a streptavidin-coated SPA bead. [3H]TTP (thymidine-5'-triphosphate) is incorporated into the primer by reverse transcription. In brief, [3H]TTP, at a final concentration of 0.5 µCi/sample, was diluted in RT assay buffer (49.5 mMTris-HCl, pH 8.0, 80 mMKCl, 10 mM $MgCl_2$, 10 mMdithiothreitol, 2.5mM EDTA, 0.05% Nonidet P-40) and added to annealed DNA/RNA bound to SPA beads. The compound being tested was added to the reaction mixture at 0.001–100 µM concentrations. Addition of 10mU of recombinant HIV RT and incubation at 37°C for 1 h resulted in the extension of the primer by incorporation of [3H]TTP. The reaction was stopped by addition of 0.2 ml of 120 mM EDTA. The samples were counted in an open window using a Beckman LS 7600 instrument and IC_{50} [RT] values (concentration at which the compound inhibits recombinant RT by 50%) were calculated by comparing the measurements to an untreated.

3.13.3. In vitro Assay for Anti-hepatitis C Virus (HCV) Activity

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS), 2mM L-glutamine, and non-essential amino acids. Stable Huh-7 cells containing the self-replicating, sub-genomic HCV replicon, which was identical in sequence to the I377neo/NS3-3/wt replicon described by Lohmann*et al.* were selected and maintained in the presence of 0.25mg/ml G418 (Invitrogen, Carlsbad, CA) and were used for anti-HCV assays. Peripheral blood mononuclear cells (PBMC) were isolated from fresh donor blood and cultured in RPMI-1640 medium (JRH Biosciences) [26].

Determination of EC_{50} of different tested compounds in HCV replicon cells was performed as follow. Briefly, $1X10^4$ replicon cells per well were plated in 96-well plates. On the following day, replicon cells were incubated at 37°C for the indicated period of time with antiviral agents serially diluted in DMEM plus 2% FBS and 0.5% dimethyl sulfoxide (DMSO). Total cellular RNA was extracted using an RNeasy-96 kit (QIAGEN, Valencia, CA), and the copy number of HCV RNA was determined using a quantitative RT-PCR (QRT-PCR) assay. Each datum point represents the average of five replicates in cell culture.

The cytotoxicity of tested compounds (CC_{50}) was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay (Promega, Madison, WI). For the cytotoxicity assay with human hepatocyte cell lines, $4X10^4$ HepG2 cells per well were used.

3.13.4. In vitro Assay for Hepatitis C Virus (HCV) NS3/4A Serine Protease Inhibitory Activity

The *in vitro* proteolytic assay for HCV NS3/4A serine protease was carried out by incubating 20 µg of purified single-chain serine protease with 20 µg of the recombinant substrate NS5ab in 100 µl of 25 mMTris. HCl (pH 7.4), 10 % glycerol, 0.5 M NaCl, 10 mM DTT and 0.5 % NP-40 at room temperature. After the reaction was terminated at various time points, there were mixed with same volume of 2×loading buffer and heated at 90°C for 10 min, then analyzed by SDS-PAGE and stained with Coomassie brilliant blue R-250 (CBB R-250). Effects of the tested serine protease inhibitors was performed by mixing single-chain serine protease with appropriate amount of PMSF or EDTA at room temperature for 30 min before they were incubated with recombinant substrate NS5ab to pursue the reaction. 45 min later, the reaction was terminated and analyzed with SDS-PAGE and CBB R-250.

3.13.5. In vivo Assay for Anti-SSPE Activity

EC_{50}, CC_{50} of the tested compounds was determined utilizing hamsters with brain infections of SubacuteS clerosing Panencephalitis (SSPE). Under ether anesthesia, 50 mL of ribavirin or the appropriate tested compound solution at dosages of 5, 10, and 20 mg/kg/day was injected for 10 days intracranially to a depth of 2 mm by using a 27-gauge needle and was placed within the subarachnoid space. At 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were sacrificed. The brains were aseptically removed, washed twice with phosphate-buffered saline (PBS), homogenized, and suspended in PBS. The suspension was centrifuged at 1600 3g for 10 min. The supernatant was collected, ethanol was added to remove proteins, and the mixture was heated at 90°C to evaporate the ethanol. The protein-free samples were used to evaluate the EC_{50}, CC_{50} in brain tissue by HPLC and bioassay.

3.13.6. In vitro Assay for Anti-H1N1 Activity

The virus for this study was Influenza A (H1N1) virus strain A/PR/8/34 (ATCC, Manassas, VA; ATCC No. VR-1469). The tested synthesized compounds were dissolved in a minimal volume of EtOH (USP grade) prior to dilution in DMEM (pH 7.4). After infection, cells were fixed with Formalde-fresh then permeabilized Approximately 100 focus-forming units (FFU) of influenza virus were incubated with dilutions of the tested synthesized compounds solution in DMEM for 1 h at room temperature and then allowed to infect confluent MDCK cells for 1 h at room temperature with EtOH (USP). The FFU's were visualized using goat anti-influenza A virus IgG polyclonal antibody, rabbit Anti-Goat IgG (H&L) horseradish peroxidase conjugated affinity purified antibody (Chemicon, Temecula, CA) and AEC chromogen substrate (Dako, Carpinteria, CA).

3.13.7. In vitro assay for H1N1 NA1 Inhibitory Activity

The NA1 inhibitory ability of the new compounds was measured using fluorogenic substrate approach. The neuraminidase activity was measured using diluted allantoic fluid harvested from influenza virus-infected embryonated eggs and the fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA; Sigma). The test compound was incubated with diluted virus-infected allantoic fluid for 10 min at room temperature followed by the addition of 200 µM substrate. The fluorescence of the released 4-methylumbelliferone was measured in Envision plate reader (Perkin-Elmer, Wellesley, MA) using excitation and emission wavelengths of 365 and 460 nm, respectively. The inhibitor IC_{50} values were obtained from the dose–response curves by plotting the percent inhibition of NA activity *vs.* inhibitor concentrations using Prism 5 (GraphPad Software, Inc., San Diego, CA).

3.13.8. Molecular Modeling

The crystal structure of the viral enzymes HIV-1 RT (PDB ID: 1IKW), HCV NS3/4A serine protease (PDB ID: 4K8B) and H1N1 NA1 (PDB ID: 3B7E) with their respective co-crystallized ligands were acquired from the protein data bank for carrying out the docking. All docking procedures were performed by MOE (Molecular Operating Environment) software 2008.10 provided by chemical computing group, Canada. Docking on the active site of the selected enzymes was done for all synthesized derivatives for comparative purposes. Docking protocol was verified by re-docking of each co-crystallized ligand in the vicinity of the active site of the enzyme by specifying a 5 Å grid. The best scoring conformation for each compound was chosen for drug-ligand interaction pose analysis. All synthesized compounds showed good interaction with the active site amino acids of the enzymes under study with good binding energy scores (S) supporting their observed inhibitory activities.

4. RESULTS AND DISCUSSION

4.1. *In vitro* anti-HIV, HIV RT Inhibitory Activities and SAR Findings

The tested compounds were screened *in vitro* against HIV-1 to determine their EC_{50} values and to determine their cytotoxic CC_{50} values (Table **1**). Regarding the anti-HIV activity, all the tested compounds were found to be very potent and displaying EC_{50} values in the nanomolar range (EC_{50} = 1.3-4.5 nM). SAR observations for this class of benzimidazoles are summarized as follows. While the thiazolylhydrazine derivative **4** showed the highest anti-HIV activity in this study (EC_{50} = 1.3 nM), its thiazolylamino analog **5** was less potent (EC_{50} = 2.4 nM) and its methylthiazolyl counterpart **7** was even less active (EC_{50} = 3.2 nM). This indicates that while three analogs bear electron donating groups ($NHNH_2$, NH_2 and CH_3 for **4**, **5** and **7**, respectively) the highest activity was observed for the one that is capable of making more hydrogen bonds. Also, with regards to hydrophilicity it seems that the hydrophilic groups ($NHNH_2$ and NH_2, in **4** and **5**, respectively) like in are more in favor of activity than the lipophilic one (CH_3 in case of **7**). Adding a diazenyl moiety to **5** furnished the(un) substitutedphenyldiazenyl derivatives **6a-c**; only **6a** (EC_{50} = 1.3 nM) with unsubstitutedphenyldiazenyl moiety exhibited better activity than its precursor **5** while **6b** and **6c** bearing a p-CH_3 and p-Cl substituent on the phenyl diazenyl were less

potent than **5**. Regarding the diazenyl derivatives of **7**, derivatives **8a-c** were all more potent than their precursor, with **8a** and **8c** being equipotent (EC_{50} = 2.3 nM) and **8b** displaying the best activity (EC_{50} = 1.4 nM) coming as the second best active anti-HIV in this series after **4** and **6a** (EC_{50} = 1.3 nM).

Concerning the isosteric pair **20** and **22**, the phenylsulfonyl analog **22** (EC_{50} = 2.9 nM) was more potent than its phenylthio counterpart **20** (EC_{50} = 4.5 nM). Formation of diazenyl derivatives of both **20** and **22** to yield **21a-c** and **23a-c**, respectively led to an improvement in anti-HIV efficacy in all cases. While 4-methylphenyl analog **21b** (EC_{50} = 2.8 nM) was the most active amongst its group **21a-c**, the unsubstituted phenyl congener **23a** (EC_{50} = 1.8 nM) elicited the best anti-HIV effect in the **23a-c** series. Finally, the quinazolinylbenzimidazole derivative **12** (EC_{50} = 3.4 nM) did not seem to exhibit higher advantage over the other members of this series in terms of anti-HIV activity.

All derivatives in this study elicited low cytotoxic profiles (CC_{50} = 26.12-295.55 μM) leading to a high safety margin as reflected in their therapeutic index (TI = CC_{50}/EC_{50}) (TI = 11355.94-54768.67) being able to affect viral inhibition without causing cell toxicity (Table **1**).

To explore these compounds potential mechanism of action, they were evaluated for their reverse transcriptase (HIV-1 RT) catalytic activity inhibition ability. It was found that all derivatives (IC_{50} values between 13.23 and 55.98 μM) were powerful inhibitors of RT. Following a similar pattern to what was observed for the anti-HIV activity, the thiazolylhydrazine derivative **4** displayed the highest RT inhibitory power (IC_{50} = 29.44 μM) followed by its thiazolylamino analog **5** (IC_{50} = 38.43 μM) and finally their methylthiazolylbioisostere **7** (IC_{50} = 55.98 μM). Also, in alignment with the anti-HIV screening results, the phenylsulfonyl derivative **22** (IC_{50} = 34.54 μM) was found to be more active than its phenylthio analog **20** (IC_{50} = 47.89 μM). Derivatization of **5**, **7**, **20** or **22** to their diazenylcounterparts **6a-c**, **8a-c**, **21a-c** and **23a-c** always caused an improvement in RT inhibition, the only exception being **6a**. Again, the quinazoline derivative **12** only showed intermediate activity among tested compounds (IC_{50} = 35.09 μM) (Table **1**).

Table 1. Anti-HIV-1 and HIV-1 RT inhibition results.

Compound No	HIV-1			HIV-1RT
	EC_{50} (nM)[a]	CC_{50} (μM)[b]	TI[c]	IC_{50} (μM)[d]
4	1.3	59.49	45764.50	29.44
5	2.4	131.43	54764.40	38.43
6a	1.3	56.91	43775.60	48.53
6b	3.2	142.89	44654.67	37.54
6c	4.3	195.80	45535.78	26.75
7	3.2	103.82	32444.85	55.98
8a	2.3	26.12	11355.94	42.09
8b	1.4	31.31	22364.03	33.55
8c	2.3	30.99	13475.94	24.87
12	3.4	117.60	34586.85	35.09
20	4.5	295.55	65677.76	47.89
21a	3.4	186.21	54768.67	36.76
21b	2.8	122.81	43859.48	24.46
21c	3.7	121.17	32749.36	13.23
22	2.9	68.61	23658.45	34.54
23a	1.8	62.19	34547.64	21.36
23b	2.8	121.68	43456.83	23.45
23c	2.7	141.33	52345.73	22.45

[a] Compound concentration required to inhibit the virus induced cell killing by 50%.

[b] Compound concentration required to achieve 50% cytotoxicity to CEM-SS cells.

[c] TI: Therapeutic index is the cytotoxic concentration of a drug for 50% of the population (CC_{50}) divided by the effective concentration for 50% of the population (EC_{50}).

[d] Compound concentration required to achieve 50% inhibition of recombinant HIV-1 RT activity.

* for EC_{50} and CC_{50}, statistical comparison of the difference between control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test, $P < 0.05$.

4.2. Anti-HCV, NS3/4A Serine Protease Inhibition, anti-SSPE Activities and SAR Findings

The success of human immunodeficiency virus (HIV) protease inhibitors in treating HIV-infected patients has raised the hope that inhibitors of HCV NS3-4A serine protease could also become effective therapy options for hepatitis C patients.

Results obtained from determination of effective concentration and cytotoxic concentration (EC_{50}& CC_{50}) of the different tested compounds in HCV replicon cells are reported in Table **2**. The most active compound in anti-HCV assay was the thiazolylhydrazine derivative **4** (EC_{50} = 0.122 nM), the amino and the methyl counterparts of which **5** (EC_{50} = 0.263 nM) and **7** (EC_{50} = 0.236 nM) were less potent anti-HCV agents. On the other hand, for the biososteres **20** and **22**, the phenylsulphonyl analog **22** (EC_{50} = 0.333 nM) was slightly more potent than the phenylthio congener **20** (EC_{50} = 0.378 nM). As for the diazenyl derivatives of **6a-c**, **8a-c**, **21a-c** and **23a-c**, except for **21a-c** all the diazenyl analogs were less potent than their respective precursors. In fact, **21b** (EC_{50} = 0.15 nM) was almost 2.5 folds more potent than its precursor **20** (EC_{50} = 0.378 nM).On the other hand, the quinazoline derivative **12** showed intermediate potency (EC_{50} = 0.486 nM) compared to the other tested compounds.

Regarding the cytotoxicity of the synthesized analogs against CEM-SS cells, they were all found to be weakly cytotoxic (CC_{50} values in the range of 0.182 to 0.78 µM) and displaying high therapeutic indices (TI values between 1109.053 and 1973.333) (Table **2**).

Investigating the HCV NS3/4A protease inhibition assay, the thiazolyl derivatives **4**, **5** and **7** followed the same order of activity as reported in the anti-HCV screening (IC_{50} = 0.026, 0.056 and 0.05 nM, respectively). Furthermore, the phenylsufonyl derivative **22** was found to be more active than its phenylthio analog **20** (IC_{50} = 0.07 and 0.08 nM, respectively). Also and in compliance with the observed anti-HCV assay results, formation of daizenyl derivatives of the precursors **5**, **7**, **20** and **22** (**6a-c**, **8a-c**, **21a-c** and **23a-c**, respectively) caused a decrease in the NS3/4a inhibition capacity of the diazenyl products except for **21a-c** (IC_{50} =

Table 2. Anti-HCV, NS3/4A serine protease inhibition and anti-SSPE results.

Compound No	HCV			NS3/4A	SSPE
	EC_{50} (nM)[a]	CC_{50} (µM)[b]	TI[c]	IC_{50} (nM)[d]	EC_{50}µM
4	0.122	0.182	1491.803	0.026	1.43
5	0.263	0.314	1193.916	0.056	2.53
6a	0.379	0.425	1121.372	0.08	3.43
6b	0.468	0.546	1166.667	0.099	4.34
6c	0.357	0.465	1302.521	0.075	5.24
7	0.236	0.314	1330.508	0.05	4.35
8a	0.374	0.438	1171.123	0.079	3.46
8b	0.485	0.569	1173.196	0.102	2.67
8c	0.597	0.780	1306.533	0.125	1.78
12	0.486	0.539	1109.053	0.107	2.89
20	0.378	0.548	1449.735	0.08	3.70
21a	0.269	0.427	1587.361	0.057	4.68
21b	0.150	0.296	1973.333	0.036	5.57
21c	0.249	0.325	1305.221	0.05	5.45
22	0.333	0.514	1543.544	0.07	6.44
23a	0.434	0.623	1435.484	0.097	6.33
23b	0.546	0.763	1397.436	0.12	6.42
23c	0.434	0.642	1479.263	0.09	6.41

[a] Compound concentration required to inhibit the virus induced cell killing by 50%.

[b] Compound concentration required to achieve 50% cytotoxicity to CEM-SS cells.

[c] TI: Therapeutic index is the cytotoxic concentration of a drug for 50% of the population (CC_{50}) divided by the effective concentration for 50% of the population (EC_{50}).

[d] Compound concentration required to achieve 50% inhibition of HCV NS3/4a serine protease activity.

* for EC_{50} and CC_{50}, statistical comparison of the difference between control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test, $P < 0.05$.

0.036-0.057 nM). As for the quinazoline analog 12, it just displayed intermediate NS3/4A inhibition ability (IC_{50} = 0.107 nM) (Table **2**).

The good results obtained in the anti-HCV screening prompted us to test these compounds against the more resistant viral strain SubacuteSclerosingPanencephalitis (SSPE) in the hamster brain model. SSPE was more resilient to chemotherapy to the synthesized derivatives which displayed EC_{50} values between 1.43 and 6.44 μM (Table **2**).

4.3. Anti-H1N1, H1N1 NA1 Inhibition Activities and SAR Findings

A viral focus reduction assay was used to characterize the in vitro anti-influenza activity of the tested compounds. Human influenza A (H1N1) virus particles were used to infect Madin-Darby canine kidney NBL-2 (MDCK) cells. The tested compounds showed clear dose-dependent inhibition of H1N1 virus infection. The 50% effective concentration (EC_{50}) of the tested compounds for inhibition of H1N1 were achieved and tabulated in Table **3**. Also, the cytotoxic activity (CC_{50}) and the calculated therapeutic indices (TI) of these derivatives were determined and recorded in Table **3**. SAR findings for the anti-H1N1 assay showed that the activity of the thiazolylhydrazine **4**, thiazolylamine **5** and methylthiazole **7** analogs follow the order **4** ≈ **7**>**5** (EC_{50} values = 2.2, 2.4 and 3.3 nM, respectively). On the other hand, the phenylsulfonyl derivative **22** (EC_{50} values = 5.4 nM) showed only a slightly better activity when contrasted with its phenylthiobioisostere **20** (EC_{50} values = 5.8 nM). With respect to the un(substituted)diazenyl derivatives **6a-c**, **8a-c**, **21a-c** and **23a-c**, no specific pattern of variation of in anti-H1N1 efficacy was followed when they were compared with their precursors **5**, **7**, **20** and **21**, respectively. For **6a-c**, while the phenyl diazenyl derivative **6a** (EC_{50} values = 2.2 nM) was more potent than its precursor **5**, the chlorophenyl**6c** analog (EC_{50} values = 3.3 nM) was equipotent and the methyl counterpart **6b** (EC_{50} values = 4.3 nM) showed lower activity than **5**. On the other hand, preparation of the aryl diazenyl derivatives of **7** furnished the series **8a-c** yielding the most

Table 3. Anti-H1N1 and NA1 inhibition results.

Compound No	H1N1			NA1
	EC_{50} (nM)[a]	CC_{50} (μM)[b]	SI[c]	IC_{50} (nM)[d]
4	2.2	3.4	1545.455	0.3
5	3.3	4.6	1393.939	0.44
6a	2.2	3.3	1500	0.3
6b	4.3	5.4	1255.814	0.57
6c	3.3	3.7	1121.212	0.44
7	2.4	3.8	1583.333	0.36
8a	1.4	2.9	2071.429	0.19
8b	2.5	3.8	1520	0.36
8c	3.6	4.1	1138.889	0.48
12	4.7	5.9	1255.319	0.65
20	5.8	7.0	1206.897	0.78
21a	4.7	6.1	1297.872	0.64
21b	4.6	6.0	1304.348	0.63
21c	5.5	7.0	1272.727	0.72
22	5.4	6.9	1277.778	0.71
23a	6.3	7.8	1238.095	0.84
23b	6.2	7.8	1258.065	0.88
23c	7.3	9.1	1246.575	0.92

[a] Compound concentration required to inhibit the virus induced cell killing by 50%.

[b] Compound concentration required to achieve 50% cytotoxicity to CEM-SS cells.

[c] TI: Therapeutic index is the cytotoxic concentration of a drug for 50% of the population (CC_{50}) divided by the effective concentration for 50% of the population (EC_{50}).

[d] Compound concentration required to achieve 50% inhibition of H1N1 NA1 activity.

* for EC_{50} and CC_{50}, statistical comparison of the difference between control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test, $P < 0.05$.

potent anti-HN1 agent in this study **8a** (EC_{50} values = 1.4 nM), yet while **8b** (EC_{50} values = 2.5 nM) was almost equipotent with **7**, **8c** was less active (EC_{50} values = 3.6 nM). Furthermore, **21a-c** all displayed variable degrees of enhanced activity (EC_{50} values = 4.7, 4.6 and 5.5 nM, respectively) relative to their precursor **20** (EC_{50} values = 5.8 nM). In contrast, **23a-c** all exhibited lower potency (EC_{50} values between 6.2 and 7.3 nM) relative to their synthetic precursor **22** (EC_{50} values = 5.4 nM). Finally and yet again, the quinazolinederivative **12** only showed intermediate activity (EC_{50} values = 4.7 nM) relative to the other members of this series.

The cytotoxicity of the tested compounds was assessed against CEM-SS cells and they all elicited CC_{50} values in the micromolar level (CC_{50}s in the range of 2.9 to 9.1 µM). This high CC_{50} value relative to the recorded EC_{50} caused the compounds to have high therapeutic indices (TIs between 1121.212 and 2071.429).

Since themechanism of action of the synthesized compounds was thought to be *via* inhibition of neuraminidase 1 (NA1) enzyme, NA1 inhibition screening of all the new compounds was carried out and results are shown in Table **3**. The pattern of activity and SAR observations noted in the NA1 catalytic activity inhibition assay was in compliance with those attained in the anti-H1N1 screening. All the tested compounds inhibited NA1 at the subnanomolar level. Comparing the three thiazolyl analogs **4**, **5** and **7**, it was found that their activities wher in the order **4**>**7**>**5** (IC_{50}s = 0.3, 0.36 and 0.44 µM, respectively). Regarding the bioisosteric pair and as expected the phenylsulfonyl analog **22** was slightly more potent than the phenylthio analog **20** (IC_{50}s = 0.71 and 0.78 µM, respectively). With respect to the (un)substitutedphanyldiazenyl derivatives **6a-c**, **8a-c**, **21a-c** and **23a-c**, no consistent pattern was observed for alteration of their activity in comparison with their respective precursors **45, 6, 20** and **22** except for the fact that **23a-c** were all less effective enzyme inhibitors than their precursor. However, the most active compound in this study was found to bethephenyldiazenyl derivative **8a** with an IC_{50} of 0.19 µM. Finally, the quinazoline derivative **12** showed just intermediate NA1 inhibitor activity compared with the other members of this class.

4.4. Molecular Docking

In an attempt to better understand the binding mode of the new derivatives with their biological targets molecular docking experiments were performed using MOE software version 2008.10 employing the default parameters. Docking study was performed for all the synthesized compounds to assess their binding interaction mode with their potential viral enzyme targets HIV RT (PDB ID: 1IKW), HCV NS3/4A serine protease (PDB ID: 4K8B) and H1N1 NA1 (PDB ID: 3B7E). For validation purposes the co-crystallized ligands were docked in the active site of their respective enzymes and rmsd values were determined. The observed rmsd values were 0.6512 Å for efavirenz in the binding site of RT, 1.1022 Å for the ligand co-crystallized with NS3/4a serine protease and 0.413 Å for oseltamivir in the active pocket of NA1. Molecular binding simulation results of the most active compound in this study against each of the tested three viral enzymes are displayed in (Figs. **1-3**).

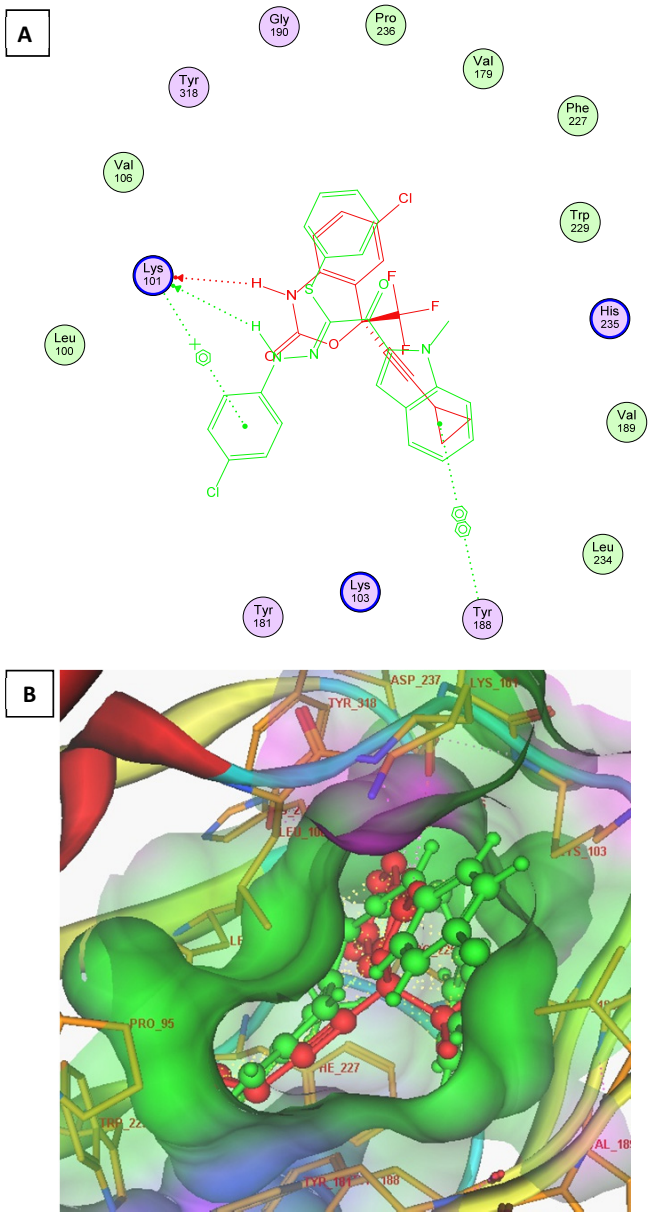


Fig. (1). 21c (green) and efazirenz (red) 2D (panel **A**) and 3D (panel **B**) overlay interactions in the active site of HIV-1 RT (hydrogen bonds are in pink and vdW interactions are in yellow).

Pertaining to the HIV RT docking results, **21c** was found to bind to more strongly to the active pocket of RT than the native ligand efavirenzas judged by their binding energy scores (S values = -13.2165 Kcal/mol and -10.9087 Kcal/mol, respectively). Also, it was observed that while efavirenz binds to RT with only one H-bond between its oxazine NH and Lys101, **21c** was able to make two interactions with Lys101, one hydrogen bond through its diazenyl NH and one pi-cationic interactions through its 4-chlorophenyl ring (Fig. **1**, panels A). The 2D and 3D overlay examination showed that **21c** binds in the same region as the

native ligand efavirenz indicating that **21c** recognizes the active site of RT like well known RT inhibitors (Fig. **1**, panels A and B).

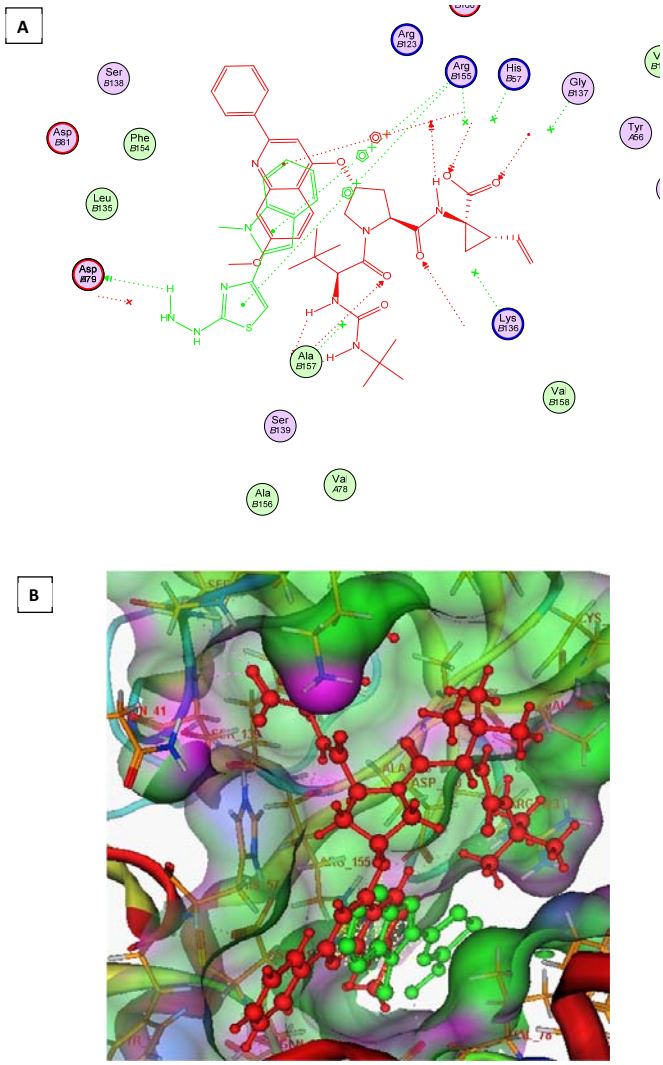


Fig. (2). 4 (green) and co-crystallized ligand (red) 2D (panel **A**) and 3D (panel **B**) overlay interactions in the active site of NS3/4A (hydrogen bonds are in pink and vdW interactions are in yellow).

Concerning the HCV NS3/4A serine protease docking results, while the native ligand was found to bind with the active site amino acids His57, Lys136, Gly137, Arg 155 and Arg157 by a network of interactions (S= -15.7227 Kcal/mol), **4** was able to form only two pi-cationic interactions through its imidazole and thiazole moieties along with Arg155 and one hydrogen bond between the hydrazine NH and Asp79 (S = -9.9177 Kcal/mol) (Fig. **2**, panels A). However, the 2D and 3D overlay inspection showed that **4** binds in the same region as the native ligand macromolecule indicating that **4** recognizes the active site of NS3/4A (Fig. **2**, panels A and B).

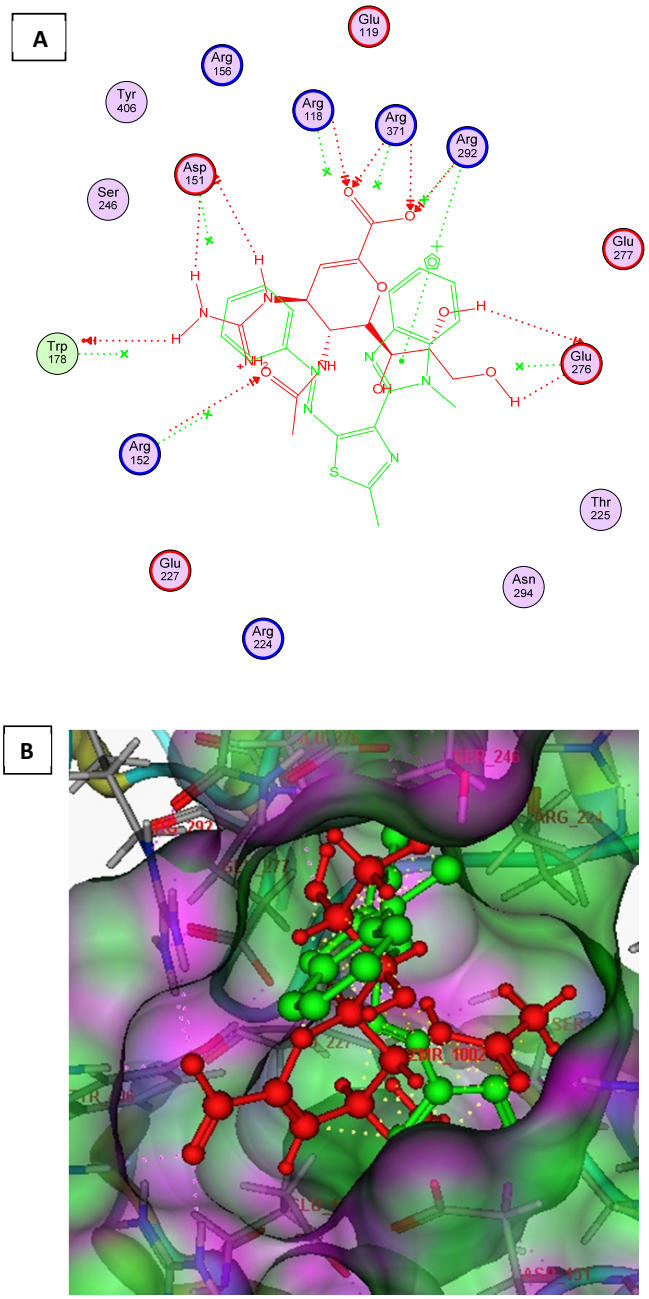


Fig. (3). 8a (green) and oseltamivir (red) 2D (panel **A**) and 3D (panel **B**) overlay interactions in the active site of N1H1 NA1 (hydrogen bonds are in pink and vdW interactions are in yellow).

With regards to the H1N1 NA1 docking findings, **8a** was found to interact with NA1 by just one pi-cationic interaction with Arg292 (S = -11.043 Kcal/mol) (Fig. **3**, panel A). Oseltamivir on the other hand binds with NA2 by six hydrogen bonds with Glu119, Asp151, Arg152, Arg292 and Arg371 (S = -23.8741 Kcal/mol) (Fig. **3**, panel A). The 2D and 3D overlay of **8a** and oseltamivir shows that **8a** can appropriately distinguish the active site of the enzyme and its

essential amino acids in a fashion comparable to oseltamivir (Fig. **3**, panels A and B, respectively). In spite of the fact that the observed docking interactions of **8a** doesn't well support the NA1 enzyme inhibition assay findings, yet the *in vitro* enzyme assay results can be explained in terms of possible water mediated hydrogen bonds acting as bridges between **4** and the active site amino acids which were not simulated *in silico*.

5. CONCLUSION

In the investigation described above, a new series of substituted benzo[d]midazoles was prepared *via* reactions of 2-bromo-1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)ethanone (**1**) or 1-(1-methyl-1*H*-benzo[d]imidazole-2-yl)-2-thiocyanatoethanone (**2**) with various reagents. Thiazolyl derivatives **5**, **6**, **7** and the diazenyl analogs of **7** (**8a-c**) as well as the phenylthio **20** and phenylsulfonyl **22** derivatives and their respective diazenyl counterparts **21a-c** and **23a-c** were prepared. Also, a quinazolinecogener (**12**) was synthesized.

In this paper, we showed that over the past decades benzo[d]midazoles have achieved an important place in the arsenal of organic chemists involved in the construction of complex molecules and biologically active molecules.

The derivatives we have synthesized were screened for antiviral potential against HIV-1, HCV, SSPE and H1N1 and have all displayed high potency against the examined viruses, indicating that they possess broad spectrum antiviral activity.

Also, the compounds were tested *in vitro* and *in silico* for their enzyme inhibition and binding abilities with the target viral enzymes HIV-1 RT, HCV NS3/4A and H1N1 NA1. These enzymes are essential enzymes for the progression of the viral life cycles of their respective enzymes. All compounds were found to be active, potent inhibitors of the investigated enzymes and displayed high degree of active site recognition ability in the *in silico* molecular modeling experiments.

Finally, the prepared molecules can be visualized as promising leads for synthesis and optimization of novel broad spectrum and potent antiviral benzo[d]imidazole agents.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] WHO: Global alert and response; http://www.who.int/csr/disease/en/, last accessed April 4th, **2014**.

[2] Sanjua´n, R.; Combe, M. Variation in RNA virus mutation rates across host cells. *PLos Pathog.,* **2014**, *10*, e1003855.

[3] M.A. Raslan. Heterocyclic Fused Rings with Bridgehead Nitrogen Atoms: Single-StepSynthesis of Azolo[1 ",2":1 ',2^pyrido[5',6':5,4] pyrimido[1,6-a]benzimidazole;Pyrido[1,2-a]benzimidazole; Pyrido [4", 5":2',3']pyrido[6',5':4,5]pyrimido-[1,6-a]benzimidazole and Polysubstituted Pyridine Derivatives. *J. Chinese Chem. Soc.,* **2000***, 47,* 961-965.

[4] Bartlett, M. S.; Edlind, T. D.; Durkin, M. M.; Shaw, M. M.; Queener, S. F.; Smith, J. W. Antimicrotubule benzimidazoles inhibit in vitro growth of *Pneumocystis carinii*. *Antimicrob. Agents. Chemother.,* **1992**, *36*, 779-782.

[5] Benes, L.; Borovansk, A.; Csoellei, J.; Rada, B. substituted 1-phenylcarbamoyl benzimidazoles with potential antiviral effect Czech. Pat. 1983, 206 956, (*Chem. Abstr*. **1984**, *101*, 7152).

[6] Gill, C.; Jadhav, G.; Shaikh, M.; Kale, R.; Ghawalkar, A.; Nagargoje, D.; Shiradkar, M. Clubbed [1,2,3]triazoles by fluorine benzimidazole: a novel approach to H37Rv inhibitors as a potential treatment for tuberculosis. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 6244-6247.

[7] Dhage, A. N.; Joshi, N. S.; Wadokar, S. G.; Kasture, A. V. *Indian Drugs,* **1986**, *23*, 601 (Chem. Abstr. 1987, 106, 168513).

[8] Gbadamassi, M.; Barascut, J. L.; Imbach, J. L.; Gayral, P. 5-Nitro-1-nonyl-1H-benzimidazol-2(3H)-one. *Eur. J. Med. Chem.,* **1988**, *23*, 225-232.

[9] Preston, P.N. Synthesis, reactions, and spectroscopic properties of benzimidazoles. *Chem. Rev.,* **1974**, *74*, 279.

[10] Maekawa, K.; Taniguchi, E.; Kuwano, E.; Shuto, Y. Pesticides derived from amino acids. *Environ. Qual. Saf. Suppl.,* **1975**, *3*, 749.

[11] D'Amico, J. the title compounds show activity as central nervous system depressants are useful as neuroleptic agent, and inhibit Apo morphine-induced vomiting in dogs J. U.S. Pat. 1965, 3 225 059 (Chem. Abstr. **1966**, *64*, 8193).

[12] Hasegawa, G.; Kotani, A. plant growth-regulating thiazolo benzimidazoles Jpn. Pat. 1974, 7 495 997 (Chem. Abstr. **1975**, *82*, 156299).

[13] Otsuka Pharmaceutical Co., Jpn. Pat. 1982, 82 109 789 (Chem. Abstr. **1982**, *87*, 182457).

[14] Altisent, M. R. C.; Constansa, J. F.; Corominas. J. P. Eur. Pat. 1992, 507 696, (Chem. Abstr. **1993**, *118*, 124568).

[15] Werner, W.; Letsch, G.; Ihn, W.; Sohr, R.; Preiss, R. Synthesis of a potential metabolite of the anticancer drug bendamustin cytostasan. *Pharmazie,* **1991**, *46*, 113-114.

[16] Petyunin, P. A.; Chaudri, A. M.; Kuznetsova, N. L. *Farm Zh. (Kiev)*, **1984**, *2*, 63 (Chem. Abstr. 1984, 101, 122559).

[17] Cox, D.; Hall, D. E.; Ingall, A. H.; Suschitzky, preparation ofbenzimidazolyl)methyl anilines as gastric acid secretion inhibitors and cytoprotectants [(J. L. Eur. Pat. 1988, 251 536, (Chem. Abstr. **1988**, *109*, 6517).

[18] Diamond, J.; Wohl, R. A. US Pat. 1984, 4 434 174 (Chem. Abstr. **1984**, *101*, 7157).

[19] Farag, A. M.; Ali, K. A. K.; El-Debss, T. M. A.; Mayhoub, A. S.; Amr, A. E.; Abdel-Hafez, N. A.; Abdulla, M. M. Design, synthesis and structure-activity relationship study of novel pyrazole-based heterocycles as potential antitumor agents. *Eur. J. Med. Chem.,* **2010**, *45*, 5887-5898.

[20] Farag, A.M.; Mayhoub, A.S.; Eldebss, T.M.A.; Amr, A.E.; Ali, K.A.K.; Abdel-Hafez N.A.; Abdulla, M.M. Synthesis and structure-activity relationship studies of pyrazole-based heterocycles as antitumor agents. *Arch. Pharm. Chem. Life,* **2010**, *343*, 384-396.

[21] Dawood, K.M.; Eldebss, T.M.A.; El-Zahabi, H.S.A.; Yousef, M.H.; Metz, Synthesis of some new pyrazole-based 1,3-thiazoles and 1,3,4-thiadiazoles as anticancer agents. P. *Eur J. Med. Chem.,* **2013**, *70*, 740-749.

[22] El-Sherif, A.A.; Eldebss, T.M.A. Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu(II), Ni(II), Mn(II), Co(II) and Zn(II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2-aminomethylthiophene. *SpectrochimicaActa Part A.,* **2011**, *79*, 1803-1814.

[23] Eldebss, T.M.A.; Farag, A.M. A convenient route for synthesis of new benzimidazole-based heterocycles. *Iranian J. Org. Chem.,* **2013**, *5*, 1005-1013.

[24] Zarling, J.M.; Moran, A.; Haffar, O.; Diegel, M.; Myers, D.E.; Kuelbeck, V.; Ledbetter, J.A.; Uckun, F.M. *Inhibition of HIV-1 replication in seropositive patients' CD4+ T-cells by pokeweed antiviral protein-monoclonal antibody conjugates. Int. J. Immunopharmacol.,* **1991**, *13*, 63-68.

[25] Lin, K.; Kwong, A. D.; Lin, C. Combination of a hepatitis C virus NS3-NS4A protease inhibitor and alpha interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in replicon cells. *Antimicrob. Agents Chemother.,* **2004**, *48*, 4784-4792.

[26] Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science,* **1999**, *285*, 110-113.

[27] Mekky, A.E.M.; Darweesh, A.F.; Salman, A.A.; Farag, A.M. Synthesis of novel thiazole and 1,3,4-thiadiazole derivatives incorporating phenylsulfonyl moiety. *Heterocycles,* **2014**, *89*, 1827-1843.

Received: September 18, 2014 Revised: July 08, 2015 Accepted: August 31, 2015