



# Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: [www.ajpamc.com](http://www.ajpamc.com)



## DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 1, 2, 4-TRIAZOLE DERIVATIVES BASED SCHIFF'S BASES BEARING BENZIMIDAZOLE MOIETY

Noha. R. M. Gabr<sup>1,2</sup>, A. Sh. El-Etrawy<sup>2</sup>, Hanan A. Abdel-Fattah\*<sup>1</sup>, Said A. H.El-Feky<sup>1</sup>

<sup>1</sup>\*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig-44519, Egypt.

<sup>2</sup>Department of Pharmaceutical Organic Chemistry, College of Pharmacy and Drug Manufacturing, Misr University for Science and Technology, Al-Motamayez District, 6<sup>th</sup> of the October, P. O. Box: 77, Egypt.

### ABSTRACT

A series of some newly Schiff-base 3a-h and 8 were synthesized by condensation of some aromatic aldehydes with 1, 2, 4-triazole derivatives 2 and 6, respectively. The biological screening of the synthesized compounds against microorganism showed that all of them possessing high activity against fungi (*Candida albicans*). Compounds 3a, 3c, 3f, 3g and 6, show high activity against gram- positive and gram- negative bacteria. Compounds 3d, 3f and 8 showed the highest potency at low  $\mu\text{g/ml}$  level against breast carcinoma (MCF-7) and colon carcinoma (HCT116) cell lines respectively. The docking calculations were carried out in order to rationalize the obtained biological results.

### KEYWORDS

Benzimidazole, 1, 2, 4-Triazole, Schiff's base, Anti-bacterial, Anti-fungal and Anti-cancer.

### Address for Correspondence:

Hanan A. Abdel-Fattah,  
Department of Pharmaceutical Organic Chemistry,  
Faculty of Pharmacy, Zagazig University, Zagazig,  
Egypt.

**Email:** [dr\\_hanan500@yahoo.com](mailto:dr_hanan500@yahoo.com)

### INTRODUCTION

Research and development of potent and effective antimicrobial agents represents one of the most important advances in therapeutics, not only in the control of serious infections, but also in the prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. Over the past decade, microbial infection became an important complication and a major cause of morbidity and mortality in immuno-compromised individuals such as those suffering from tuberculosis, cancer and AIDS and in organ transplantation cases<sup>1</sup>.

Antimicrobial agents are considered “miracle drugs” that are our leading weapons in the treatment of infectious diseases. Antimicrobial resistance is the ability of certain microorganism to withstand attack by antimicrobials, and the uncontrolled rise in resistant pathogens threatens lives and wastes limited healthcare resources. Life-threatening infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world. Consequently, the development of newer antimicrobial agents will remain an important challenging task for medicinal chemists<sup>2</sup>. So, there is an urgent need for identification of novel lead structures for the designing of new, potent and less toxic agents which ideally shorten the duration of therapy and are effective against resistant strains<sup>3</sup>. Schiff’s bases are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to presence of imine (N=CH-) which imports in elucidating the mechanism of transformation and racemization reaction in biological system<sup>4</sup>. These compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation<sup>5</sup>. In particular, triazoles and their heterocyclic derivatives have been reported to be used as drugs and to have considerable biological activities such as analgesic, anthelmintic, antitubercular, plant growth regulating, antiviral, antifungal, antibacterial and anticancer<sup>6-12</sup>. Schiff bases of 1,2,4-triazoles possess extensive biological activities<sup>13-17</sup>. On the other hand, benzimidazole and its derivatives have a pharmaceutical and biological activities such as, antimicrobial, antioxidant, antiviral, antihypertensive, antiprotozoal, anti-inflammatory and molluscicidal agents<sup>18-26</sup>. Due to immense pharmacological significance of the Schiff bases, triazoles and benzimidazole derivatives and in continuation of our effort to find potential biologically active molecules, herein we report synthesis and design of some Newly 1,2,4-triazole derivatives based Schiff’s bases bearing benzimidazole moiety and evaluate their activities against bacteria, fungi and tumor.

## MATERIALS AND METHODS

### Experimental

All melting points are uncorrected, that were determined using a Kofler block instrument. IR spectra were recorded with a Perkin-Elmer model 1720 FTIR (KBr), <sup>1</sup>H, <sup>13</sup>CNMR spectra were recorded with Bruker AC 300 FT NMR spectrometer at 300MHz with TMS as an internal standard. MS spectra were recorded with a Shimadzu QP-2010 Plus. The elemental analysis and the evaluation of cytotoxicity against (MCF7) cell line and (HCT-116) cell line were carried at The Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. The antimicrobial evaluation was carried in the microbiology department, Faculty of pharmacy, Zagazig University, Zagazig, Egypt.

Ethyl 2-(1*H*-benzo[*d*]imidazol-1-yl) acetate (1) and 2-(2-(1*H*-benzo[*d*]imidazol-1-yl)acetyl)-*N*-arylhydrazinecarbothioamide (4,5) were prepared according to reported methods<sup>12,27,28</sup>.

### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-amino-4*H*-1,2,4-triazole-3-thiol (2)<sup>29</sup>

To a solution of the ester derivative **1** (10 mmol) in sodium methoxide (10 mmol) in methanol (25 ml) was added an equivalent amount of thiocarbohydrazide (10 mmol). The mixture was refluxed for 10 hours. The reaction mixture was evaporated to dryness; the residue was dissolved in 40 ml H<sub>2</sub>O and acidified with conc. hydrochloric acid to pH ~ 4-5. The precipitate was collected, washed with cold water, dried and crystallized from ethanol to afford **2**.

Yield: 83 %, m.p. 229-232°C as reported<sup>30</sup> IR (KBr,  $\nu$  cm<sup>-1</sup>), 3300, 3251, 3149 (NH, NH<sub>2</sub>), 3086, 3055 (CH, aromatic), 2927, 2856 (CH, aliphatic), 1616 (C=N), 1577(C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 5.58 (s, 2H, NCH<sub>2</sub>), 5.65 (s, 2H, NH<sub>2</sub> D<sub>2</sub>O exchangeable), 7.18-7.67 (m, 4H, Ar-H,), 8.27 (s, 1H, C2 imidazole), 14.20 (s, 1H, NH, D<sub>2</sub>O exchangeable). MS: *m/z* (%) 247 (13.64) M<sup>+</sup>+1, 158 (21.77), 119 (100). Microanalysis for: C<sub>10</sub>H<sub>10</sub>N<sub>6</sub>S (246.07), Calcd: % C, 48.77; H, 4.09; N,34.12; Found:%C,48.89; H, 4.13; N,34.29.

### Procedure for the Preparation of 3a-h

A mixture of **2** (5 mmol), aromatic aldehyde (5 mmol), and glacial acetic acid (5 drops) in ethanol (10 ml) was refluxed for 6 hours. The solid products were collected, dried, and crystallized from ethanol to give **3a-h** in 67-92 % yields.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(benzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione (3a)

Yield 83 %; m.p 209-211 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3128, (NH), 3056 (CH aromatic), 2977,2928 (CH aliphatic), 2741 (SH), 1615 (C=N), 1558 (C=C), 1478 (C-N),1241(C=S); <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.08 (s, 2H, NCH<sub>2</sub>), 7.54-7.90 (m, 9H, Ar-H), 9.60 (s, 1H, C2 imidazole), 10.11 (s, 1H, N=CH) 14.18 (s, 1H, NH, D<sub>2</sub>O exchangeable); MS: *m/z* (%) 335 (69.23) M<sup>+</sup>+ 1, 334 (89.74) M<sup>+</sup>, 332(80.77), 327 (100); Microanalysis: for: C<sub>17</sub>H<sub>14</sub>N<sub>6</sub>S (334.3). Calcd: % C, 61.06; H, 4.22; N, 25.13. Found: % C, 61.19; H, 4.18; N, 25.24.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(2-chlorobenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione (3b)

Yield 67 %; m.p 248-250 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3396, (NH), 3057, 3068 (CH aromatic)2947, 2895 (CH aliphatic), 2709 (SH), 1602 (C=N), 1598 (C=C), 1462 (C-N), 1276 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 5.80 (s, 2H, NCH<sub>2</sub>), 7.21-8.33 (m, 8H, Ar-H), 8.77 (s, 1H, C2 imidazole), 10.87 (s, 1H, N=CH) 14.10 (s, 1H, NH, D<sub>2</sub>O exchangeable); MS: *m/z* (%) 370 (15.20) M<sup>+</sup> + 1, 302 (18), 164 (21.2), 136 (54.5), 116 (84.80), 115 (60.60), 51(100). Microanalysis: for: C<sub>17</sub>H<sub>13</sub>ClN<sub>6</sub>S (368.8). Calcd: % C, 55.36; H, 3.55; N, 22.78. Found: % C, 55.49; H, 3.58; N, 22.89.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(4-chlorobenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione (3c)

Yield 83 %; m.p 245-247 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3124, (NH), 3054 (CH aromatic) 2922 (CH aliphatic), 2734 (SH), 1597 (C=N), 1488 (C=C), 1450 (C-N), 1269 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.04 (s, 2H, NCH<sub>2</sub>), 7.47-7.94 (m, 8H, Ar-H), 9.36 (s, 1H, C2 imidazole), 10.18 (s, 1H, N=CH) 14.14 (s, 1H, NH, D<sub>2</sub>O exchangeable). <sup>13</sup>CNMR(75MHz,DMSO-

*d*<sub>6</sub>), $\delta$ ppm:56.01,113.13,115.66, 126.22, 129.32, 130.37, 137.46, 142.97, 154.62, 161.24, 162.25, 192.09.

MS: *m/z* (%) 369 (9.74) M<sup>+</sup>, 368 (11.04), 231 (24.68), 137 (100), 102 (65.10), 75 (41.70), Microanalysis: for; C<sub>17</sub>H<sub>13</sub>ClN<sub>6</sub>S (368.8). Calcd: % C, 55.36; H, 3.55; N, 22.78. Found: % C, 55.43; H, 3.59; N, 22. 91.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(2-bromobenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione 3d

Yield 91 % m.p 224-226 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3117, NH, 3051 (CH aromatic) 2912 (CH aliphatic),2751 (SH), 1552 (C=N), 1496 (C=C), 1447 (C-N), 1268 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.08 (s, 2H, NCH<sub>2</sub>), 7.55-8.14 (m, 8H, Ar-H), 9.59(s, 1H,C2 imidazole), 10.91 (s, 1H, N=CH) 14.20 (s, 1H, NH D<sub>2</sub>O exchangeable). MS: *m/z* (%) 414 (24.14) M<sup>+</sup>+1, 413 (27.97), M<sup>+</sup>, 287 (37.16), 275 (38.31), 139 (38.31), 74 (40.61), 63 (100). Microanalysis: for; C<sub>17</sub>H<sub>13</sub>BrN<sub>6</sub>S (413.2) Calcd: % C, 49.40; H, 3.17; N, 20.33 Found: % C, 49.51; H, 3.19; N, 20. 40.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(4-bromobenzylideneamino)-2*H*-1,2,4-triazole-3(4*H*)-thione(3e)

Yield 92 %, m.p 235-237 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3123, NH, 3055 (CH aromatic) 2924 (CH aliphatic), 2750(SH) , 1592 (C=N), 1554 (C=C), 1482 (C-N), 1267(C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.08 (s, 2H, NCH<sub>2</sub>), 7.62-8.03 (m, 8H, Ar-H), 9.68 (s, 1H, C2 imidazole), 10.18 (s, 1H, N=CH) 14.18 (s, 1H, NH, D<sub>2</sub>O exchangeable). MS: *m/z* (%) 414 (49.52) M<sup>+</sup>+1, 413 (72.38) M<sup>+</sup>, 396 (66.67), 135 (100). Microanalysis: for C<sub>17</sub>H<sub>13</sub>BrN<sub>6</sub>S (413.2). Calcd: % C, 49.40; H, 3.17; N, 20.33. Found: % C, 49.53; H, 3.22; N, 20.51.

#### 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(4-methylbenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione(3f)

Yield 76 %; m.p. 249-251 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3111, (NH), 3055, 3084 (CH aromatic)2985, 2939(CH aliphatic), 2708 (SH), 1616 (C=N), 1602 (C=C), 1423 (C-N), 1282(C=S);<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.41 (s, 3H, CH<sub>3</sub>), 5.75 (s, 2H, NCH<sub>2</sub>), 7.20-7.80 (m, 8H, Ar-H), 8.30(s, 1H, C2

imidazole), 9.96 (s, 1H, N=CH)14.0 0(s, 1H, NH D<sub>2</sub>O exchangeable). MS: *m/z* (%) 350 (3.60) M<sup>+</sup>+2, 349 (2.40) M<sup>+</sup> + 1, 348 (7) M<sup>+</sup>, 158 (11.70), 116 (73.60). Microanalysis: for C<sub>18</sub>H<sub>16</sub>N<sub>6</sub>S (348.4). Calcd: % C, 62.05; H, 4.63; N, 24.12. Found: % C, 62.17; H, 4.69; N, 24.30.

**5-((1*H*-benzo[*d*]imidazol-1-yl)methyl)-4-(3,4,5-trimethoxybenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione (3*g*)**

Yield 89%.m.p 151-153 °C. IR(KBr,cm<sup>-1</sup>) 3103,(NH),3041 (CH aromatic)2974 ,2930 ( CH aliphatic) , 2738(SH),1573(C=N), 1491 (C=C), 1455 (C-N),1230(C=S) <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)δ ppm 3.87(s, 9H,3OCH<sub>3</sub>), 6.08 (s,2H, NCH<sub>2</sub>), 7.18-7.92(m, 6H,Ar-H,), 8.84 (s, 1H,C2 imidazole) ,9.67 (s,1H,N=CH) 14.16 ( s, 1H, NH, D<sub>2</sub>O exchangeable), MS :*m/z* 426 (0.87)M<sup>+</sup>+2, 425 (2.73)M<sup>+</sup>+1, 424 (8.72)M<sup>+</sup>,406(4.33) 231(35.75), 193(100). Microanalysis: for: C<sub>20</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub>S (424.4). Calcd: %C, 56.59; H, 4.75; N, 19.80 Found: %C, 56.73; H, 4.82; N, 19.97.

**5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(4-nitrobenzylideneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione( 3*h*)**

Yield 76 %; m.p. 225-227 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3120, (NH), 3042 (CH aromatic),2918 (CH aliphatic), 2737 (SH), 1557 (C=N), 1481 (C=C),1517, 1344 (NO<sub>2</sub>) 1255(C=S); <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.07 (s, 2H, NCH<sub>2</sub>), 7.55-8.40 (m, 8H, Ar-H), 9.46 (s, 1H, C2 imidazole), 10.50 (s, 1H, N=CH) 14.24 (s, 1H, NH D<sub>2</sub>O exchangeable); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 61.33 , 113.35 , 115.36 , 124.14 , 126.19 , 129.79 , 131.29 , 137.87 , 142.86 , 149.51 , 158. 81 , 162.33. MS: *m/z* (%) 381 (0.62) M<sup>+</sup>+2, 380 (2.24) M<sup>+</sup> + 1, 379 (8.79) M<sup>+</sup>, 376 (0.14), 264 (0.46), 231 (100), 118 (91.04). Microanalysis: for C<sub>17</sub>H<sub>13</sub>N<sub>7</sub>O<sub>2</sub>S (379.4). Calcd: % C, 53.82; H, 3.45; N, 25.84. Found % C, 54.02; H, 3.43; N, 25.97.

**5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-*N*-aryl-4*H*-1,2,4-triazol-3,4-diamine (6,7)**

**General procedure**

To a suspension thiosemicarbazide **4**, **5** (10 mmol) in methanol (10 ml), hydrazine hydrate (4 mmol, 2.2 ml) was added. The reaction mixture was heated in water bath for 12 hours then pour into crushed ice.

The solid product was collected, and crystallized from ethanol.

**5-[(1*H*-benzo[*d*]imidazol-1-yl) methyl]-*N*-phenyl-4*H*-1,2,4-triazol-3,4-diamine ( 6)**

Yield: 79 %; m.p. 281-283 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3421, 3246 (NH, NH<sub>2</sub>), 3029 (CH aromatic), 2915 (CH aliphatic), 1587 (C=N), 1494 (C=C), 1414 (C-N). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.42 (s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 5.49 (s, 2H, NCH<sub>2</sub>), 7.19-7.63 (m, 9H, Ar-H), 7.71 (s, 1H, C2 imidazole), 13.90 (s, 1H, NH D<sub>2</sub>O exchangeable). MS: *m/z* (%) 307 (62.07) M<sup>+</sup> + 2, 305 (74.71) M<sup>+</sup>, 292 (100), 291 (64.37).

Microanalysis: for C<sub>16</sub>H<sub>15</sub>N<sub>7</sub> (305.3). Calcd: % C, 62.94; H, 4.95; N, 32.11. Found: % C, 63.08; H, 5.03; N, 32.39.

**5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-*N*-(4-methoxy phenyl)-4*H*-1,2,4-triazol-3,4-diamine (7)**

Yield 77 %; m.p. 273-276 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3431, 3180 (NH, NH<sub>2</sub>), 3090 (CH aromatic), 2969, 2904 (CH aliphatic), 1603 (C=N), 1514 (C=C), 1453 (C-N). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.37 (s, 2H, NH<sub>2</sub> , D<sub>2</sub>O exchangeable) 3.89 (s, 3H, OCH<sub>3</sub>), 5.51 (s ,2H, NCH<sub>2</sub>), 7.09-7.71 (m, 8H, Ar-H), 7.79 (s, 1H, C2 imidazole), 13.94 ( s, 1H, NH, D<sub>2</sub>Oexchangeable); <sup>13</sup>CNMR(75MHz,DMSO-*d*<sub>6</sub>), $\delta$ ppm: 55.48 ,60.10 , 114.62 , 119.41 , 121.73 , 122.50 , 125.30 , 129.06 , 133.58 , 143.91 , 148. 02, 159.85

MS: *m/z* (%) 337 (59.77) M<sup>+</sup> + 2, 335 (80.46) M<sup>+</sup>,319 (59.77), 285 (68.97). Microanalysis: for C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O (335.36). Calcd: % C, 60.8; H, 5.11; N, 29.24. Found: % C, 61.04; H, 5.17; N, 29.39.

**5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-(2-chlorobenzylideneamino)-*N*-phenyl-4*H*-1,2,4-triazol-3-amine ( 8 )**

To a solution of compound **6** (3.05 g, 10 mmol) in absolute ethanol (10 ml) equivalent amount of 2-chlorobenzaldehyde (1.1 g, 10 mmol) was added with catalytic amount of glacial acetic acid (2 drops) and the reaction mixture was heated under refluxed temperature for 6 hours, the solid product was collected and crystallized from ethanol.

Yield 86 %; m.p. 213-215 °C. IR (KBr,  $\nu$  cm<sup>-1</sup>): 3130 (NH), 3061 (CH, aromatic), 2950 (CH, aliphatic), 1671 (C=N), 1609 (C=C), 1432 (C-N). <sup>1</sup>H

NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 5.70 (s, 2H, NCH<sub>2</sub>), 7.44-7.60 (m, 13H, Ar-H), 8.16 (s, 1H, C2 imidazole), 8.96 (s, 1H, N=CH) 9.72 (s, 1H, NH, D<sub>2</sub>O exchangeable). MS: *m/z* (%) 428(5.19) M<sup>+</sup>, 427(7.79), 241 (47.05), 75 (100). Microanalysis: for C<sub>23</sub>H<sub>18</sub>ClN<sub>7</sub> (427.89). Calcd: % C, 64.56; H, 4.24; N, 22.91. Found: % C, 64.67; H, 4.74; N, 22.91.

## BIOLOGICAL SCREENING

### Antimicrobial activity test

All the newly synthesized compounds were evaluated for *in vitro* antimicrobial activity against Gram positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, Gram negative bacteria as *Pseudomonas aeruginosa* and *Escherichia coli* and Fungi such as *Candida albicans* at concentration 50 mg/ml by agar well diffusion method<sup>31,32</sup> as modified from NCCLS. Mueller-Hinton agar plates were surface-inoculated with the tested strains suspensions adjusted to match 0.5 McFarland standard and the inoculate were spread over the surfaces of the plates using sterile cotton swabs. After drying of the plates, cups (10 mm diameter) were punched in the agar and 100  $\mu$ l of the samples in DMF or the antimicrobial agents were added into the wells. The plates were incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The test was repeated three times and the mean inhibition zones were calculated. A total of five standard microbial strains were used in this study obtained from the Egyptian Pharmaceutical Industries Company (EPICO). DMF used as solvent control, nutrient agar was employed as culture media. The activity was compared with Cefotaxim as positive control for bacteria and Nystatin for fungi. The results were represented in (Table 1). All the newly synthesized compounds showed promising antibacterial and antifungal activities.

### The anticancer activity (*In vitro* Antitumor activity)<sup>33-35</sup>

The cell lines were grown as monolayers in growth medium supplemented with 10% inactivated fetal calf serum and 50 $\mu$ g/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate (Falcon, NJ,

USA) incubated for 24h at 37°C in a humidified incubator with 5%CO<sub>2</sub>. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100  $\mu$ l from different dilutions of tested compound in fresh maintenance medium and incubated at 37°C. A control of untreated cells was made in the absence of the tested compound. Three wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation and the percentage of viability was calculated as  $[1-(OD_t/OD_c)] \times 100\%$  where OD<sub>t</sub> is the mean optical density of wells treated with the tested compounds and OD<sub>c</sub> is the mean optical density of untreated cells. The cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 $\mu$ g/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were subcultured two to three times a week. The results were represented in Table No.2.

## DOCKING

### Cancer cell lines assays

#### Molecular Modelling Job

The ligands were sketched and subjected to geometry optimization by running energy minimization using Sybylx1.1 (2010) program suite. Parameters of energy minimization as follows: Charges: Gasteiger-Marsili Charges; Force Field: Tripos; Termination: Gradient energy change, 1.1 Kcal/ Å ; RMS displacement: 0.001Å; Non-bonded cutoff: 8.000Å; Dielectric function: distant dependent; Dielectric constant: 1.00; Iteration: 100. The crystal structure of epidermal growth factor receptor with erlotinib (Tarceva<sup>TM</sup>) (PDB code: 1M17) and the crystal structure of CDK2 in complex with ATP (PDB code:1HCK) were retrieved from

protein data bank (PDB). Ligands were docked into receptor active site of both epidermal growth factor receptor (EGFR) and cyclindependent kinase 2(CDK2) along with EGFR inhibitor and ATP respectively. Docking of the ligands was performed using Gold suite v5.2.0 (2010).In the docking process of EGFR, erlotinib and water molecules except HOH10 were removed from the binding site. Concerning docking job using CDK2, ATP and Mg metal were removed. Other parameters were set as default according to gold suite docking protocol.

## RESULTS AND DISSCUSION

### Chemistry

The title compounds were synthesized as shown in (Schemes 1 and 2) according reported methods with appropriate modifications<sup>14,15,27-30</sup>. The starting material, ethyl 2-(1*H*-benzo[*d*]imidazol-1-yl) acetate (1) was synthesized by reacting benzo[*d*]imidazole and ethyl 2-chloroacetate in presence of anhydrous potassium carbonate in acetone. The treatment of the compound 1 with thiocarbonylhydrazide in the presence of sodium methoxide in methanol afforded 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4-amino-4*H*-1,2,4-triazole-3-thiol (2). 4-Arylideneamino-5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-4*H*-1,2,4-triazole-3-thiol derivatives (3a-h) were synthesized by condensation of 2 with aromatic aldehydes in the presence of catalytic amount of glacial acetic acid in ethanol at reflux temperature (Scheme 1). Thiosemicarbazide derivatives 4,5 were obtained by treating compound 1 with hydrazine hydrate in ethanol at refluxed temperature and then, treated with phenylisothiocyanate derivatives in ethanol<sup>17</sup>. The reaction of the thiosemicarbazide derivatives 4,5 with hydrazine hydrate in methanol under reflux give the corresponding 6, 7. The condensation of compound 6 with 4-chlorobenzaldehyde in the presence of catalytic amount of glacial acetic acid. In ethanol give 5-[(1*H*-benzo[*d*]imidazol-1-yl)methyl]-*N*<sup>4</sup>-(2-chlorobenzylidene)-*N*<sup>3</sup>-phenyl-4*H*-1,2,4-triazole-3,4-diamine (8) (Scheme 2).

IR spectra of the title compounds show the absence of NH<sub>2</sub> and C=O absorption bands in the IR spectra confirmed that the title compounds 3a-h and 8 were obtained via condensation. The C-H stretching

vibration bands of (C=CH) and (CH<sub>2</sub>) groups are at 3030-3088 and 2920-2990 cm<sup>-1</sup>, respectively. The characteristic stretching vibrations of the products are at 1550-1620 cm<sup>-1</sup> (C=N) and 1230-1280 cm<sup>-1</sup> (N=C-S).

In the <sup>1</sup>H-NMR spectra of the key intermediate compounds 2, 6, and 7 we observed an absorption at δ 5.65, 5.49, 5.51 ppm (s, 2H, -NH<sub>2</sub>) respectively. In all the title compounds 3a-h and 8, the above absorption disappeared and additional resonances assigned to the -N=CH- signals were observed at δ = 9.93- 10.91 ppm (compounds 3a-h) because the proton is deshielded by the C=S, while compound 8 assigned N=CH signal at δ 8.96 ppm. In addition, they revealed the characteristic signals of aromatic and benzimidazole protons in the region 7.16-8.37 ppm, and Ph-NH proton in region 13.91, 13.94 and 9.72 ppm D<sub>2</sub>O-exchangeable assignable, compounds 6,7and8 respectively. A downfield signal appearing at δ 14.16 -14.24 ppm D<sub>2</sub>O-exchangeable assignable, compounds 2 and 3a-h is attributed to the -NH-C=S moiety. In the <sup>13</sup>C-NMR spectra δ 168-162, 161-158, 149-145 ppm due to N-C=N, N-C=S and Ph-CH=N groups, respectively.

The mass spectra (ESI MS) showed the presence of peak at definite m/z value in accordance to the molecular ion peak.

## BIOLOGICAL SCREENING

### Antimicrobial activity evaluation

The new synthesized compounds have been investigated against microorganisms representing Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*), Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Candida albicans*). The results obtained in Table No.1 showed that all the tested compounds show high activity against fungi. Moreover compounds 3a, 3c, 3f, 3g and 6, show high activity against gram- positive and gram- negative bacteria while, compound 3d show moderate activity. Compound 3b showed mild activity against gram-negative bacteria, but no activity against gram-positive bacteria. In addition, compounds 7 and 3h show moderate activity against gram-negative bacteria, but no activity against gram-positive.

Finally, compound 3e showed moderate activity against gram-negative bacteria, but weak activity against gram-positive.

#### **The anticancer activity (*In vitro* Antitumor Activity)**

Some of the newly synthesized compounds have been evaluated for their Potential cytotoxicity against two carcinoma cell lines, namely MCF-7 and HCT-116 cells. The results obtained in Table No.2 showed that, compounds 8 and 3f significantly active against both breast cancer cell line (MCF7) and Colon cancer cell line HCT-116, compound 3g show moderate activity against the two cell lines, compound 3d is highly active against breast cancer cell line but moderately active against colon cancer cell line, compound 3b possess moderate activity against colon cancer cell line but weakly active against breast cancer cell line, compound 7 is less active against the two cell lines, compound 3c is less active against breast cancer cell line but inactive against colon cancer cell line. In addition, compound 6 is less active against colon cancer cell line but inactive against breast cancer cell line. Finally compound 3h is completely inactive against both carcinoma cell lines.

#### **DOCKING**

##### ***In vitro* anticancer activity and structure activity relationship**

Target compounds were assessed for *in vitro* anticancer assay against colon (HCT116) and breast (MCF7) cancer cell lines. The following structure-affinity relationships can be drawn from the *in vitro* antitumor activity data presented in (Table 2).

These experimental data show that ligands 3d and 3f show the highest affinity against breast cancer (12 and 12.1 $\mu$ g/ml) respectively, where as ligand 8 is the most active analogue among the synthesized analogues against colon cancer (10.5 $\mu$ g/ml). Considering antitumor activity of the target ligands against breast cancer, replacement of methyl group at 4-position (3f) with bromo group at 2-position of hydrophobic phenyl moiety (3d) retained the inhibitory activity. On the other hand the substitution of phenyl moiety with chloro, and nitro group at 4 position (3c, and 3h), chloro group

at 2 position (3b) and 3,4,5 tri methoxy (3g) led to loss of activity. The absence of substituted phenyl fragment at position 4 of triazole skeleton ligands (6 and 7) led to loss of antitumor activity. Introduction of extra hydrophobic group at position 4 of the triazole scaffold (8) decreased the activity against breast cancer. According to the antitumor activity results and structural variation against breast cancer, the minimal structural requirements for antitumor activity are as follows; hydrophobic scaffold (triazole ring) with hydrogen bonding acceptor(N<sup>1</sup>) and donor group(NH), two hydrophobic moieties (phenyl and benzimidazole ring). This pharmacophoric postulation was in consistence with the reported results for other antitumor azolidine pharmacophore<sup>36-39</sup>.

Regarding, the inhibitory activity of synthesized derivatives against colon cancer, only ligand 8 show antitumor activity. Removal of phenyl hydrophobic moiety at position 4 (6 and 7) or anilino fragment at position 3 (3a-d and 3f,g) led to loss of the inhibitory activity against colon cancer. Thus, we could postulate that besides the hydrophobic core (triazole ring), the three hydrophobic fragments (anilino, phenyl, benzimidazole ring) are essential for antitumor activity.

##### **Computer Based Analysis of the Structure Activity Relationships**

The anti-cancer activities of the compound 3d over breast cancer(MCF-7) and 8 over colon cancer(HCT116) cells, in which epidermal growth factor receptor (EGFR) and Cyclin dependant kinase2(CDK2) is highly expressed, respectively. (Fricker, 2006; Madhusudan and Ganesan, 2004; Cockerill and Lackey,2002;) <sup>40-42</sup> encouraged us to perform molecular docking into the ATP binding site of EGFR and CDK2 to predict if this compounds has analogous binding mode to the EGFR and CDK2 inhibitors. We assumed that the active target compounds might demonstrate antiproliferative activity against breast cancer (MCF-7) and colon cancer (HCT116) cell lines through inhibition of EGFR and CDK2 respectively. Regarding docking of ligand 3d into EGFR-TK, the complex model illustrated that, this compound interacts with EGFR-TK in a fashion similar to

erlotinib where the triazole scaffold binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR-TK forming interaction with Ala719, Gln767, Leu768, Leu694, Leu820, Met769, Thr830 and Val702 residues. The essential interactions were conserved between compound 3d and EGFR-TK where the NH at position 2 of triazole ring interacts with the backbone CO of Gln-767 via a hydrogen bond (2.44 Å) Also, forked hydrogen bond formed between the N-1 of the triazole ring and water (HOH-10) molecule (2.33 Å), Thr766 (2.90 Å). Benzimidazole fragment form good hydrophobic contact with Ala719, Glu738, Leu764, Pro770, Leu753, Lys721, Met742, Thr766, Thr830 and Val702. 2-Bromophenyl at position 4 interacts with hydrophobic side chains of Cys773, Gly772, Leu694, Leu768, Leu820 and Val702 (Figure No.1 A and B).

Concerning docking of ligand 3f (Figure No.2A), the complex model, show binding mode similar to Ligand 3d. Thus could explain the similar inhibitory activity.

On the other hand, docking of ligands, 3b, c,h and g showed decreasing in hydrophobic contact area with Cys773, Gly772, Leu694, Leu768, Leu820 and Val702. The hydrogen bonding with backbone CO of Gln-767, (HOH-10) molecule and Thr766 are consisted. Although the hydrogen bonding interactions are retained, the markedly decreasing in hydrophobic contact area led to loss of activity of these derivatives. When ligand 6, 7 (Figure No.2B) docked in EGFR-TK, the essential hydrogen bonding with backbone CO of Gln-767, a water (HOH-10) molecule-mediated and Thr766 are lost. Also, the hydrophobic interaction with Cys773, Glu738, Leu753 and Thr830 lost. Losing of important hydrogen bonding and hydrophobic interactions illustrated the inactivity of these ligands against breast cancer. Concerning the binding of ligand 8 (Figure No.2C), the hydrogen bonding interactions are lost. Interestingly, new hydrophobic

interaction formed between anilino group and Phe699 Thus could compensate for loss of hydrogen bonding interaction and retain the activity of ligand 8 (13.7µg/ml).

On the other hand, when ligand 8 (Figure No.3) docked into the ATP binding site of cyclindependent kinase 2(CDK2), forked hydrogen bond formed between NH<sub>2</sub> of Gln131 and N(1) triazole (2.57 Å) and N1 of benzimidazole ring (2.30 Å). Triazole scaffold imersed in hydrophobic pocket formed by, Asp85, Gln131, Ileu10, Lys33, Lys89, Lys129 and Val18. Benzimidazolidine fragment flanked by hydrophobic pocket made by Gln131, Gly13, Lys33, Lys129 and Val18. Important hydrophobic contacts formed between anilino moiety and Ala31, Asp85, Glu85, Ileu10, Leu134, Lys89, Phe82 and Val18. Hydrophobic benzylidene moiety interacts with Asp85, Gln131, Lys89 and Lys129. The forked hydrogen bond hydrophobic interactions could be attributed to the activity of ligand 8 and essential against colon cancer. The results of this molecular docking study can support the postulation that our active compound may inhibit the growth of colon cell lines through inhibition of Cyclin Dependant kinase 2.

Docking score is taken as the negative of the sum of the component energy terms including the following four components:

- 1-Protein-ligand hydrogen bond energy.
  - 2-protein-ligand Van der Waals energy.
  - 3-ligand internal Van der Waals energy.
  - 4-Ligand torsional strain energy (internal torsional).
- As evident from the experimental data and molecular docking studies, the pharmacophoric features essential for the antitumor activity of this series are as follows ; Hydrophobic scaffold (triazole ring) with hydrogen bonding acceptor(N1) and donor group(NH), two hydrophobic moieties (phenyl and benzimidazole ring).



**Table No.1: Diameter (mm) of inhibition zones against the corresponding standard strains of different microorganisms**

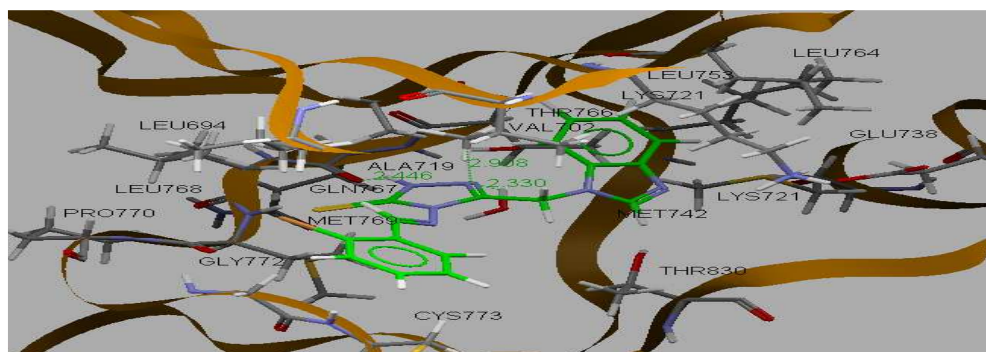
S.No	Tested Compounds	G +ve bacteria		G -ve bacteria		Fungi
		<i>Staph. aureus</i> ATCC 6538	<i>Staph. epidermidis</i> ATCC 12228	<i>Pseud. aeruginosa</i> ATCC 9027	<i>Esch. coli</i> ATCC 10536	<i>Candida albicans</i> ATCC 10231
1	3a	30	28	28	28	27
2	3b	14	12	26	28	31
3	3c	29	28	28	29	30
4	3d	27	25	26	27	32
5	3e	20	19	25	25	25
6	3f	27	26	29	30	30
7	3g	30	28	30	31	32
8	3h	18	18	22	23	29
9	6	32	31	31	32	33
10	7	16	16	22	24	29
11	Cefotaxime	30	28	30	35	-
12	Nystatin	-	-	-	-	24
13	DMF	-	-	-	-	-

**Table No.2: The IC<sub>50</sub> (µg/mL) of some of the selected new compounds against Breast cancer cell line (MCF7) and Colon cancer cell line HCT-116**

S.No	Tested Compounds	Breast cancer cell line MCF-7(µg)	Colon cancer cell line HCT-116(µg)
1	3b	46.9	25
2	3c	34.4	50>
3	3d	12	28.5
4	3f	12.1	20.4
5	3g	27.8	23.4
6	3h	50>	50>
7	6	50>	49.1
8	7	48.1	42.9
9	8	13.7	10.5
10	Doxorubicin(Standard)	0.426	0.469

**Table No.3: Docking score of the most active compounds**

S.No	Compounds	Docking score
1	Erlotinib	67.3
2	3d	64.2
3	ATP	70.8
4	8	55.57



**Figure No.1A: Docking of compound 3d**

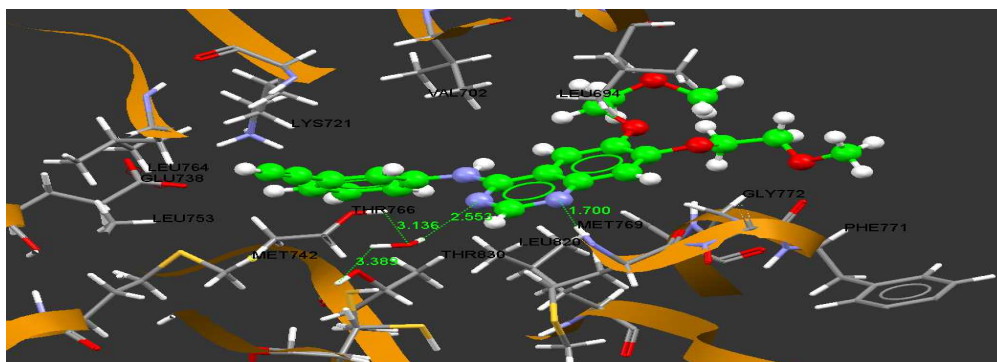


Figure No.1B: Erlotinib into the active site of epidermal growth factor receptor

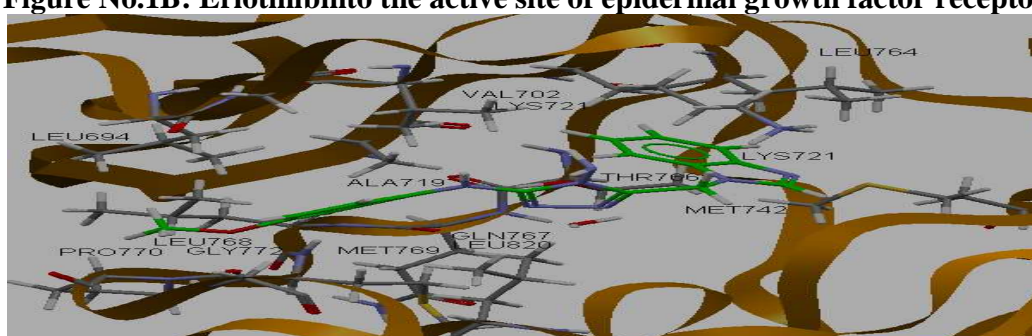


Figure No.2A: Binding mode of ligand 3f illustrate the similar interactions formed with 3d

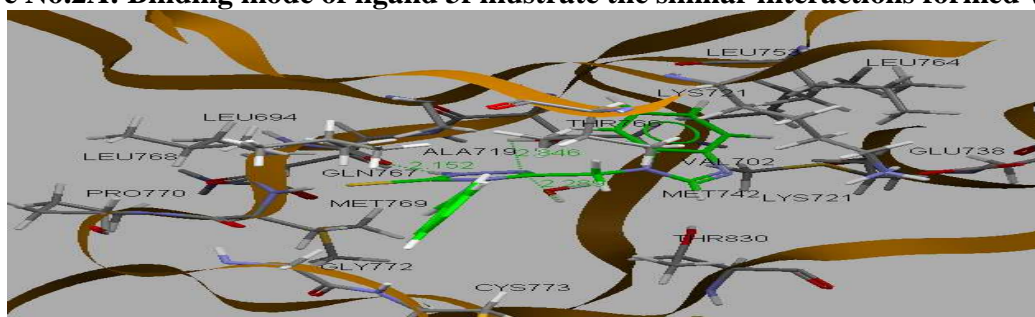


Figure No.2B: The complex model of ligand 6 shows the absence of hydrogen bonding with backbone CO of Gln-767, water (HOH-10) molecule-mediated and NH of Thr766 is lost

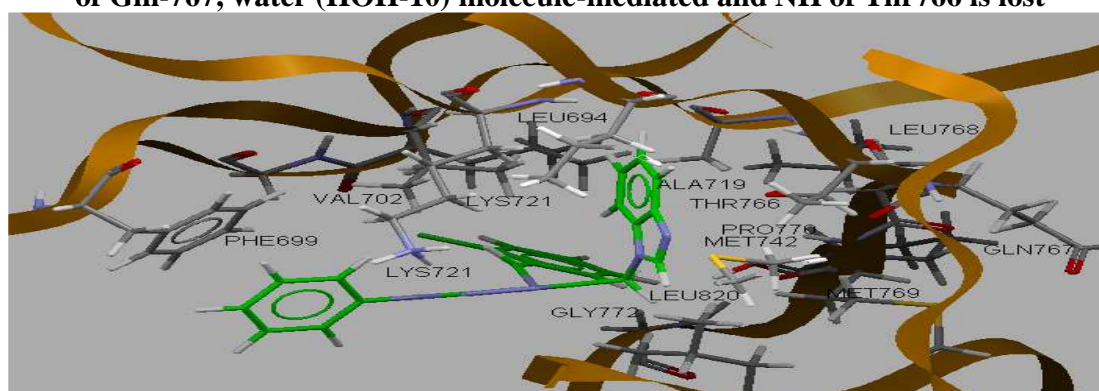


Figure No.2C: The binding mode of ligand 8 illustrates the formation of new hydrophobic interaction Phe699

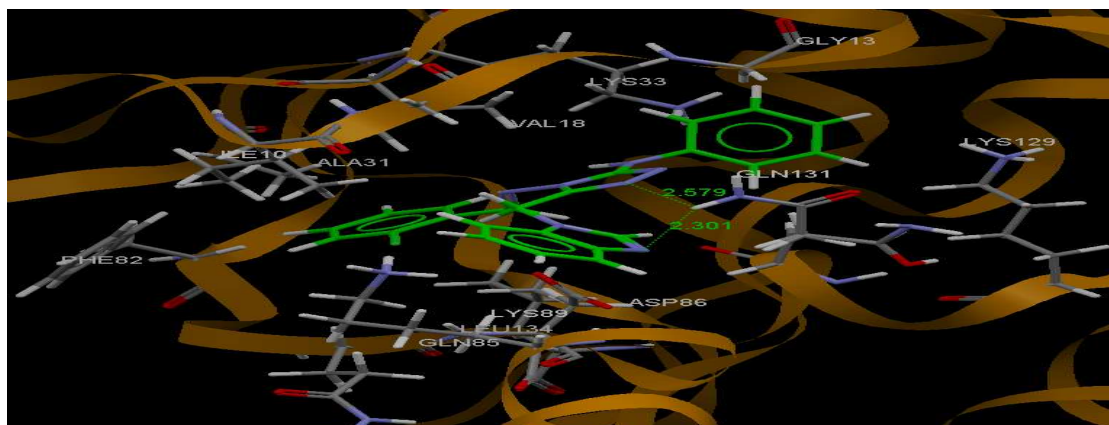
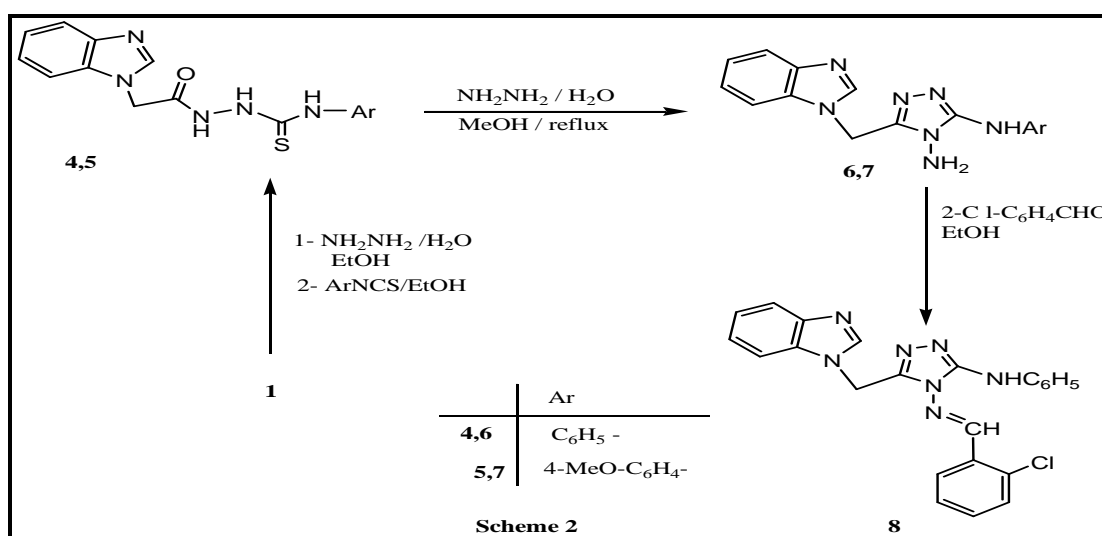
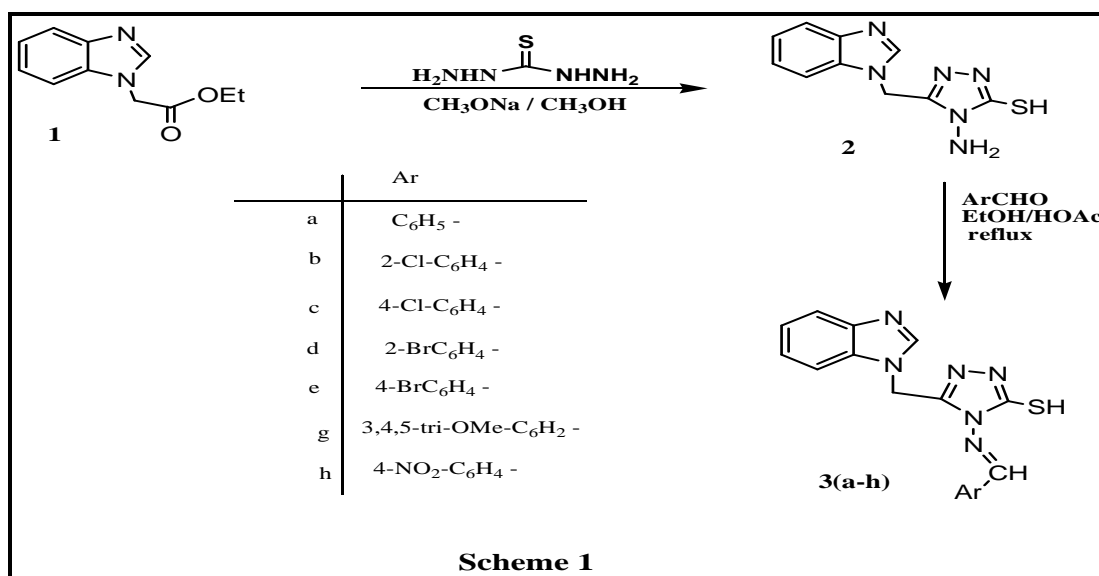


Figure No.3: The binding mode of ligand 8 in the ATP binding site of cyclindependent kinase 2(CDK2)



## CONCLUSION

Newly synthesized Schiff-base 3a-h and 8 of 1,2,4-triazole derivatives attached to benzimidazole moiety and biological screening against microorganism showed that all of them have high activity against fungi. Compounds 3a, 3c, 3f, 3g and 6, show high activity against gram- positive and gram- negative bacteria. Compounds 3d, 3f and 8 showed the highest potency at low  $\mu\text{g/ml}$  level against breast MCF-7 and colon HCT116 cell lines respectively. Docking calculations were carried out to aid the interpretation the results. Develop of promising anticancer compounds containing substituted triazole scaffold.

## ACKNOWLEDGEMENT

We wish to sincerely thank assistant lecture Nader Shawky Mohamed, Microbiology department, Faculty of Pharmacy, Zagzig University for performing the antimicrobial screening. We wish to express our appreciation to Regional center for Mycology and Biotechnology, Al-Azhar University, Cairo, for cytotoxic activity evaluation and elemental analysis. We would like to thank demonstrator Botros Yousef, Pharmaceutical chemistry department, Faculty of Pharmacy, Sinai University for performing docking study.

## CONFLICT OF INTEREST

None declared.

## BIBLIOGRAPHY

1. Turan-Zitouni G, Kaplancikli Z A, Yildiz M T, Chevallet P and Kaya D. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl) acetamido] thio-4H-1,2,4-triazole derivatives, *Eur. J. Med. Chem.*, 40, 2005, 607-613.
2. Verma A and Saraf S K. 4-Thiazolidinone - A biologically active scaffold, *Eur. J. Med. Chem.*, 43, 2008, 897-905.
3. Murphy S T, Case H L, Ellsworth E, Hagen S, Huband M, Jonnides T and Limberakis C. et al. The synthesis and biological evaluation of novel series of nitrile-containing fluoroquinolones as antibacterial agents, *Bioorg. Med. Chem. Lett.*, 17, 2007, 2150-2155.
4. Rajavel R, Vadivu M S and Anitha C. Synthesis, physical characterization and biological activity of some Schiff bases complexes, *E.J. Chem.*, 5(3), 2008, 620-626.
5. Mohamed G G, Omar M M and Hindy A M. Metal complexes of Schiff bases: preparation, characterization, and biological activity, *Turk. J. Chem.*, 30(3), 2006, 361-382.
6. El-Sayed W A, Ali O M, Hathoot M M and Abdel-Rahman A. H. Synthesis and Antimicrobial activity of New Substituted Fused 1,2,4-Triazole Derivatives, *Z.Naturforsch.*, 65c, 2010, 22-28.
7. Nadkarni B A, Kamat V R and Khadse B G. Synthesis and anthelmintic activity of 3, 6-disubstituted-7H-s-triazolo [3,4-b]-1,3,4-thiadiazine, *Arzneim. Forsch.*, 51, 2001, 569-573.
8. Holla B S, Akberali P M and Shivananda M K. Studies on nitrophenylfuran derivatives: part XII. Synthesis, characterization, antibacterial and antiviral activities of some nitrophenyl-furfurylidene-1, 2, 4- triazolo[3,4-b]-1, 3, 4-thiadiazine, *Farmaco*, 56, 2001, 919-927.
9. Hui X P, Zhang Y, Xu P F, Wang Q, Zhang Q. and Zhang Z Y. Syntheses and antibacterial activities of novel 3-substituted-6-(4-chlorophenyl)-7-(1H-1,2,4-triazole-1-yl) 1,2,4-triazolo [3,4-b]-1, 3, 4 -thiadiazine, *Chin. J. Org. Chem.*, 25, 2005, 700-704.
10. Holla B S, Rao B S, Sarojini B K, Akberali P M and Kumari N S. Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents, *Eur. J. Med. Chem.*, 41, 2006, 657-663.
11. Zhou S, Zhang L, Jin J, Zhang A, Lei X, Lin J, He J and Zhang H. Synthesis and Biological Activities of Some Novel Triazolothiadiazines and Schiff Bases Derived from 1, 2, 4-Triazole, *Phosphorus, Sulfur Silicon Relat. Elem.*, 182, 2007, 419-432.
12. Abdel-Fattah H A, El-Etrawy A S and Gabr N R. Synthesis and Biological Evaluation of Some

- New 1,3,4-Oxa, Thiadiazole and 1,2,4-Triazole Derivatives attached to Benzimidazole, *Int. J. Pharm. Chem*, 4(3), 2014, 112-118.
- Li Z, Gu Z, Yin K, Zhang R, Deng Q and Xiang J. Synthesis of substitutedphenyl-1,2,4-triazol-3-thione analogues with modified D-glucopyranosyl residues and their antiproliferative activities, *Eur. J. Med. Chem.*, 44, 2009, 4716-4720.
  - Holla B S, Veerendra B, Shivananda M K and Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles, *Eur. J. Med. Chem.*, 38, 2003, 759-767.
  - Bayrak H, Demirbas A, Karaoglu S A and Demirbas N. Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities, *Eur. J. Med. Chem.*, 44, 2009, 1057-1066.
  - Bagihalli G B, Avaji P G, Patil S A and Badami P S. Synthesis, spectral characterization, *in vitro* anti-bacterial, antifungal and cytotoxic activities of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases, *Eur. J. Med. Chem.*, 43, 2008, 2639-2649.
  - Bekircan O and Bektas H. Synthesis of new bis-1,2,4-triazole derivatives, *Molecules*, 11, 2006, 469-477.
  - Bayomi S M, Maarouf A R, Abdel-Aziz N I. and Mohamed A A B. Synthesis, modeling, preliminary antitumor and antimicrobial evaluation of new benzimidazole, *J. of Amer. Science*, 9(10), 2013, 42-48.
  - Ozden S, Atabey D, Yildiz S and Goker H. Synthesis and potent antimicrobial activity of some novel methyl or ethyl 1H- benzimidazole-5- carboxylate derivatives carrying amide and amidine groups, *Bioorg. Med. Chem.*, 13, 2005, 1587-1597.
  - Nofal Z M, Fahmy H H and Mohamed H S. Synthesis, antimicrobial and molluscicidal activities of new benzimidazole derivatives, *Arch. Pharm. Res.*, 25(1), 2002, 28-38.
  - Kus C, Ayhan-Kilcigil G, Eke B C and Iscan M. Synthesis and antioxidant properties of some novel benzimidazole derivatives on lipid peroxidation in the rat liver, *Arch. Pharm. Res.*, 27(2), 2004, 156-163.
  - Porcari A R, Devivar R V, Kucera L S, Drach J C and Townsend L B. Design, synthesis and antiviral evaluation of 1-(substituted benzyl)-2-substituted-5,6-dichloro benzimidazoles as nonnucleosides analogues of 2,5,6-trichloro-1-( $\beta$ -D-ribofuranosyl) benzimidazole, *J. Med. Chem.*, 41, 1998, 1252-1262.
  - Tewari A K and Mishra A. Synthesis and antiviral activities of N-substituted benzimidazole derivatives, *Ind. J. Chem.*, 45B, 2006, 489-493.
  - Kumar J R, Jawahar J and Pathak D P. Synthesis of benzimidazole derivatives as antihypertensive agents, *E. J. Chem.*, 3(4), 2006, 278-285.
  - Navarrete-Vazquez G., Cedillo R., Hernandez-Campos A, Yopez L, Hernandez-Luis F., Valdez J. and Morales R. *et al.* Synthesis and antiparasitic activity of 2-(Trifluoromethyl)-benzimidazole derivatives, *Bioorg. Med. Chem. Lett.*, 11, 2001, 187-190.
  - Achar K C S., Hosamani K M. and Seetharama reddy H R. *In vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives, *Eur. J. Med. Chem.*, 45, 2010, 2048-2054.
  - Gwada J., Khadar A M A., Kalluraya B and Kumari N S. Microwave assisted synthesis of 1,3,4-oxadiazoles carrying benzimidazole moiety and their antimicrobial properties, *Ind. J. Chem.*, 49B, 2010, 1130-1134.
  - Patel R V, Patel P K, Kumari P, Rajani D P and Chikhalia K H. Synthesis of benzimidazolyl 1,3,4-oxadiazol-2ylthio-N-phenyl benzothiazol yl) acetamides as antibacterial, antifungal and antituberculosis agents, *Eur. J. Med. Chem.*, 53, 2012, 41-51.
  - El-Etrawy A S, Abdel-Megied A E S, El-Berrawy S R A, Abdel-Rahman A A H. Synthesis and Antimicrobial Evaluation of Some 1,2,4-Triazole Derivatives Attached to C-

- Furyl Glycosides, *Arab. J. Chem.*, In Press, 2012.
30. Nitin P M. and Gowda P T. Synthesis and antifungal activity of some substituted benzimidazole analogues, *IRJP*, 3(7), 2012, 189-194.
31. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M100-S12. Wayne, PA, NCCLS, 2002.
32. Barry A L. The antimicrobial susceptibility test: Principle and practices, Edited by Illuslea and Febiger (Philadelphia USA) (1976) (180), *Biol Abstr.*, 64, 1977, 251-83.
33. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, 65, 1983, 55-63.
34. Gangadevi V and Muthumary J. Preliminary studies on cytotoxic effect of fungal taxol on cancer cell lines, *African Journal of Biotechnology*, 6, 2007, 1382-1386.
35. Wilson A P. Cytotoxicity and viability assays in animal cell culture: A Practical Masters, (J. R. W.) Oxford University Press, 2000.
36. Abdel-Aziz A A M, El-Azab A S, El-Subbagh H I, Al-Obaid A M, Alanazi A M and Al-Omar M A. Design, synthesis, single-crystal and preliminary antitumor activity of novel arenesulfonylimidazolidin-2 ones, *Bioorg. Med. Chem. Lett.*, 22, 2012, 2008-2014.
37. El-Deeb I M, Bayoumi S M, El-Sherbeny M A and Abdel-Aziz A A M. Synthesis and antitumor evaluation of novel cyclic arylsulfonyleureas: ADME-T and pharmacophore prediction, *Eur. J. Med. Chem.*, 45, 2010, 2516-2530.
38. Carmi C, Cavazzoni A, Zuliani V, Lodola A, Bordi F, Plazzi P V, Alfieri R R, Petronini P G. and Mor M. 5-Benzylidene-hydantoin as new EGFR inhibitors with antiproliferative activity, *Bioorg. Med. Chem. Lett.*, 16, 2006, 4021-4025.
39. Zuliani V, Carmi C, Rivara M, Fantini M, Lodola A, Vacondio F, Bordi F, Plazzi P V, Cavazzoni A, Galetti M, Alfieri R R, Petronini P G and Mor M. 5-Benzylidene-hydantoin: Synthesis and antiproliferative activity on A549 lung cancer cell line, *Eur. J. Med. Chem.*, 44, 2009, 3471-3479.
40. Fricker J. Tyrosine kinase inhibitors: the next generation, *Lancet. Oncol.*, 7, 2006, 621.
41. Madhusudan S and Ganesan T S. Tyrosine kinase inhibitors in cancer therapy, *Clin Biochem*, 37, 2004, 618-635.
42. Cockerill G S and Lackey K E. Small Molecule Inhibitors of the Class 1 Receptor Tyrosine Kinase Family, *Curr. Top. Med. Chem.*, 2, 2002, 1001-1010.

**Please cite this article in press as:** Hanan A. Abdel-Fattah et al. Design, Synthesis and Biological Evaluation of Some 1, 2, 4-Triazole Derivatives Based Schiff's Bases Bearing Benzimidazole Moiety, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(2), 2015, 62 - 75.