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# SYNTHESIS, CYTOTOXICITY AND DOCKING SIMULATION OF BIOACTIVE [1,2,4]TRIAZOLO[3,4-*a*]DIHYDROISOQUINOLINE CHALCONE DERIVATIVES

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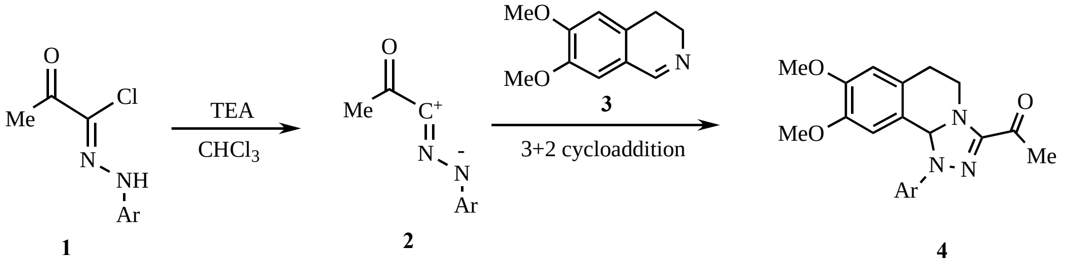
**Abstract** – 1-(1-Aryl-8,9-dimethoxy-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-*a*]-isoquinolin-3-yl)ethan-1-ones **4** were prepared *via* reaction of *C*-acetylmethanohydrazonoyl chlorides **1A,B** with 6,7-dimethoxy-3,4-dihydroisoquinoline **3**. Treatment of the latter products **4** with 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde derivatives **5** in ethanol in the presence of sodium hydroxide solution at room temperature afforded chalcones **6**. Cytotoxic assay was performed for *in vitro* antitumor screening against caucasian breast adenocarcinoma (MCF7) and hepatocellular carcinoma (HepG2) cell lines. Chalcones **6Ab** and **6Ba** have promising cytotoxic effects against MCF7 with $IC_{50}$ values 8.0 and 7.5 µg/mL, respectively. Molecular docking using Mcule.com was carried out, for the most potent compounds **6Ab** and **6Ba**, against two protiens which are EGFR and DHFR.

## INTRODUCTION

α,β-Unsaturated ketonic compounds such as chalcones are considered as secondary metabolites that cause them to have several bioactivities including antimalarial,[1] antioxidant,[2,3] antiviral,[4] antibacterial,[5,6] anti-inflammatory,[2,7,8] antiplatelet,[9] analgesic[10] and anticancer.[3,6,11,12] Moreover, heterocyclic compounds containing nitrogen such as pyrazoles have powerful pharmacological activities like anticonvulsant,[13,14] antidepressant,[15,16] antimalarial,[17] antimicrobial,[18,19] antibacterial,[20,21] antifungal[22,23] and antitumor activities.[24-28] Also, [l,2,4]triazolo[3,4-*a*]isoquinolines showed pharmacological activities that include anti-inflammatory, antidepressant, and cardiovascular.[29] In continuation of our work in the synthesis of the bioactive heterocyclic compounds,[6,30-32] we report herein the synthesis of new chalcones incorporating both [l,2,4]triazolo[3,4-*a*]isoquinolines and pyrazoles moieties.

## RESULTS AND DISCUSSION

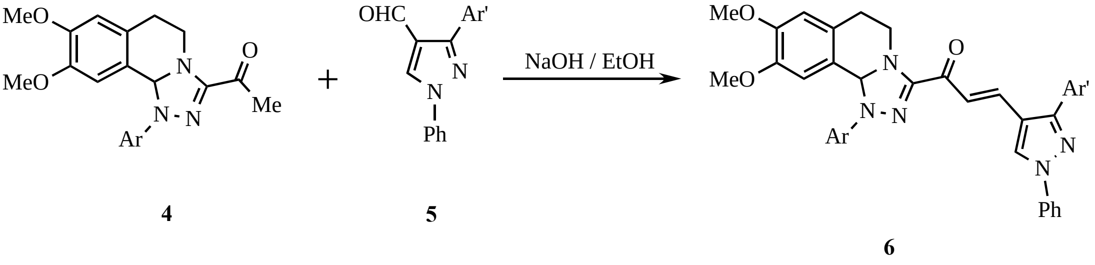
Nitrilimines **2**, produced *in situ* by reaction of *C*-acetylmethanohydrazonoyl chlorides **1A,B** with triethylamine in chloroform, reacted with 6,7-dimethoxy-3,4-dihydroisoquinoline **3** to give the corresponding 1-(1-aryl-8,9-dimethoxy-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)ethan-1-ones **4** (Scheme 1).[30]



Ar : A = 4-Me$C_6H_4$, B = 4-MeO$C_6H_4$

Scheme 1. Synthesis of 1-(1-aryl-8,9-dimethoxy-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)ethan-1-ones **4**

Claisen–Schmidt condensation of the latter products **4** with 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde derivatives **5** in ethanol in the presence of sodium hydroxide solution at room temperature afforded chalcones **6** (Scheme 2). Products **6** were identified on the basis of their elemental analysis and spectral data. For example, $^1$H NMR spectrum of compound **6Ac** revealed four singlet signals at $\delta$ 2.34, 3.61, 3.86 and 3.88 corresponding to one Me and three MeO groups, respectively. Also, it showed four multiplet signals at 2.75-2.90 (2H of $CH_2$), $\delta$ 3.82-3.84 (1H of $CH_2$), $\delta$ 4.16-4.18 (1H of $CH_2$) and $\delta$ 6.59-8.34 corresponding to aminal, enone and aromatic protons. Its $^{13}$C NMR spectrum showed 32 signals for asymmetric carbon atoms. Also, its mass spectrum showed a molecular ion peak at *m/z* 625.



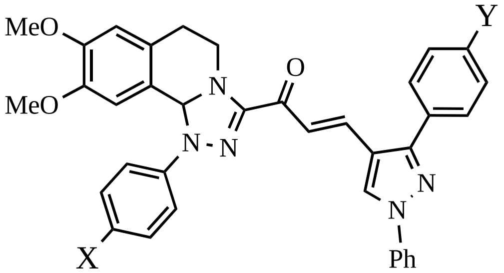
Ar : A = 4-Me$C_6H_4$, B = 4-MeO$C_6H_4$
Ar' : a = $C_6H_5$, b = 4-Me$C_6H_4$, c = 4-MeO$C_6H_4$, d = 4-Cl$C_6H_4$

Scheme 2. Synthesis of tetrahydro-([1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)-3-arylprop-2-en-1-ones **6**

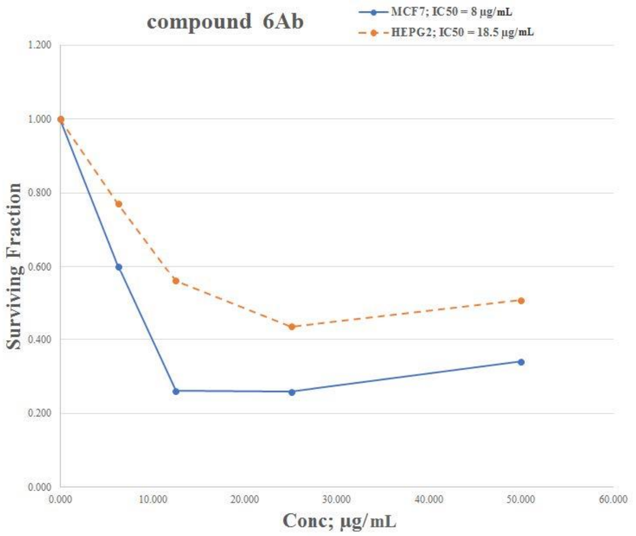
## Antitumor screening

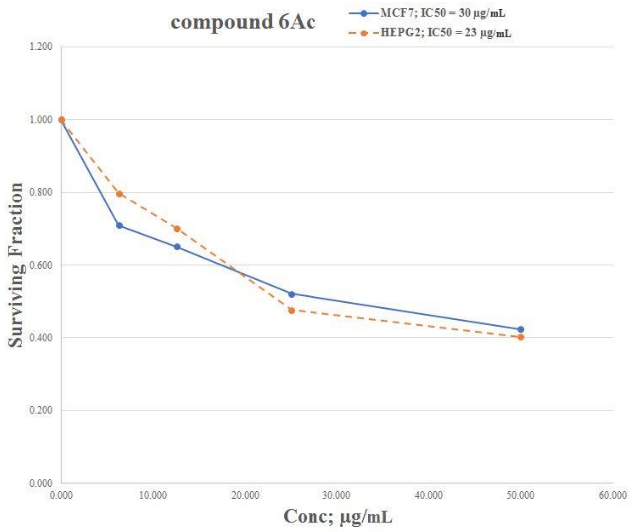
The newly synthesized compounds **6A,B** were subjected to *in vitro* antitumor screening against different human cancer cell lines namely: MCF7 and HepG2. Data illustrated in Table 1 showed that two new chalcones **6Ab** and **6Ba** have promising cytotoxic effect against MCF7 with $IC_{50}$ values 8.0 and 7.5 µg/mL, respectively, which were compatible with that obtained by the previous literature[30] where methoxy groups enhanced anticancer activity. Moreover, compound **6Bc** showed no activity (NA) against MCF7. According to Shier scale, the other tested compounds showed moderate to weak antitumor activities with respect to doxorubicin. Also, all chalcones showed very weak activities against HepG2. The results in Table 1 illustrates that, in entry 6, the two electron donating groups of compound **6Bc** (X, Y = OMe) led to decrease in the antitumor activities, while for compound **6Ad** (X = Me; Y = Cl), entry 3, the presence of one electron donating group and another electron withdrawing group, led to increase in the antitumor activities. Accordingly, compound **6Ba** (X = OMe; Y = H) showed higher activity in comparison with compound **6Ab** (X = Me; Y = Me) against MCF7 with $IC_{50}$ values 7.5 and 8.0 µg/mL, respectively.

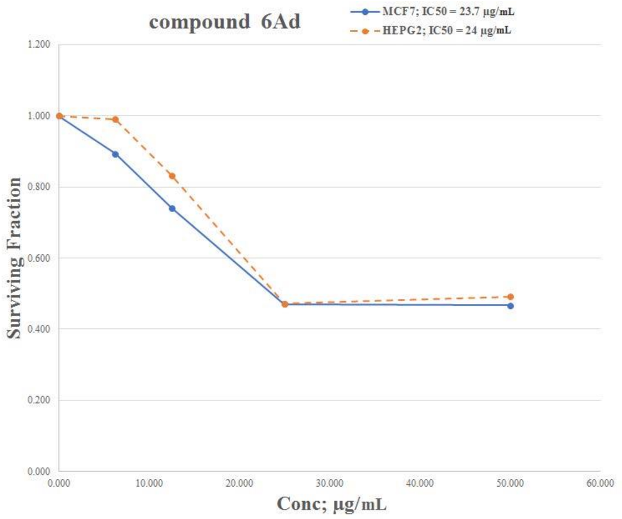
Table 1. $IC_{50}$ of the tested compounds **6A,B** for their *in vitro* antitumor activity against MCF7 and HepG2

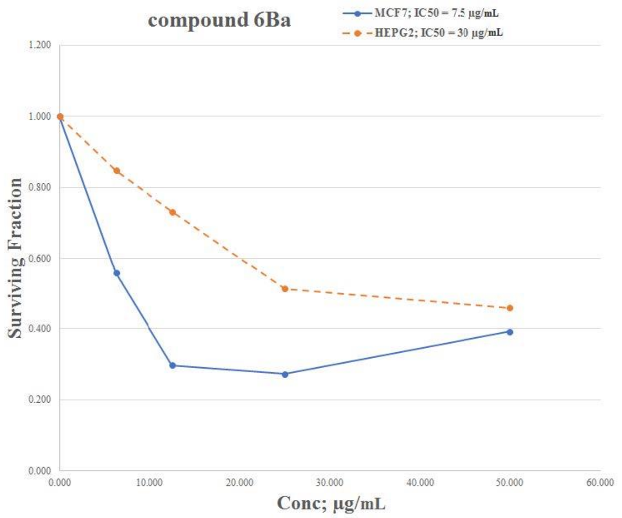


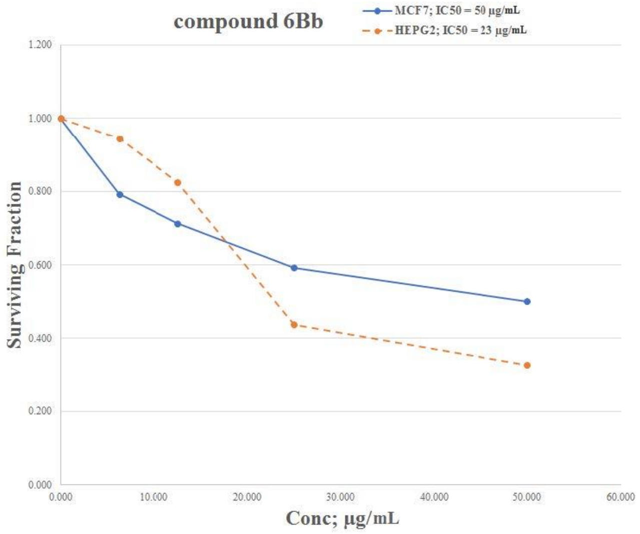
| | | | | **$IC_{50}$ (µg/mL)** | |
|---|---|---|---|---|---|
| **Entry** | Compounds | X | Y | MCF7 | HepG2 |
| **Doxorubicin** | | -- | -- | 3.68 | 4.2 |
| **1** | **6Ab** | Me | Me | 8.0 | 18.5 |
| **2** | **6Ac** | Me | OMe | 30.0 | 23.0 |
| **3** | **6Ad** | Me | Cl | 23.7 | 47 |
| **4** | **6Ba** | OMe | H | 7.5 | 30 |
| **5** | **6Bb** | OMe | Me | 50 | 23 |
| **6** | **6Bc** | OMe | OMe | NA | 40 |

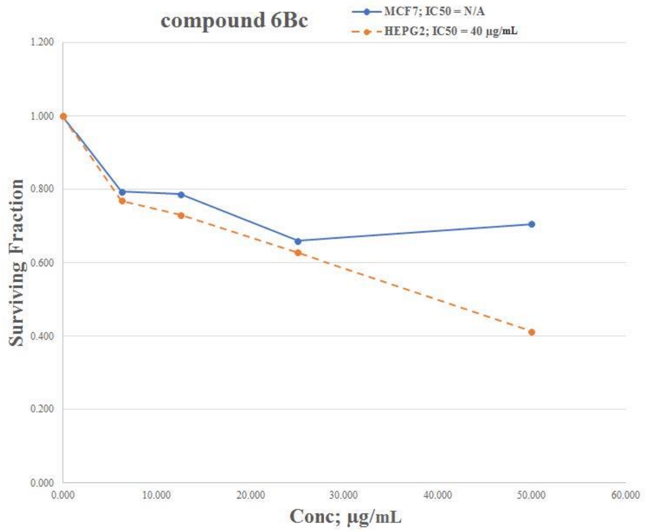

compound 6Ab
MCF7; IC50 = 8 μg/mL
HEPG2; IC50 = 18.5 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000


compound 6Ac
MCF7; IC50 = 30 μg/mL
HEPG2; IC50 = 23 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000


compound 6Ad
MCF7; IC50 = 23.7 μg/mL
HEPG2; IC50 = 24 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000


compound 6Ba
MCF7; IC50 = 7.5 μg/mL
HEPG2; IC50 = 30 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000


compound 6Bb
MCF7; IC50 = 50 μg/mL
HEPG2; IC50 = 23 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000


compound 6Bc
MCF7; IC50 = N/A
HEPG2; IC50 = 40 μg/mL
Surviving Fraction
Conc; μg/mL
0.000
0.200
0.400
0.600
0.800
1.000
1.200
0.000
10.000
20.000
30.000
40.000
50.000
60.000

## Docking study

Docking studies visualization was performed using PLIP (Protein-Ligand Interaction Profiler).[33] We performed these studies to anticipate the binding mode between the ligand and the binding site of target proteins which are epidermal growth factor receptor (EGFR) and dihydrofolate reductase (DHFR), two of the human proteins which are highly expressed in most tumor cells to validate and specify the mechanism of action in the context of anticancer activity. Proteins were selected and downloaded from the Protein Data Bank (PDB ID: 4i23 and 1DLS, respectively). The proteins were optimized by removing water molecules and cofactors, deleting the standard ligand present in the crystal structure and adding hydrogen atoms.

Compound **6Ab** achieved eleven binding modes with DHFR protein including eight hydrophobic and three hydrogen bonds while another eleven binding modes were achieved by compound **6Ba** with the same protein including ten hydrophobic interactions and one hydrogen bond (Figures 1 and 3, Table 2). Docking studies of the active compound **6Ab** into the active domain of EGFR protein revealed eight interactions, seven hydrophobic interactions and one hydrogen bonding. The hydrophobic interactions were found to be with Leu170, Leu171, Leu168, Val229, Leu813, Trp1538 and Leu2632 with bond distances 3.93, 3.66, 3.86, 3.61, 3.58, 3.77, 3.94 Å, respectively. The eight interactions are the hydrogen bonding between Asp2734 and oxygen atom with bond distance 3.64 Å and angle 110.87 (Figure 2). All these binding modes confirmed our suggestion that 1-(8,9-dimethoxy-1-(*p*-tolyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)-3-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)prop-2-en-1-one **6Ab** may have a strong anticancer activity and play a role in inhibiting the cancer progression. Regarding the binding mode of compound **6Ba** toward the same protein, it showed ten interactions, nine of them are hydrophobic interactions (Figure 4, Table 2) and the other binding is a hydrogen bond between nitrogen sharing formal positive charge atom (guanidinium, amidinium) and Arg2771 with bond distance 2.97 Å and angle 132.44. The hydrophobic interactions were found to be with Leu170, Val229, Ala354, Lys369, Leu619, Leu712, The732, Leu753 and Leu1281 with bond distances 3.60, 3.92, 3.81, 3.54, 3.55, 3.48, 3.52, 3.57 and 3.54 Å, respectively (Figure 4). All these binding modes confirmed our suggestion that triazoloisoquinoline and pyrazole groups played an important role in the enhanced binding activity of both compounds into the active site of the target proteins and hence inhibit cancer progression.

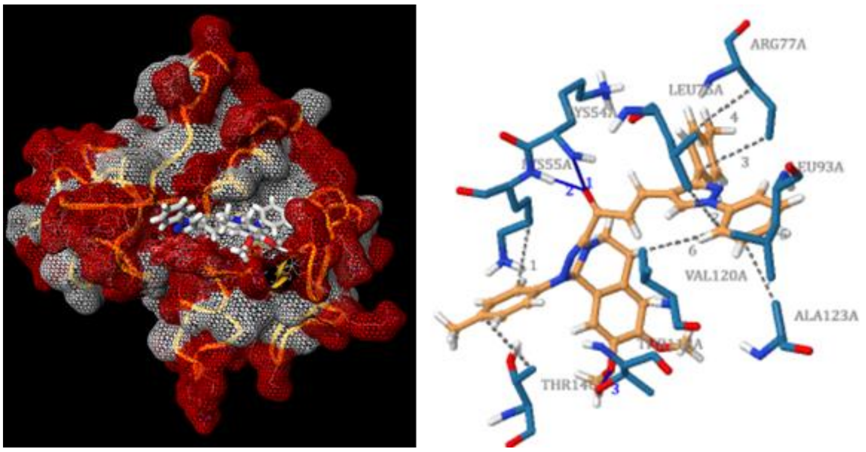


Figure 1. 3D and 2D dimensional representation of compound **6Ab** into the active site of dihydrofolate reductase (DHFR)

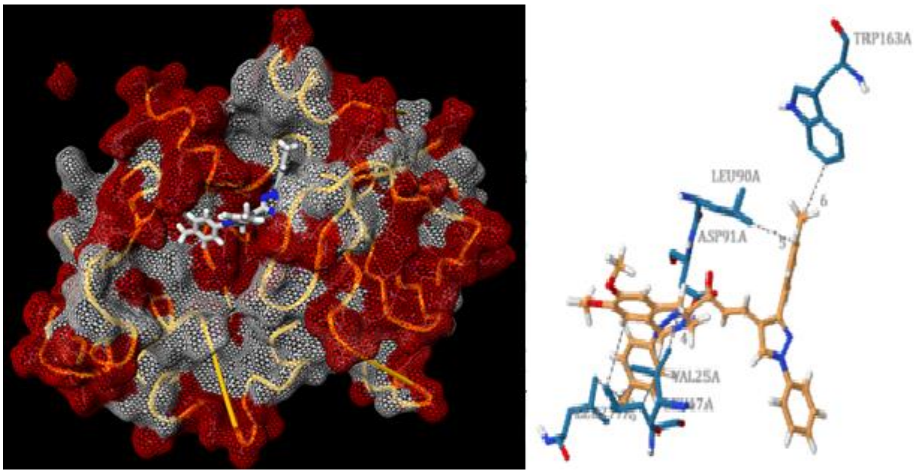


Figure 2. 3D and 2D dimensional representation of compound **6Ab** into the active site of human epidermal growth factor receptor (EGFR)

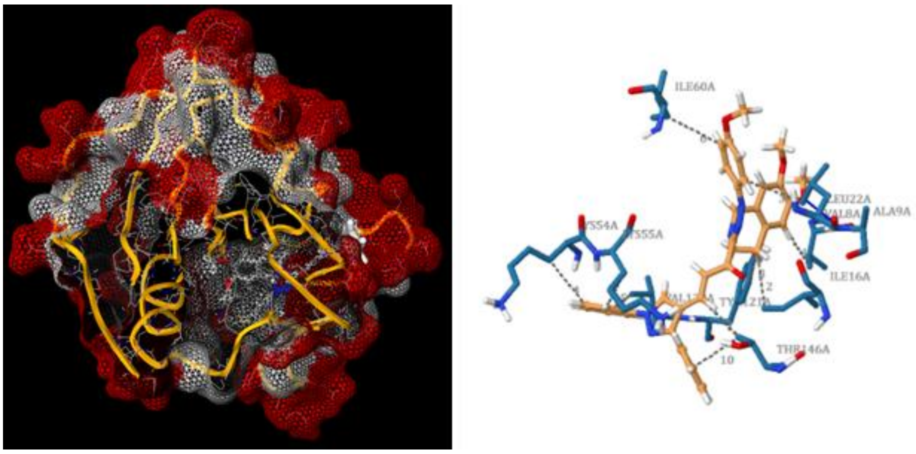


Figure 3. 3D and 2D dimensional representation of compound **6Ba** into the active site of dihydrofolate reductase (DHFR)

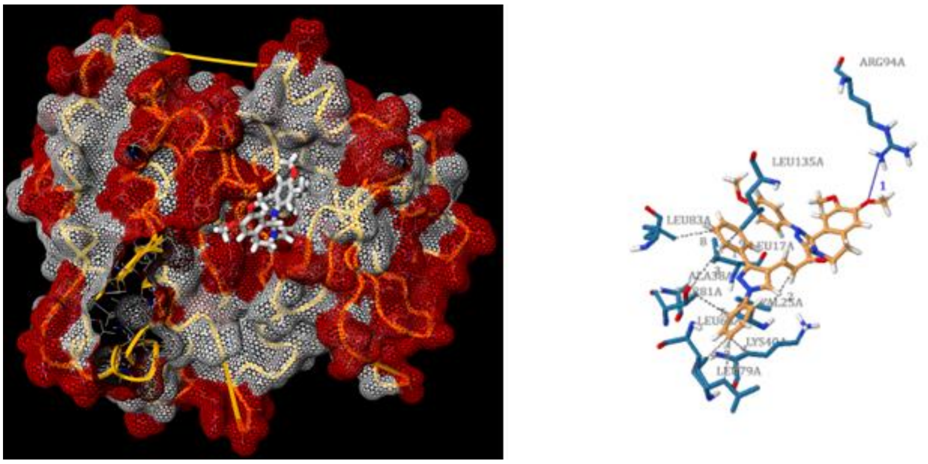


Figure 4. 3D and 2D dimensional representation of compound **6Ba** into the active site of human epidermal growth factor receptor (EGFR)

Table 2. Summarized list of the contacts in atomic-level detail. Each entry contains detailed information on protein residues, participating ligand atoms and geometry of the interaction (e.g., the distance of interacting atoms).

| **Hydrophobic interaction** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Compound number** | **Protein target** | **Index** | **Residue** | **Amino acid** | **Distance** | **Ligand atom** | **Protein atom** |
| **6Ab** | **DHFR** | 1 | 55A | Lys | 3.84 | 1815 | 509 |
| | | 2 | 75A | Leu | 3.56 | 1797 | 726 |
| | | 3 | 77A | Arg | 3.69 | 1802 | 743 |
| | | 4 | 77A | Arg | 3.75 | 1804 | 741 |
| | | 5 | 93A | Leu | 3.55 | 1799 | 883 |
| | | 6 | 120A | Val | 3.76 | 1797 | 1101 |
| | | 7 | 123A | Ala | 3.38 | 1798 | 1134 |
| | | 8 | 146A | Thr | 3.71 | 1812 | 1370 |
| | **EGFR** | 1 | 17A | Leu | 3.93 | 2738 | 170 |
| | | 2 | 17A | Leu | 3.66 | 2765 | 171 |
| | | 3 | 17A | Leu | 3.86 | 2763 | 168 |
| | | 4 | 25A | Val | 3.61 | 2730 | 229 |
| | | 5 | 90A | Leu | 3.58 | 2758 | 813 |
| | | 6 | 163A | Trp | 3.77 | 2759 | 1538 |
| | | 7 | 277A | Leu | 3.94 | 2766 | 2632 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **6Ba** | **DHFR** | 1 | 8A | Val | 3.66 | 1781 | 66 |
| | | 2 | 16A | Ile | 3.53 | 1779 | 134 |
| | | 3 | 22A | Leu | 3.81 | 1813 | 186 |
| | | 4 | 54A | Lys | 3.69 | 1798 | 494 |
| | | 5 | 55A | Lys | 3.83 | 1797 | 507 |
| | | 6 | 60A | Ile | 3.73 | 1810 | 566 |
| | | 7 | 120A | Val | 3.4 | 1800 | 1101 |
| | | 8 | 121A | Tyr | 3.91 | 1779 | 1112 |
| | | 9 | 146A | Thr | 3.19 | 1790 | 1370 |
| | | 10 | 146A | Thr | 3.28 | 1807 | 1370 |
| | **EGFR** | 1 | 17A | Leu | 3.6 | 2759 | 170 |
| | | 2 | 25A | Val | 3.92 | 2742 | 229 |
| | | 3 | 38A | Ala | 3.81 | 2756 | 354 |
| | | 4 | 40A | Lys | 3.54 | 2753 | 369 |
| | | 5 | 68A | Leu | 3.55 | 2752 | 619 |
| | | 6 | 79A | Leu | 3.48 | 2752 | 712 |
| | | 7 | 81A | Thr | 3.52 | 2754 | 732 |
| | | 8 | 83A | Leu | 3.57 | 2757 | 753 |
| | | 9 | 135A | Leu | 3.54 | 2756 | 1281 |

| **Hydrogen bonding** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Protein target** | **Index** | **Residue** | **Amino acid** | **Distance H-A** | **Distance D-A** | **Donor angle** | **Protein donor** | **Side chain** | **Donor atom** | **Acceptor atom** |
| **6Ab** | **DHFR** | 1 | 54A | Lys | 2.77 | 3.18 | 105.29 | Yes | No | 490 [Nam] | 1788 [O2] |
| | | 2 | 55A | Lys | 1.84 | 2.8 | 159.96 | Yes | No | 503 [Nam] | 1788 [O2] |
| | | 3 | 118A | Thr | 2.31 | 3.03 | 132.38 | Yes | Yes | 1085 [O3] | 1816 [O2] |
| | **EGFR** | 1 | 91A | Asp | 3.64 | 4.07 | 110.87 | Yes | Yes | 822 [O3] | 2734 [N2] |
| **6Ba** | **DHFR** | 1 | 9A | Ala | 2.34 | 2.85 | 111.05 | Yes | No | 68 [Nam] | 1818 [O2] |
| | **EGFR** | 1 | 94A | Arg | 2.97 | 3.74 | 132.44 | Yes | Yes | 856 [Ng+] | 2771 [O2] |

## EXPERIMENTAL

Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ or DMSO-$d_6$ as the solvent on Varian Gemini NMR spectrometer at 300 MHz and 75 MHz, respectively, using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses were performed at the Micro analytical centre, Cairo University.

***Synthesis of tetrahydro-([1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-arylprop-2-en-1-ones*** **(6)**:

A mixture of compound **4** (1 mmol) and the appropriate pyrazole-4-carbaldehydes **5** (1 mmol) was dissolved in EtOH (20 mL) containing 5 mL of NaOH (20%). The reaction mixture was stirred at room temperature for 8 h, then poured over ice containing HCl. The obtained solid product was then filtered, washed with water, dried, and crystallized from proper solvent to afford chalcones **6**.

***(E)-1-(8,9-Dimethoxy-1-(p-tolyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)prop-2-en-1-one*** **(6Ab)**. Orange crystals; mp 139-140 °C (MeCN); yield (85%); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.35 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 2.75-2.90 (m, 2H, $CH_2$), 3.62 (s, 3H, $CH_3O$), 3.82-3.84 (m, 1H, CH-isoquinoline), 3.86 (s, 3H, $CH_3O$), 4.16-4.18 (m, 1H, CH-isoquinoline), 6.58-8.35 (m, 19H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 20.67, 21.35, 27.93, 42.18, 55.79, 55.93, 78.65, 108.35, 111.08, 114.23, 114.87, 119.31, 121.95, 126.55, 127.06, 127.82, 128.65, 129.41, 129.49, 129.76, 130.06, 130.73, 131.63 132.61, 139.46, 141.88, 147.39, 148.75, 149.64, 153.69, 159.98, 179.73; MS (EI, 70 eV) *m/z* (%): 609 ($M^+$, 11.52), 321 (15.75), 91 (41.91), 77 (63.06), 57 (100). Anal. Calcd for $C_{38}H_{35}N_5O_3$ (609.73): C, 74.86; H, 5.79; N, 11.49. Found: C, 74.79; H, 5.87; N, 11.38.

***(E)-1-(8,9-Dimethoxy-1-(p-tolyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one*** **(6Ac)**. Orange crystals; mp 156-158 °C (MeCN); yield (83%); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.34 (s, 3H, $CH_3$), 2.75-2.90 (m, 2H, $CH_2$), 3.61 (s, 3H, $CH_3O$), 3.82-3.84 (m, 1H, CH-isoquinoline), 3.86 (s, 3H, $CH_3O$), 3.88 (s, 3H, $CH_3O$), 4.16-4.18 (m, 1H, CH-isoquinoline), 6.59-8.34 (m, 19H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 20.66, 27.90, 42.15, 55.31, 55.76, 55.90, 78.61, 108.28, 111.02, 114.16, 114.83, 118.17, 119.24, 121.86, 124.73, 126.53, 127.01, 127.78, 128.12, 129.47, 129.76, 130.01, 130.70, 132.56, 139.40, 141.97, 147.45, 148.61, 149.58, 153.76, 159.94, 179.65; MS (EI, 70 eV) *m/z* (%): 625 ($M^+$, 13.15), 321 (17.54), 85 (32.38), 77 (57.12), 57 (100). Anal. Calcd for $C_{38}H_{35}N_5O_4$ (625.73): C, 72.94; H, 5.64; N, 11.19. Found: C, 73.09; H, 5.32; N, 11.08.

***(E)-3-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-(8,9-dimethoxy-1-(p-tolyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one*** **(6Ad)**. Light orange crystals; mp

176-178 °C (DMF+EtOH); yield (78%); IR ($\nu_{max}$, cm$^{-1}$) $\nu$ 1651 (CO); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.35 (s, 3H, CH$_3$), 2.73-2.96 (m, 2H, CH$_2$), 3.61 (s, 3H, CH$_3$O), 3.80-3.84 (m, 1H, CH-isoquinoline), 3.86 (s, 3H, CH$_3$O), 4.19-4.21 (m, 1H, CH-isoquinoline), 6.60-8.34 (m, 19H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 20.69, 27.90, 42.15, 55.78, 55.92, 78.66, 108.27, 111.04, 113.03, 114.90, 118.33, 119.34, 122.45, 126.89, 127.30, 127.71, 128.13, 128.94, 129.82, 130.04, 130.86, 131.72, 134.66, 135.40, 139.29, 141.84, 147.47, 148.71, 149.53, 152.62, 179.53; MS (EI, 70 eV) *m/z* (%): 631 (M$^{+2}$, 34.07), 629 (M$^+$, 21.54), 321 (42.60), 91 (60.38), 77 (100). Anal. Calcd for $C_{37}H_{32}ClN_5O_3$ (630.15): C, 70.52; H, 5.12; Cl, 5.63; N, 11.11. Found: C, 70.39; H, 5.17; Cl, 5.54; N, 10.99.

***(E)-1-(8,9-Dimethoxy-1-(4-methoxyphenyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-one*** **(6Ba)**. Deep red; mp 149-150 °C (MeCN); yield (85%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.66-2.92 (m, 2H, CH$_2$), 3.60 (s, 3H, CH$_3$O), 3.71-3.78 (m, 1H, CH-isoquinoline), 3.83 (s, 3H, CH$_3$O), 3.86 (s, 3H, CH$_3$O), 4.25-4.29 (m, 1H, CH-isoquinoline), 6.57-8.36 (m, 20H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 27.88, 42.37, 55.57, 55.71, 55.96, 79.66, 108.47, 111.42, 114.75, 117.71, 118.31, 119.16, 121.90, 126.45, 127.14, 127.26, 127.57, 128.40, 128.83, 129.18, 129.21, 133.77, 138.21, 138.28, 139.41, 147.33, 148.75, 149.51, 153.89, 155.23, 179.58; MS (EI, 70 eV) *m/z* (%): 611 (M$^+$, 28.58), 337 (22.82), 233 (26.73), 77 (100), 55 (52.02). Anal. Calcd for $C_{37}H_{33}N_5O_4$ (611.70): C, 72.65; H, 5.44; N, 11.45. Found: C, 72.53; H, 5.35; N, 11.60.

***(E)-1-(8,9-Dimethoxy-1-(4-methoxyphenyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)prop-2-en-1-one*** **(6Bb)**. Red; mp 168-170 °C (MeCN); yield (82%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.44 (s, 3H, CH$_3$), 2.66-2.92 (m, 2H, CH$_2$), 3.60 (s, 3H, CH$_3$O), 3.71-3.79 (m, 1H, CH-isoquinoline), 3.83 (s, 3H, CH$_3$O), 3.86 (s, 3H, CH$_3$O), 4.25-4.29 (m, 1H, CH-isoquinoline), 6.57-8.34 (m, 19H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 21.33, 27.93, 42.22, 55.62, 55.75, 55.90, 79.70, 108.59, 111.22, 114.62, 117.55, 118.36, 119.28, 122.01, 126.47, 127.03, 127.21, 128.43, 128.65, 129.39, 129.47, 130.23, 132.41, 138.09, 138.40, 139.46, 147.41, 148.68, 149.65, 154.01, 155.10, 179.53; MS (EI, 70 eV) *m/z* (%): 625 (M$^+$, 30.62), 380 (34.62), 337 (36.96), 247 (40.64), 77 (100). Anal. Calcd for $C_{38}H_{35}N_5O_4$ (625.73): C, 72.94; H, 5.64; N, 11.19. Found: C, 72.99; H, 5.56; N, 11.33.

***(E)-1-(8,9-Dimethoxy-1-(4-methoxyphenyl)-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one*** **(6Bc)**. Deep red; mp 172-174 °C (DMF); yield (82%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.72-2.96 (m, 2H, CH$_2$), 3.60 (s, 3H, CH$_3$O), 3.75-3.79 (m, 1H, CH-isoquinoline), 3.83 (s, 3H, CH$_3$O), 3.86 (s, 3H, CH$_3$O), 3.88 (s, 3H, CH$_3$O), 4.25-4.29 (m, 1H, CH-isoquinoline), 6.57-8.33 (m, 19H, -NCHN-, olefinic-H and Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 27.84, 42.37, 55.20, 55.71, 55.83, 55.97, 78.75, 108.63, 111.17, 114.03, 114.56, 115.32, 118.19, 119.09, 120.12, 124.58, 126.57, 127.21, 127.94, 128.00, 129.39, 131.52, 132.73, 139.29, 141.71, 147.49, 148.52, 149.50, 151.69, 153.64, 159.79, 179.82; MS (EI, 70 eV) *m/z* (%): 641 ($M^+$, 13.69), 337 (19.42), 263 (16.45), 77 (65.70), 57 (100). Anal. Calcd for $C_{38}H_{35}N_5O_5$ (641.73): C, 71.12; H, 5.50; N, 10.91. Found: C, 71.22; H, 5.63; N, 10.84.

## Cytotoxicity assay

The cells were propagated in DMEM supplemented with 10% heat-inactivated FBS, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% $CO_2$ and were subcultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability. The cells were seeded in a 96-well plate at cell concentration of $1\times10^4$ cells per well in 100 µL of growth medium. A fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% $CO_2$ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without a test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37 °C, various concentrations of the sample (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colourimetric method.[34]

## Molecular modeling

The most active compounds **6Ab, 6Ba** in this series were designed to predict the protein-ligand interactions at the active site. The protein structure of EGFR (Epidermal growth factor receptor) and dihydrofolate reductase enzyme (DHFR) were downloaded from the Protein Data Bank (PDB ID: 4i23 and 1DLS, respectively). The proteins were ready for the modelling investigation using Mcule.com. By the end, the final data was visualized using PLIP (Protein-Ligand Interaction Profiler). Proteins were prepared for docking, and other instructions were described according to the previous literatures.[30,33,35]

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