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# Enhancement to synthesize, design and dock of novel EGFR inhibitors containing pyrazolo[3,4-*d*]pyrimidine cores of expected anticancer activity

**Eman K.A.Abdelall[1,2]*, Rania B.Bakr[1,2], Mohamed K.Abdel-Hamid[1,2], Manal M.Kandeel[1,2]**
[1]Pharmacutical Organic Chemistry Department, Faculty of Pharmacy, Beni Suef University, Beni Suef, (EGYPT)
[2]Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, (EGYPT)
E-mail: emankamal954@yahoo.com

## ABSTRACT

Several novel pyrazolo [4,3-*d*]pyrimidine series **(5,6,9,10,12)** were prepared by treatment of the hydrazide **(4)** with some different commercially available reagents such as isocyanates, isothiocynates, aromatic acids, a series of aromatic aldehydes and certain acid anhydrides respectively. All newly synthesized compounds were confirmed by elemental analysis, spectral data, and subsequent reactions whenever possible. Some of the prepared compounds were screened for cytotoxic activity. Their molecular simulation docking to protein tyrosine kinase (EGFR), using Erlotinib (Tarceva™) as a lead compound was also included. 

## KEYWORDS

Pyrazolo[3,4-*d*]pyrimidine;
Antitumor;
MCF-7;
Docking study;
EGFR.

## INTRODUCTION

Epidermal growth factor receptor (EGFR) family that has four members: HER2 (human epidermal growth factor receptor-2 and its relativies HER1, HER3, HER4, like other EGFR members, is a transmemberanous glycoprotein (P185 neu) with interensic tyrosine kinase activity encoded by HER2 protogen that located on long arm chrosome17(17q21)[1,2]. Ligand activitating tyrosine kinases of growth factor receptors undergo receptor autophosphorylation and phosphorylate their cellular substrates to carry out signal transduction in the cells via ATP[3,4]. The over expression of some growth factor receptors with tyrosine kinase has been found in human cancers as breast and liver cancers[5-7]. Inhibition of tyrosine kinases in the signaling pathways that regulating proliferation and diffusion of tumer celles is one of the most promising strategies in cancer chemotherapy. Additionally several pyrazolo[3,4-*d*]pyrimidines have received a great attention due to their wide pharmacological activities such as antiviral[8], antibacterial[9-11], antifungal[12,13], antituberculosis[14], antidiabetic[15] and herbicidal[16]. Also, they were proved to be effective as inhibitor of inflammatory mediators in intact cells[17]. Moreover, several reports stated that pyrazolopyrimidine derivatives could act as anticancer by inhibition of different types of protein tyrosine kinases such as epidermal growth factor receptor protein tyrosine kinase (EGFR)[1,18,19], cyclin-dependent kinases (CDK)[20] and Src[21,22]. These finding suggest that inhibition of EGFR could be an optional new point of intervention in prevention the onset and spread of cancer. herein certain substituted pyrazolo[3,4-*d*]pyrimidine

skeleton have been designed, synthesized and docked to EGFR with the objective that these new compounds might show EGFR inhibitory activity and subsequently would have anticancer activity.A number of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials. For example, anilinoquinazoline-containing compounds erlotinib I (Tarceva™)[23,24] and gefitinib II (Iressa™)[25,26] have been approved for the chemotherapeutic treatment of patients with advanced non small lung cancer. Also, lapatinib (Tykerb™)[27-29] was approved for treatment of Human epidermal growth factor receptor2 (HER2) positive advanced or meta-

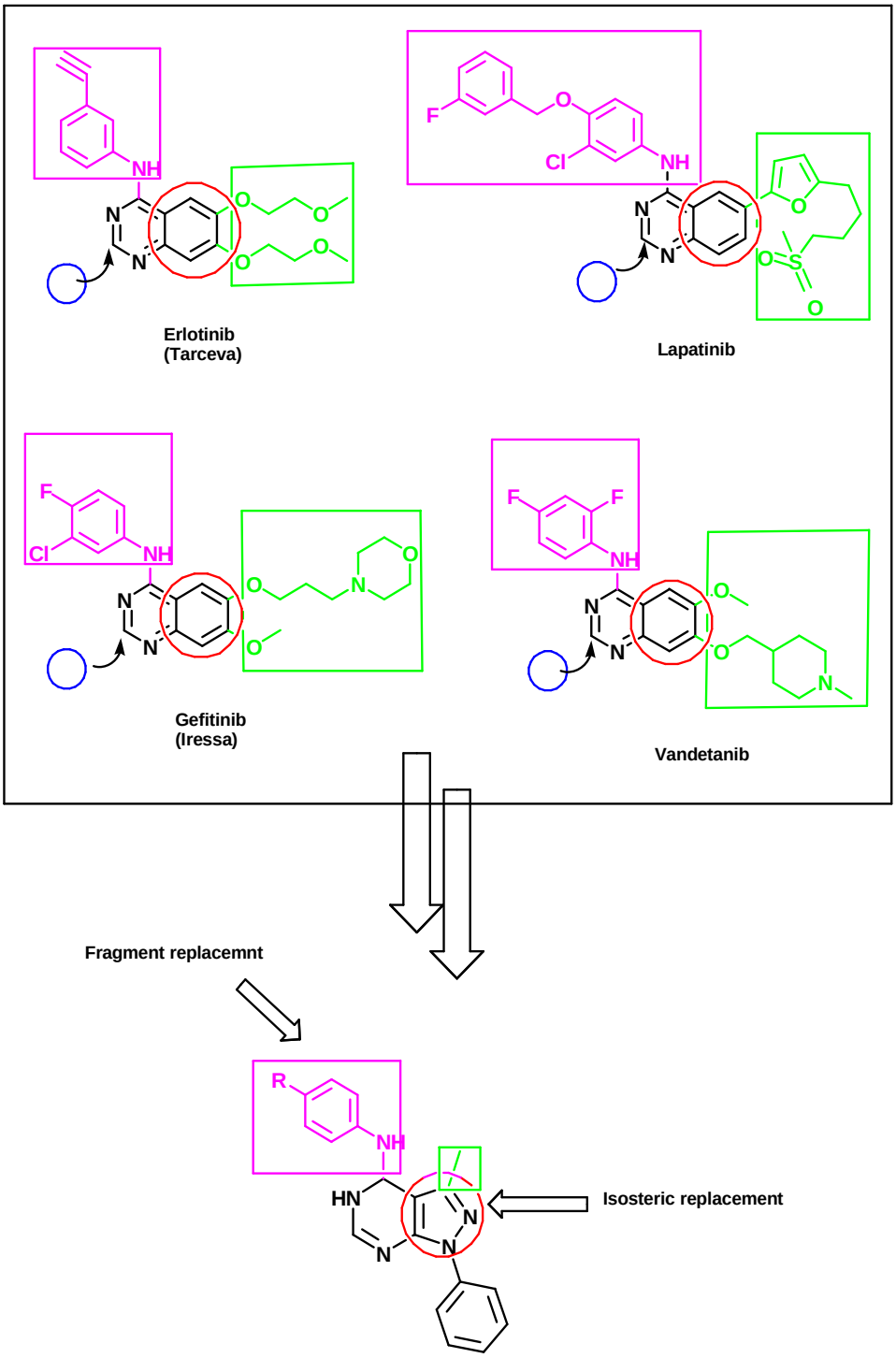



**Figure 1 : Planned design of new pyrazolo[3,4-*d*]pyrimidine derivatives for cytotoxic activity**

static breast cancer.

Erlotinib I, gefitinib II and lapatinib are considered as 4-substituted amino pyrimidine pharmacophoric core compounds that bind to the hinge region of the kinase enzyme. On the basis of the bioisosterism between benzene and pyrazole which is well known and due to their structure similarity to natural purine bases that closely related to ATP base, isomeric pyrazolopyrimidine cores were evaluated as isosteres for 4-anilinoquinazoline core and led to new models of kinase inhibitors(Figure 1). Pyrazolopyrimidines III were considered as potent and selective kinase inhibitors[30]. Structural graft will be done on C4 $NH_2$ group of pyrimidine ring either to expand the hydrophobic moiety or to add hydrogen bond donor-acceptor pair. The isosteric modification in the pharmacophoric based models and structural graft will allow designing new kinase inhibitors.

## EXPERIMENTAL SECTION

### Chemistry

Melting points were determined on Graffin apparatus and are uncorrected. IR spectra were recorded on Shimadzu 435 Spectrometer, using KBr discs and values were represented in $cm^{-1}$. $^1$H-NMR spectra were carried out on Varian Gemini 200 or 300 MHZ Spectrometer, at the Microanalytical Center, Cairo University, Egypt using TMS as internal standard and chemical shifts were recorded in ppm on δ scales. Mass spectra run on Hewlett Packard 5988 Spectrometer, at the Microanalytical center, Cairo University, Egypt and National Research Center, Cairo, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo, Egypt. Progress of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp. 4-Chloro-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine **(2)**[35] were prepared according to reported procedures.

### Ethyl 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzoate (3)

A mixture of 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(2)** (2.30 gm, 0.01 mol), benzocaine (1.65 gm, 0.01 mol) and sodium iodide (0.2 gm) in isopropyl alcohol (20 ml) was heated under reflux for 2 h. The reaction mixture after cooling was neutralized to litmus paper with sodium carbonate solution (20%). The formed precipitate was collected by filtration, washed with water and crystallized from ethanol to afford crystals of **(3)**.

**(3)**: yellow (Yield: 2.9 gm). m.p. 240-242°C; $\nu_{max}$/$cm^{-1}$ (KBr): 3328 (NH); 3124-3044 (CH aromatic); 2983 (CH aliphatic); 1682 (C=O); 1632, 1613, 1596 (C=N and C=C). $^1$HNMR: δ= 1.29 (t, *J*= 7 Hz, 3H, $CH_2$-C<u>H</u>$_3$); 4.28 (q, *J*= 7 Hz, 2H, C<u>H</u>$_2$$CH_3$); 7.37-7.40 (t,1H, ArH ); 7.53-7.61 ArH (t, 2H, ArH); 7.97 (d, *J*= 9 Hz, 2H, ArH); 8.07 (d, *J*= 9 Hz, 2H, ArH); 8.18-8.22 (d,2H, ArH); 8.63 (s, 1H, pyrazole H); 8.65 (s, 1H, pyrimidineH); 10.49 (s, 1H, NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 359 (M)$^+$ (100).

### 4-(1-Phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (4)

A mixture of the ester **(6)** (3.59 gm, 0.01 mol) and hydrazine hydrate (99.9%) (0.5 ml, 0.01 mol) in butanol (30 ml) was heated under reflux for 2 h. The white precipitate formed was filtered while hot, dried and crystallized from dioxane to yield white crystals of **(4)**.

**(4)**: white (Yield: 2.8 gm; 82%). m.p. 260-261°C, $\nu_{max}$/$cm^{-1}$ (KBr): 3463-3309 ($NH_2$ & NH); 1638 (C=O); 1611, 1566, 1529 (C=N and C=C); $^1$HNMR: δ= 4.47 (s, 2H, $NH_2$, $D_2O$ exchangeable); 7.37-7.40 (t, 1H, ArH); 7.54-7.60 (t, 2H, ArH); 7.86 (d, *J*= 9 Hz, 2H, ArH); 7.96 (d, *J*= 9 Hz, 2H, ArH); 8.19-8.22 (d, 2H, ArH); 8.60 (s, 1H, pyrazole H); 8.62 (s, 1H, primidine H); 9.69 (s, 1H, O=C-NH, $D_2O$ exchangeable); 10.38 (s, 1H, NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 345 (M)(17.77). (calcd.for $C_{18}H_{15}N_7O$: C, 62.60; H, 4.38; N, 28.39. Found: C, 62.89; H, 4.61; N, 28.62).

### General procedure for the synthesis of (5a,b)

A mixture of acid hydrazide **(7)** (3.45 gm, 0.01 mol) and the appropriate isocyanate (0.01 mol) in dioxane (20 ml) was heated under reflux for 4 h. The obtained solid was filtered, washed with dioxane, dried and crystallized from acetic acid to yield **(5a,b)**

### *N*-Phenyl-2-[4-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)benzoyl]hydrazine carboxamide (5a)

**(5a)**:White (yield 85%). m.p. > 300 °C; $\nu_{max}$/$cm^{-1}$

(KBr): 3351-3190 (4NH); 3101-3038 (CH aromatic); 1715, 1637 (2C=O); 1614, 1599, 1580 (C=N and C=C). $^1$HNMR: δ= 6.94-6.99 (t, 1H, ArH); 7.23-7.29 (t, 2H, ArH); 7.35-7.40 (t, 1H, ArH); 7.46-7.49 (d, 2H, ArH); 7.56-7.61 (t, 2H, ArH); 7.97 (d, *J*= 8.7 Hz, 2H, ArH); 8.04 (d, *J*= 8.7 Hz, 2H, ArH); 8.14 (s, 1H, NH-NH, $D_2O$ exchangeable); 8.20-8.23 (d, 2H, H ArH); 8.63(s, 1H, pyrazole H); 8.64 (s, 1H, pyrimidine H); 8.84 (s,1H, NH-NH, $D_2O$ exchangeable); 10.22 (s, 1H, O=C-NH-Ar, $D_2O$ exchangeable); 10.45 (s, 1H, NH, $D_2O$ exchangeable); (calcd.for $C_{25}H_{20}N_8O_2$: C, 64.65; H, 4.34; N, 24.12. Found: C, 65.00; H, 4.50; N, 24.00).

### N-(4-Chlorophenyl)-2-[4-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)benzoyl] hydrazinecarboxamide (5b)

**(5b)**: White (yield 85%). m.p. > 300 °C; $v_{max}$/cm$^{-1}$ (KBr): 3329-3208 (4NH); 3115-3027 (CH aromatic); 1652, 1631 (2C=O); 1605, 1577, 1563 (C=N and C=C); $^1$HNMR: δ= 7.30-7.42 (m, 3H, ArH); 7.53-7.63 (m, 4H, ArH); 7.98 (d, *J*= 8 Hz, 2H, ArH); 8.05 (d, *J*= 8Hz, 2H, ArH); 8.20 (s, 1H, NH-NH, $D_2O$ exchangeable); 8.25-8.28 (d, 2H, ArH); 8.64 (s, 1H, pyrazole H); 8.66 (s, 1H, pyrimidine H); 9.05 (s,1H, NH-NH, $D_2O$ exchangeable); 10.27 (s, 1H, O=C-NH-Ar, $D_2O$ exchangeable); 10.48 (s, 1H, NH, $D_2O$ exchangeable) MS (70ev): *m/z* = 498 (M)(10). (calcd.for $C_{25}H_{19}ClN_8O_2$: C, 60.18; H, 3.84; N, 22.46. Found: C, 60.40; H, 3.80; N, 22.20).

### General procedure for the synthesis of (6a,b)

A mixture of acid hydrazide **(7)** (3.45 gm, 0.01 mol), the appropriate isothiocyanate (0.01 mol) and few drops triethylamine in absolute ethanol (20 ml) was heated under reflux for 6 h. The solid was filtered while hot, washed with ethanol and crystallized from ethanol to yield **(6a,b)**.

### *N*-Ethyl-2-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzoyl]hydrazine carbothioamides (6a)

**(6a)**: White (yield 60%). m.p. 240-242 °C; $v_{max}$/cm$^{-1}$ (KBr): 3219-3127 (4NH); 1664 (C=O, amidic); 1614, 1566, 1501 (C=N and C=C); 1185 (C=S). $^1$HNMR: δ=1.06 (t, *J*= 7.2 Hz, 3H, $CH_2CH_3$); 3.47 (q, *J*= 7.2 Hz, 2H, $CH_2CH_3$); 7.38-7.41 (t, 1H, ArH); 7.55-7.61 (t, 2H, ArH); 7.96 (d, *J*= 9 Hz, 2H, ArH); 7.99 (s,1H, NH-$CH_2CH_3$, $D_2O$ exchangeable); 8.02 (d, *J*= 9 Hz, 2H, ArH); 8.20-8.23 (d, 2H, ArH); 8.62 (s, 1H, pyrazole H); 8.63 (s, 1H, pyrimidine H); 9.10 (s, 1H, NH-NH-C=S, $D_2O$ exchangeable); 10.20 (s, 1H, O=C-NH, $D_2O$ exchangeable); 10.40 (s, 1H, NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 432 (M)(1). (calcd.for $C_{21}H_{20}N_8OS$: C, 58.32; H, 4.66; N, 25.91. Found: C, 62.49; H, 4.20; N, 23.32).

### *N*-Phenyl-2-[4-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino) benzoyl] hydrazinecarbothio-amides (6b)

**(6b)**: White (Yield 60%). m.p. > 300 °C; $v_{max}$/cm$^{-1}$ (KBr): 3308-3118 (4NH); 1648 (C=O, amidic); 1613, 1594, 1561 (C=N and C=C); 1192 (C=S); $^1$HNMR: δ= 7.15-7.17 (m, 1H, ArH ); 7.33-7.41 (m, 3H, ArH); 7.46-7.48 (d, 2H, ArH); 7.56-7.61 (m, 2H, ArH); 7.95 (d, *J*= 8.7Hz, 2H, ArH); 8.01 (d, *J*= 8.7Hz, 2H, ArH); 8.20-8.23 (d, 2H, ArH); 8.63 (s, 1H, pyrazole H); 8.64 (s, 1H, pyrimidine H); 9.67 (s, 1H, S=C-NH-Ar, $D_2O$ exchangeable); 9.80 (s, IH, NH-NH-C=S, $D_2O$ exchangeable); 10.45 (s, 2H, O=C-NH and NH, $D_2O$ exchangeable). (calcd.for $C_{25}H_{20}N_8OS$: C, 62.49; H, 4.20; N, 23.32. Found: C, 61.99; H, 4.62; N, 23.00).

General procedure for the synthesis of **(7a,b)**

The appropriate thiosemicarbazide derivative **(6a,b)** (3.4 mmol) was stirred for 10 min in ice cold concentrated sulfuric acid (10 ml), then left for another 10 min at room temperature. The resulting solution was poured onto ice-cold water and made alkaline to pH 8 with aqueous ammonia (30%). The precipitated product was filtered, washed with water then crystallized from isopropyl alcohol to yield **(7a,b)**

### *N*-Ethyl-5-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]-[1,3,4]thiadiazol-2-amines (7a)

**(7a)**: White (yield 56%). m.p. > 300 °C; $v_{max}$/cm$^{-1}$ (KBr): 3270, 3200 (2NH); 3100-3060 (CH aromatic); 1563, 1532, 1509 (C=N and C=C). $^1$HNMR: δ= 1.18 (t, *J*=7.2 Hz, 3H, $CH_2CH_3$); 3.28 (q, *J*=7.2 Hz, 2H, $CH_2CH_3$); 7.37-7.40 (m, 1H, ArH); 7.55-7.60 (m, 2H, ArH); 7.78 (d, *J*=8.4 Hz, ArH); 7.86 (s,1H, NH-$CH_2CH_3$, $D_2O$ exchangeable); 8.01 (d, *J*= 8.4 Hz, 2H, ArH); 8.20-8.23 (d, 2H, ArH); 8.60 (s, 1H, pyrazole H); 8.62 (s, 1H, pyrimidine H); 10.40 (s, 1H, NH,

$D_2O$ exchangeable); MS (70ev): *m/z* = 414 (M) (100). (calcd. for $C_{21}H_{18}N_8S$: C, 60.85; H, 4.38; N, 27.03. Found: C, 61.24; H, 4.06; N, 27.24).

### *N*-Phenyl-5-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]-[1,3,4]thiadiazol-2-amines (7b)

**(7b)**: White (yield 85%). m.p. > 300 °C; $\nu_{max}/cm^{-1}$ (KBr): 3312, 3215 (2NH); 3100-3034 (CH aromatic); 1611, 1571, 1525 (C=N and C=C). $^1$HNMR: δ= 7.31-7.37 (m, 4H, ArH &NH-phenyl, $D_2O$ exchangeable); 7.37-7.38 (d, 2H, ArH); 7.51-7.58 (m, 5H, ArH); 7.86 (d, *J*= 6.3 Hz, 2H, ArH); 8.17-8.19 (d, 2H, ArH); 8.55 (s, 1H, pyrazole H); 8.56 (s, 1H, pyrimidine H); 10.34 (s, 1H, NH, $D_2O$ exchangeable); (calcd. for $C_{25}H_{18}N_8S$: C, 64.92; H, 3.92; N, 24.23. Found: C, 65.28; H, 4.42; N, 24.05).

### General procedure for the synthesis of (8a,b)

A mixture of the appropriate thiosemicarbazide derivative **(6a,b)** (0.005 mol) was heated under reflux for 6 h in a mixture of piperidine (2ml) and water (5ml). The reaction mixture was poured onto ice-cold water and the mixture was neutralized with acetic acid (20%). The solid obtained was filtered, dried and crystallized from the appropriate solvent(acetone and ethanol respectively) to give **(8a,b)**.

### 4-Ethyl -5-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]-4*H*-[1,2,4]triazole-3-thiols (8a)

**(8a)**: White (yield 60%). m.p. > 300 °C ;; $\nu_{max}/cm^{-1}$ (KBr): 3427 (NH); 3105-3050 (CH aromatic); 1614, 1590, 1563 (C=N and C=C)$^1$HNMR: δ=1.17 (t, *J*= 7.2 Hz, 3H, $CH_2C\underline{H}_3$); 4.06 (q, *J*= 7.2 Hz, 2H, $C\underline{H}_2CH_3$); 7.38-7.41 (m, 1H, ArH); 7.55-7.60 (m, 2H, ArH); 7.72 (d, *J*=7.2 Hz, 2H, ArH); 8.12 (d, *J*= 7.2 Hz, 2H, ArH); 8.22-8.23 (d, 2H, ArH); 8.62 (s, 1H, pyrazol H); 8.65 (s, 1H, pyrimidine H); 10.48 (s, 1H, NH, $D_2O$ exchangeable); 13.80 (s, 1H, SH, $D_2O$ exchangeable). (calcd. for $C_{21}H_{18}N_8S$: C, 60.85; H, 4.38; N, 27.03. Found: C, 60.38; H, 4.47; N, 26.54).

### 4-Pheyl -5-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]-4*H*- [1,2,4]triazole-3-thiols (8b)

**(8b)**: Yield 78%; m.p. > 300 °C; $\nu_{max}/cm^{-1}$ (KBr): 3312 (NH); 3082-3015 (CH aromatic); 1627, 1595, 1566 (C=N and C=C); $^1$HNMR: δ= 7.31-7.38 (m, 5H, ArH); 7.51-7.58 (m, 5H, ArH); 7.85 (d, *J*=8.7 Hz, 2H, ArH); 8.17-8.19 (d, 2H, ArH); 8.55 (s, 1H, pyrazol H); 8.57 (s, 1H, pyrimidne H); 10.34 (s, 1H, NH, $D_2O$ exchangeable); 14.15 (s, 1H, SH, $D_2O$ exchangeable). (calcd. for $C_{25}H_{18}N_8S$: C, 64.92; H, 3.92; N, 24.23. Found: C, 64.60; H, 3.92; N, 24.50).

### General procedure for the synthesis of (9a,b)

A mixture of the acid hydrazide **(4)** (0.69 gm, 0.002 mol) and the respective aromatic acid (0.002 mol) in phosphorous oxychloride (10 ml) was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto crushed ice and neutralized to litmus paper with sodium carbonate solution (20%). The formed precipitate was filtered, washed with water, dried and crystallized from ethanol to yield the title compounds **(9a,b)**.

### [4-(5-Phenyl-[1,3,4]oxadiazol-2-yl)phenyl]-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin -4-yl)amine (9a)

**(9a)**: White (yield 60%). m.p. 260-261 °C; $\nu_{max}/cm^{-1}$ (KBr): 3417 (NH);1592, 1567, 1508 (C=N and C=C). ; 3463-3309 ($NH_2$ & NH); 1638 (C=O); 1611, 1566, 1529 (C=N and C=C); $^1$HNMR: δ= 6.84-7.02 (m, 2H, ArH); 7.34-7.40 (m, 2H, ArH); 7.54-7.60 (m, 2H, ArH); 7.83-7.86 (d, 2H, ArH); 8.09 (d, *J*= 8.7 Hz, 2H, ArH); 8.19 (d, 2H, *J*= 8.7 Hz, ArH); 8.30-8.33 (d, 2H, ArH); 8.56 (s, 1H, pyrazole H); 8.63 (s, 1H, pyrimidine H); 8.96 (s, 1H, NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 431 (M)(16.64). (calcd.for $C_{25}H_{17}N_7O$: C, 69.60; H, 3.97; N, 22.72. Found: C, 69.49; H, 4.15; N, 23.11).

### 2-{5-[4-(1-Phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4ylamino)-phenyl]-[1,3,4]oxadiazol-2-yl}-phenol (9b)

**(9b)**: Yellow (yield 90%; m.p. 295 °C (dec.); $\nu_{max}/cm^{-1}$ (KBr): 3302 (NH); 3202 (OH); 3100-3000 (CH aromatic); 1612, 1564, 1500 (C=N and C=C); $^1$HNMR: δ= 3.57 (s, 1H, OH, $D_2O$ exchangeable); 7.00-7.16 (m, 2H, ArH); 7.36-7.46 (m, 2H, ArH); 7.53-7.72 (m, 2H, ArH); 7.88-7.92 (d, 1H, ArH); 8.06 (d, *J*= 8 Hz, 2H, ArH); 8.19 (d, *J*= 8 Hz, 2H, ArH); 8.26-8.30 (d, 2H, ArH); 8.59 (s,1H, pyrazol H); 8.63 (s, 1H, pyrimidine H); 10.85 (s,1H, NH, $D_2O$ exchange-

able); MS (70ev): *m/z* = 447 (M)(40). (calcd.for $C_{25}H_{17}N_7O_2$: C, 67.11; H, 3.83; N, 21.91. Found: C, 67.41; H, 3.80; N, 21.48.

**General procedure for the synthesis of (10a-e)**

A mixture of acid hydrazide **(4)** (1.73 gm, 0.005 mol) and the appropriate carbonyl compound (0.005 mol) in acetic acid (15ml) was heated under reflux for 2 h. The formed precipitate was filtered, washed with water, dried and crystallized from the appropriate solvent to yield **(10a-e)**.

**4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino) benzohydrazide (10a)**

**(10a)**: Crystallization solvent is ethanol, White (yield 77%). m.p. > 300 °C; $\nu_{max}$/cm$^{-1}$ (KBr): 3332, 3250 (2NH); 3100-3027 (CH aromatic); 1650 (C=O); 1610, 1563, 1529 (C=N and C=C). $^1$HNMR: δ=7.39-7.47 (m, 4H, ArH); 7.56-7.73 (m, 4H, ArH); 7.98 (d, *J*= 8 Hz, 2H, ArH); 8.06 (d, *J*= 6.6 Hz, 2H, ArH); 8.21-8.23 (d, 2H, ArH); 8.48 (s, 1H, N=CH azomethine); 8.64 (s, 1H, pyrazole H); 8.66 (s, 1H, pyrimidine H); 10.47 (s,1H, NH, $D_2O$ exchangeable); 11.78 (s, 1H, O=C-NH, $D_2O$ exchangeable); (calcd. for $C_{25}H_{19}N_7O$: C, 64.27; H, 4.42; N, 22.62. Found: C, 69.55; H, 4.55; N, 22.41).

***N*-(4-hydroxybenzylidene)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzo-hydrazide (10b)**

**(10b)**: Crystallization solvent is ethanol, White (yield 89%); $\nu_{max}$/cm$^{-1}$ (KBr): 3464, 3224 (2NH); 1638 (C=O); 1601, 1574, 1562 (C=N and C=C). m.p. > 300 °C; $^1$HNMR: δ= 6.82-6.85 (d, 2H, ArH); 7.37-7.40 (m, 1H, ArH); 7.55-7.57 (m, 4H, ArH '); 7.95 (d, *J*= 7.8 Hz, 2H, ArH); 8.04 (d, *J*= 7.8 Hz, 2H, ArH); 8.19-8.22 (d, 2H, ArH); 8.36 (s, 1H, N=CH azomethine); 8.63 (s, 1H, pyrazol H); 8.65 (s, 1H, pyrimidine H); 9.90 (s,1H, OH, $D_2O$ exchangeable); 10.45 (s,1H, NH, $D_2O$ exchangeable); 11.58 (s, 1H, O=C-NH, $D_2O$ exchangeable; (calcd. for $C_{25}H_{19}N_7O_2$: C, 66.81; H, 4.26; N, 21.81. Found: C, 67.13; H, 4.30; N, 21.89).

***N*-(4-chlorobenzylidene)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (10c)**

**(10c)**: Crystallization solvent is ethanol, White (yield 83%; m.p. > 300 °C; $\nu_{max}$/cm$^{-1}$ (KBr): $^1$HNMR (300MHz, DMSO): δ=7.37-7.40 (t, 1H, ArH); 7.55-7.61 (t, 2H, ArH); 7.97-8.02 (m, 4H, ArH); 8.09 (d, *J*= 8.7Hz, 2H, ArH); 8.20 (d, *J*= 8.7Hz, 2H, ArH); 8.28-8.31 (d, 2H, ArH); 8.63 (s, 1H, N=CH azomethine); 8.64 (s, 1H, pyrazole H); 8.69 (s, 1H, pyrimidine H); 10.55 (s,1H, NH, $D_2O$ exchangeable); 12.11 (s, 1H, O=C-NH, $D_2O$ exchangeable) ppm; IR (KBr, cm-$^1$): 3466, 3218 (2NH); 1661 (C=O); 1613, 1598, 1579 (C=N and C=C). Anal. Calcd. for $C_{25}H_{18}ClN_7O$: C, 64.17; H, 3.88; N, 20.95. Found: C, 64.31; H, 3.67; N, 20.57.

***N*-(4-dimethylaminobenzylidene)4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzo-hydrazide (10d)**

**(10d)**: Crystallization solvent is acetic acid, Yellow (yield 83%; m.p. > 300 °C; $\nu_{max}$/cm$^{-1}$ (KBr): 3434, 3213 (2NH); 1655 (C=O); 1613, 1602, 1576 (C=N and C=C); $^1$HNMR: δ= 2.45 (s, 6H, $N(CH_3)_2$); 7.37-7.57 (m, 3H, ArH); 8.14 (d, *J*= 7.2 Hz, 4H, ArH ); 8.35 (d, *J*= 7.2 Hz, 4H, ArH); 8.46-8.48 (d, 2H, ArH); 8.53 (s, 1H, N=CH azomethine); 8.64 (s, 1H, pyrazole H); 8.69 (s, 1H, pyrimidine H); 10.55 (s,1H, NH, $D_2O$ exchangeable); 12.11 (s, 1H, O=C-NH, $D_2O$ exchangeable); (calcd. for $C_{25}H_{18}ClN_7O$: C, 68.05; H, 5.08; N, 23.51. Found: C, 67.97; H, 5.15; N, 23.22).

***N*-(4-nitroaminobenzylidene)4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzo-hydrazide (10e)**

**(10e)**: Crystallization solvent is acetic acid, Yellow (yield 95%); m.p. > 300 °C; $\nu_{max}$/cm$^{-1}$ (KBr): 3325, 3228 (2NH); 1662 (C=O); 1614, 1582, 1564 (C=N and C=C). $^1$HNMR: δ= 7.35-7.40 (t, 1H, ArH); 7.51 (d, *J*= 7.2 Hz, 2H, ArH); 7.54-7.60 (t, 2H, ArH); 7.74 (d, *J*= 7.2 Hz, 2H, ArH); 7.97 (d, *J*= 8.7 Hz, 2H, ArH ); 8.06 (d, *J*= 8.7 Hz, 2H, ArH); 8.20-8.23 (d, 2H, ArH); 8.47 (s, 1H, N=CH azomethine); 8.63 (s, 1H, pyrazole H); 8.66 (s, 1H, pyrimidne H); 10.50 (s,1H, NH, $D_2O$ exchangeable); 11.86 (s, 1H, O=C-NH, $D_2O$ exchangeable) ; MS (70ev): *m/z* = 478 (M) (5).(calcd. for $C_{21}H_{18}N_8S$: C, 62.76; H, 3.79; N, 23.42. Found: C, 62.90; H, 3.79; N, 23.51).

**General procedure for the synthesis of (11a-c)**

A mixture of equimolar of the appropriate

azomethine derivative **(10a,c,e)** and mercaptoacetic acid (0.01 mol of each ) in dry benzene (100 ml) was heated under reflux using water separator until the theoretical amount of water was collected. The solvent was evaporated under reduced pressure and the residue was crystallized from ethanol to afford **(11a-c)**

### *N*-(4-Oxo2-phenyl-thiazolidin-3-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-benzamide (11a)

**(11a)**: White (yield 70%); m.p. 228-230 °C; $\nu_{max}$/ $cm^{-1}$ (KBr): 3336, 3216 (2NH); 2921-2852 (CH aliphatic); 1700 (C=O, exocyclic); 1644 (C=O, amidic); 1566, 1527, 1499 (C=N and C=C). $^1$HNMR: δ=3.77 (dd, $J_1$= 15.9 Hz, $J_2$= 8.7 Hz, 2H, $CH_2$ of thiazolidinone ring); 5.95 (s, 1H, CH of thiazolidinone ring); 7.35-7.42 (m, 4H, ArH); 7.49 (d, *J*= 6.3 Hz, 2H, ArH); 7.53-7.59 (t, 2H, ArH); 7.79 (d, *J*= 9 Hz, 2H, ArH "); 7.98 (d, *J*= 9 Hz, 2H, ArH); 8.18 (d, *J*= 8.4 Hz, 2H, ArH); 8.62 (s, 1H, pyrazole H); 8.64 (s, 1H, pyrimidine H); 10.4 (s, 1H, NH, $D_2O$ exchangeable); 10.64 (s, 1H, O=C-NH, $D_2O$ exchangeable); (calcd. for $C_{27}H_{21}N_7O_2S$: C, 63.89; H, 4.17; N, 19.32. Found: C, 63.47; H, 4.47; N, 19.02).

### *N*-(2-(4-chlorophenyl)-4-Oxothiazolidin-3-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzamide (11b)

**(11b)**: White (yield 62%); m.p. 232-235 °C; $\nu_{max}$/ $cm^{-1}$ (KBr): 3303, 3212 (2NH); 2919-2856 (CH aliphatic); 1704 (C=O, exocyclic); 1644 (C=O, amidic); 1569, 1511, 1492 (C=N and C=C).$^1$HNMR : δ= 3.78 (dd, $J_1$= 16.8 Hz, $J_2$= 9.3 Hz, 2H, $CH_2$ of thiazolidinone ring); 5.95 (s, 1H, CH of thiazolidinone ring); 7.34-7.39 (m, 3H, ArH); 7.51 (d, *J*= 8.4 Hz, 2H, ArH); 7.56-7.59 (t, 2H, ArH); 7.79 (d, *J*= 7.5 Hz, 2H, ArH); 7.99 (d, *J*= 7.5 Hz, 2H, ArH); 8.18-8.21 (d, 2H, ArH); 8.60 (s, 1H, pyrazole H); 8.63 (s, 1H, pyrimidine H); 10.48 (s,1H, NH, $D_2O$ exchangeable); 10.65 (s, 1H, O=C-NH, $D_2O$ exchangeable); MS (70ev): *m/z* =541 (M)(3).(calcd. for $C_{27}H_{20}ClN_7O_2S$: C, 59.83; H, 3.72; N, 18.09. Found: C, 60.03; H, 4.00; N, 17.79).

### *N*-(2-(4-nitrophenyl)-4-Oxothiazolidin-3-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino ) benzamide (11c)

**(11c)**: Yellow (yield 63%); m.p. 260-262 °C; $\nu_{max}$/ $cm^{-1}$ (KBr): 3345, 3218 (2NH); 1688 (C=O, exocyclic); 1645 (C=O, amidic); 1602, 1568, 1526 (C=N and C=C). $^1$HNMR: δ= 3.82 (dd, *J*= 15.9 Hz, $J_2$= 3 Hz, 2H, $CH_2$ of thiazolidinone ring); 6.10 (s, 1H, CH of thiazolidinone ring); 7.38-7.40 (t, 1H, ArH); 7.55-7.60 (t, 2H, ArH); 7.80 (d, *J*= 8.7 Hz, 4H, ArH); 8.00 (d, *J*= 8.7 Hz, 2H, ArH); 8.19-8.23 (d, 2H, ArH); 8.24 (d, *J*= 8.7 Hz, 2H, ArH); 8.61 (s, 1H, pyrazole H); 8.63 (s, 1H, pyrimidineH); 10.45 (s,1H, NH, $D_2O$ exchangeable); 10.72 (s, 1H, O=C-NH, $D_2O$ exchangeable); (calcd. for $C_{27}H_{20}N_8O_4S$: C, 58.69; H, 3.65; N, 20.28. Found: C, 58.89; H, 4.00; N, 20.41).

### General procedure for the synthesis of (12a-c)

A mixture of acid hydrazide **(4)** (3.45 gm, 0.01 mol) and the appropriate acid anhydride (0.01 mol) in glacial acetic acid (10 ml) was heated under reflux for 3 h. After cooling, the solution was poured onto ice-cold water while stirring. The formed precipitate was filtered, dried and crystallized from ethanol to give **(12a-c)**.

### *N*-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino) benzamide (12a)

**(12a)**: White (yield: 2.97 gm; m.p. > 300 °C; $\nu_{max}$/ $cm^{-1}$ (KBr): 3290, 3213 (2NH); 1793, 1728 (2C=O of anhydride moiety); 1683 (C=O, amidic); 1610, 1596, 1576 (C=N and C=C); $^1$HNMR: δ= 7.36-7.41 (t, 1H, ArH); 7.56-7.61 (t, 2H, ArH); 7.96-8.00 (d, 2H, CH=CH); 8.02 (d, *J* = 8.7 Hz, 2H, ArH); 8.11 (d, *J*= 8.7 Hz, 2H, ArH); 8.20-8.23 (d, 2H, ArH); 8.65 (s, 1H, pyrazole H); 8.66 (s, 1H, pyrimidine H); 10.54 (s, 1H, NH, $D_2O$ exchangeable); 11.20 (s, 1H, O=C-NH, $D_2O$ exchangeable). (calcd.for $C_{22}H_{15}N_7O_3$: C, 62.12; H, 3.55; N, 23.05. Found: C, 62.25; H, 3.45; N, 22.73).

### *N*-(2,5-Dioxo-pyrrolidin-1-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzamide (12b)

**(12b)**: Yellow (yield: 82%); m.p. > 300 °C; $\nu_{max}$/$cm^{-1}$ (KBr): 3481, 3269 (2NH); 1795, 1731 (2C=O of anhydride moiety); 1692 (C=O, amidic); 1609, 1598, 1577 (C=N and C=C); $^1$HNMR: δ= 2.86 (s, 4H, $CH_2$-$CH_2$); 7.36-7.40 (t, 1H, ArH); 7.55-7.61 (t, 2H, ArH); 7.97 (d, *J*= 9 Hz, 2H, ArH); 8.09 (d, *J*= 9 Hz, 2H, ArH); 8.20-8.22 (d, 2H, ArH); 8.63 (s, 1H, pyrazole

H); 8.65 (s, 1H, pyrimidine H); 10.50 (s, 1H, NH, $D_2O$ exchangeable); 10.95 (s, 1H, O=C-NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 427 (M)(16.1). (calcd.for $C_{22}H_{17}N_7O_3$: C, 61.82; H, 4.01; N, 22.94. Found: C, 62.31; H, 4.34; N, 22.70).

### *N*-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino) benzamide (12c)

**(12c)**: White (yield 65%); m.p. > 300 °C; $\nu_{max}/cm^{-1}$ (KBr): 3360, 3224 (2NH); 1792, 1738 (2C=O of anhydride moiety); 1670 (C=O, amidic); 1611, 1577, 1562 (C=N and C=C); $^1$HNMR: δ= 7.35-7.42 (t, 1H, ArH); 7.55-7.62 (t, 2H, ArH); 7.99-8.02 (m, 6H, ArH, H4"', ArH); 8.07 (d, *J*= 8.2 Hz, 2H, ArH); 8.20-8.24 (d, 2H, ArH); 8.66 (s, 1H, pyrazole H); 8.67 (s, 1H, pyrimidine H); 10.55 (s, 1H, NH, $D_2O$ exchangeable); 11.28 (s, 1H, O=C-NH, $D_2O$ exchangeable); MS (70ev): *m/z* = 475 (M)(6). (calcd.for $C_{26}H_{17}N_7O_3$: C, 65.68; H, 3.60; N, 20.62. Found: C, 65.81; H, 3.89; N,20.34

### Docking studies

### Preparation for docking

Molecular modelling was performed using the X-ray crystal structure of built[23] of erlotinib with EGFR aidedbwith 'Molecular Operating Environment (MOE) version 2008.10'.

### Validation of docking procedure

Docking of the co-crystallized ligand should be carried out to study the scoring energy (s), root mean standard deviation (rmsd) and amino acid interactions. The root mean square deviation (RMSD) which is measure of superposing was 0.67Aº for the lead compound. Docking was performed using London d G force and refinement of the results was done using Force field energy.

### Preparing compounds for docking

Preparation of the synthesized compounds for docking was achieved *via* their 3D structure built by MOE. Certain procedures should be taken before docking which include: 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligand. The previous measures were taken and docking for the synthesized compounds was applied.

### Antitumor activity

The breast tumor cell line was obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection (ATCC) and was maintained at the NationalCancer Institute, Cairo, Egypt, by serial subculturing. Doxorubicin was used in this experiment as a positive control. The tested compounds were dissolved in 20% DMSO in concentration 1mg/mL. Serial dilutions were made reaching final concentration of the compounds to 0, 5, 12.5, 25 and 50µg/mL. Previous experiments have shown that DMSO at this concentration does not modify the cellular activities that we are analyzing. All chemicals used in this study are of high analytical grade.They either obtained from (Sigma-Alderich or Biorad)

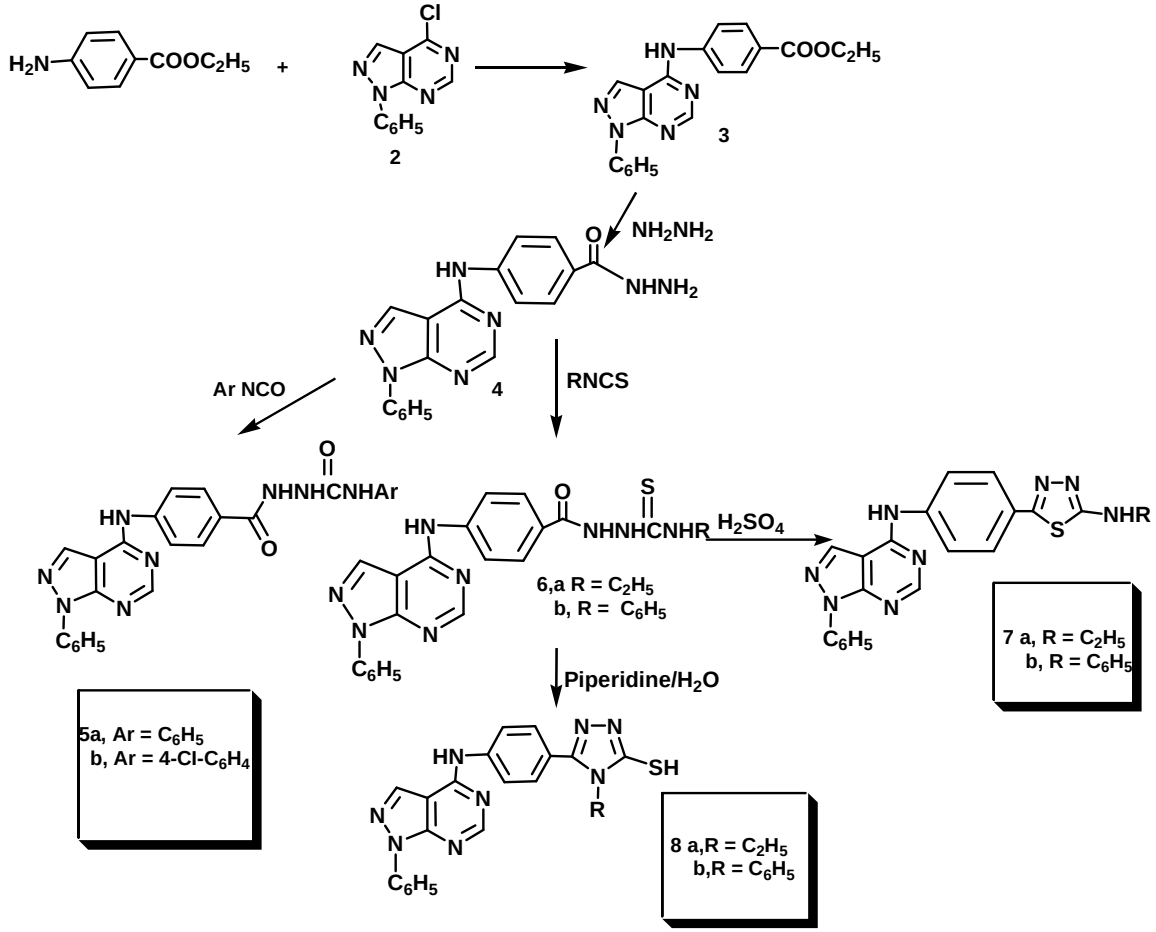
### Measurement of potential cytotoxic activity

The cytotoxic activity was measured *in vitro* on human breast tumor cell line (MCF-7) using sulforhodamine-B stain (SRB) assay applying the method of Skehan, *et al*[34]. Cells were plated in 96 multiwell plates (104 cell/well) for 24 hour before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the compound under test (0, 5, 12.5, 25, and 50µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.Monolayer cells were incubated with the compounds for 48 hours at 37oC and in atmosphere of 5% CO2. After 48 hours cell was fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain w

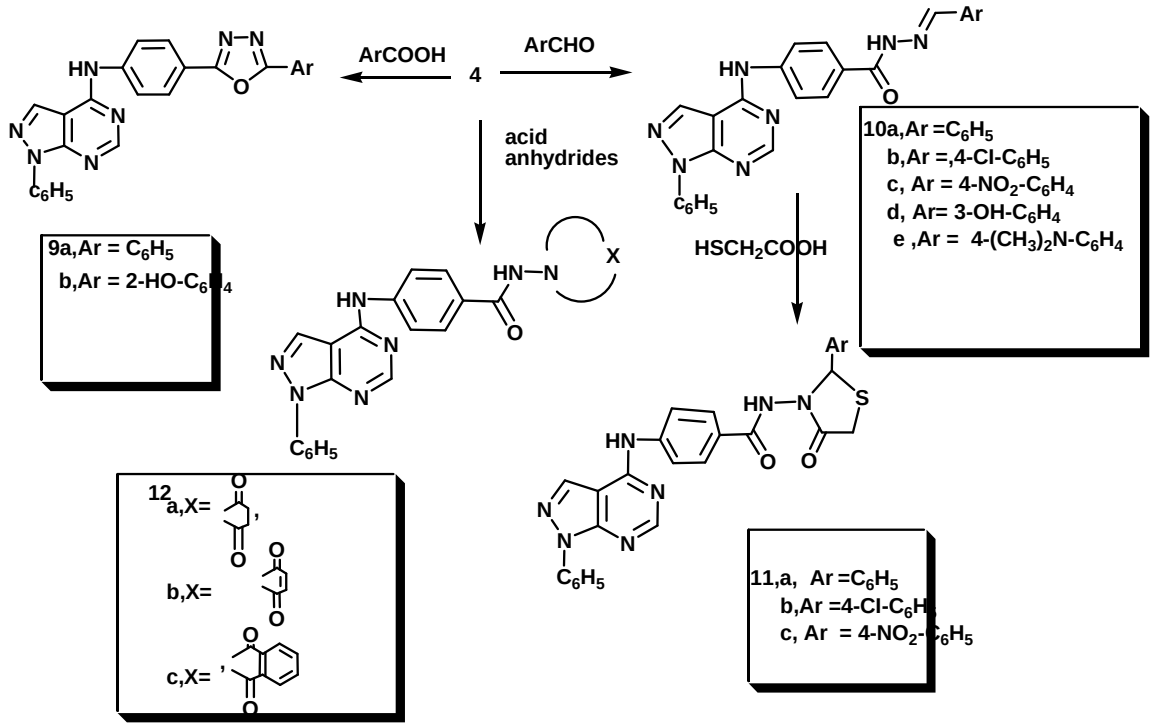
## RESULTS AND DISCUSSION

### Chemistry

Benzocaine was alkylated with 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(2)** in isopropyl alcohol to get ethyl 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino) benzoate ester **(3)**. The previous ester was converted to its hydrazide **(4)** by treatment with hydrazine hydrate in butanol and this hydrazide **(4)** is considered as the key intermediates that through it all functionally changed pyrazolo[3,4-*d*]pyrimidines were obtained as described in (Scheme1 &2).




**Scheme 1**




**Scheme 2**

Upon heating of hydrazide **(4)** with some of isocyanates and isothiocynates giving rise to series 5 and series 6 respectively. Both of series 5 and 6 include a five spacer atoms [(O=)C-N-N-C(=O or S)-N ]between aminophenyl moiety and another sided aryl. This modification might enhance bitter interaction with receptor that contains many centers for either hydrogen bond donner or acceptor centers. Additionally cyclization of **(6)** either in presence of acid or base catalyst resulted in thiadiazoles **(7a,b)** and the triazolo derivtivies **(8a, b)** respectively. Both of these series closely mimic to the reported pyrazolopyrimidines III and so expected to higher reactivity additionally, oxadiazole derivatives **(9a)** and **(9b)** were obtained by heating of **(4)** with definite acids in presence of phosphorous oxychloride. In derivatives **(9)** the aminophenyl moiety is separated from the other aryl through the oxadiazole, three aromatic rings are coplanner and maintain linearity of these compounds **(9)** and this linearity might result in more depth interaction with hydrophobic pocket (Scheme1).

Further the key intermediate **(4)** was heated with different aldhydes giving arylidine derivatives **(10)**. More over some of these arylidine were cyclized with mercaptoacetic acid to the corresponding thiazolidinone **(11)**. Finally, refluxing compound **(4)** with certain acid anhydrides in glacial acetic acid gave *N*-Substituted-4-(1-phenyl-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-ylamino)benzamides **(12a-c)**. Series **(11)** and **(12)** the modification of structures is through presence of angular amidic function para to the aminophenyl moiety and would result in another interaction with receptor and also differs from the other modification in scheme 1. (Scheme2). All prepared compound physical characteristic and its spectral data is illustrated in the experimental section.

## Molecular docking

Aberrant catalytic activity of many PKs via mutation or overexpression plays an important role in numerous pathological conditions including cancer[31,32] Protein kinase inhibitors have been widely used to probe the role of protein phosphorylation in cellular signaling and constitute an important new class of potential therapeutic agents in the management of cancer. We used the BLAST server at NCBI to search the protein Data Bank the top ranked structure was EGFR with a considerable sequence identity. Inspection of the three dimensional structure of erlotinb receptor complex was obtained from Protein Data Bank (PDB entry: 1M17) at Research Collaboration for Structural Bioinformatics (RCSB) protein database[33].

In 2002 the crystal structure of the kinase domain from the epidermal growth factor receptor (EGFR) was detected by Stamos et al[23] with erlotinib as an anti-cancer agent. In this crystal structure there are two H-bonding interactions between The N1 of the quinazoline (of erlotinib) accepts an H-bond from the Met 769 amide nitrogen, The other quinazoline nitrogen atom (N3) is not within H-bonding distance of the Thr766 side chain (4.1 Å), but a water molecule bridges this gap. The aryloxy and the phenyl near the amino group are perfectly positioned to enter two deep hydrophobic pockets with a (docking score -21.35).These hydrophobic interactions within these selectivity pockets combined with the additional hydrogen bond interactions are the key features for several potent kinase inhibitors. Our goal was to explore virtual screening of a diverse of the synthesized pyrazolpyrimidine derivatives, using more closely related crystal structure build by Stamos et al[23] as a templetate for the possibility of discovering novel hits that may be developed into drug leads. The prepared compounds were docked manually and a rigid receptor/flexible ligand approach was adopted that used five potential energy maps combining hydrophobicity, electrostatic, hydrogen bond formation and van der waal parameters. The docking scores were displayed in energy terms; the higher the score (in negative terms) the better the binding affinity. The results of the docking study were listed in TABLE 1.

**TABLE 1 : The percentage of cell survival and $IC_{50}$ values (the concentration required to produce 50%inhibition of cell growth)**

| Compound | Survival fraction (%) Conc. (μM) | | | | $IC_{50}$(μM) |
|---|---|---|---|---|---|
| | 5 | 12.5 | 25 | 50 | |
| Doxorubicin | 0.82 | 0.17 | 0.18 | 0.20 | 5.47 |
| 5b | 0.19 | 0.17 | 0.18 | 0.20 | -ve |
| 7b | 0.47 | 0.48 | 0.55 | 0.67 | 10.39 |
| 9a | 0.89 | 0.40 | 0.27 | 0.36 | 25.52 |
| 9b | 0.54 | 0.40 | 0.51 | 0.49 | 15.45 |
| 11c | 0.65 | 0.39 | 0.51 | 0.49 | 16.30 |
| 12a | 0.46 | 0.36 | 0.25 | 0.31 | 10.94 |
| 12c | 0.60 | 0.25 | 0.24 | 0.41 | 21.89 |

Full Paper

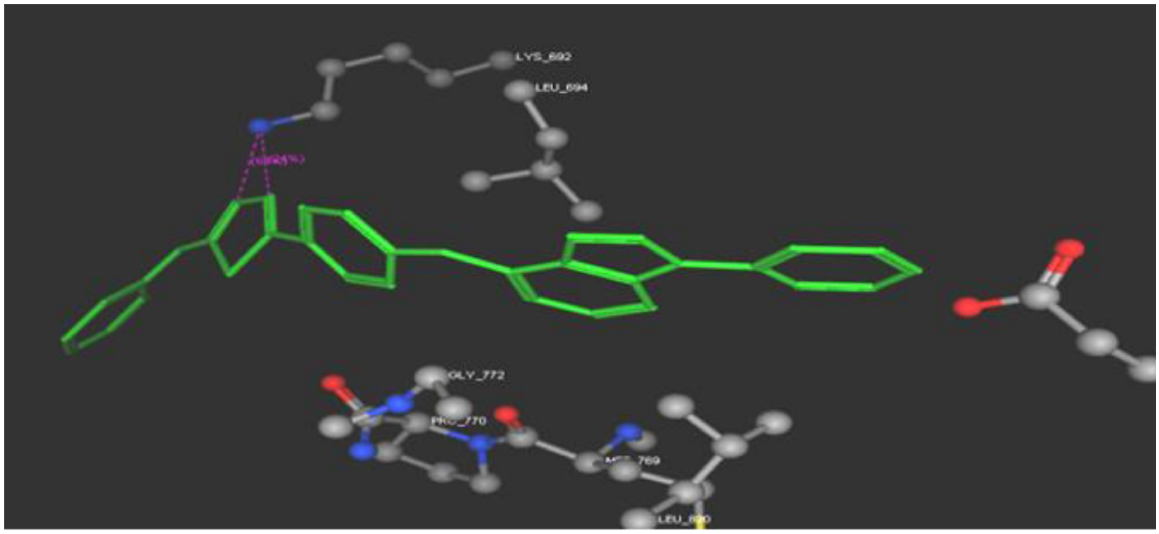
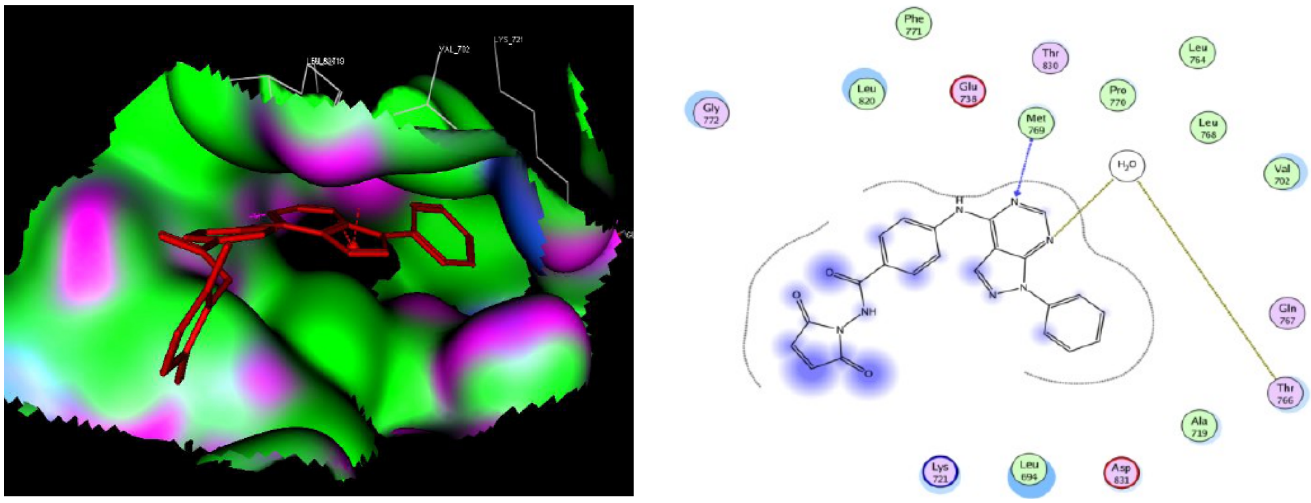



**Figure 2 : Represents of 7b (2D in ball and stick manner) with the binding site of EGFR using Moe software, the dotted lines in pink represent the new H- bonding interactions with Lys692 acid residues**




**(A)** **(B)**

**Figure 3 : (A) 3D represention of 12a with build template using MOE software showing the hydrophobic areas (green colored) near N1. (B) 2D showed the hydrogen bond bond with Thr766, and Met769**

## Biology study

From the synthesized compounds, those compounds having the highest binding energy score (Kcal/mol) and those showed new or no interaction were subjected to in vitro cytotoxic activity. Seven compounds were tested for their anticancer activity using human breast carcinoma cell line (MCF-7)using the procedure of Skehan etal[35], and their results: The percentage of cell survival was calculated as follows: Survival fraction= O.D. (treated cells)/O.D. (control cells) The $IC_{50}$ values (the concentration required to produce 50%inhibition of cell growth) were calculated using sigmodial dose response curve fitting models (GraphPad, Prizm software incorporated). Each concentration was repeated 3 times. Human breast cancer cell line (MCF7) was sensitive to the antiproliferative effect of the prepared compounds in different concentrations. From the synthesized compounds, those compounds having the highest binding energy score (Kcal/mol) were subjected to in vitro cytotoxic activity and their result listed in TABLE 2.

The theoretical docking of the synthesized compounds showing their mode of interaction on enzyme receptor and their binding scores are listed in TABLE 1. And searching for the coincidence between biology data (TABLE 2) and docking results we exclude that MCF-7 cells appeared to be sensitive to the activity of most of new compounds.

**TABLE 2 : Docking results**

| Comp NO. | Number of H-bonds | Atoms of compound forming H-bonds | Amino acid Residues forming-bonds (H-bond length in A°) | BindingEnergy Score Kcal/mol |
|---|---|---|---|---|
| Erlotinib | 2 | N1,N4 | Met769 (2.70), Thr766 (2.78) | -21.35 |
| 5a | 1 | C=O | Asp788 (2.51) | -10.50 |
| 5b | 0 | | _ | -11.08 |
| 7a | 1 | NH | Lys692 (2.51) | -15.79 |
| 7b | 2 | 2N of thiadiazole ring | Lys692 (2.60), Lys692 (2.93) | -19.79 |
| 8a | 2 | N5,N7 | Met769 (3.17), Thr766 (2.69) | -16.24 |
| 8b | 2 | N5,N7 | Met769 (1.99), Thr766 (2.87) | -18.75 |
| 9a | 1 | N2 | Lys721 (3.12) | -17.19 |
| 9b | 3 | N5,N7, OH | Met769 (3.02), Thr766 (2.90), Glu780 (1.30) | -17 .86 |
| 11a | 2 | N5,N7 | Met769 (3.11), Thr766 (2.87) | -16.11 |
| 11b | 2 | N5,N7 | Met769 (3.13), Thr766 (2.88) | -15.66 |
| 11c | 2 | N5,N7 | Met769 (2.97), Thr766 (2.77) | -18.75 |
| 12a | 2 | N5,N7 | Met769 (3.04), Thr766 (2.84) | -20.95 |
| 12b | 2 | N5,N7 | Met769 (2.98), Thr766 (2.93) | -17.16 |
| 12c | 1 | C=O | Lys692 (2.42) | -18.88 |

**Binding Energy Score (Kcal/mol): energy of interaction of the ligand in the active site; $IC_{50}$ (μM): the concentration required to produce 50% inhibition of cell growth.**

**Each repeated for three times and average was taken**

In fact, six of seven tested compounds showed higher activity with $IC_{50}$ range from10.39-89.21 μM but all are less potant than the reference doxorubicin except compound (namely **(5b)**) showed inhibition activity towards cells more than 50%. In an attempts to rationalize the relationship between the cytotoxic activity and the presence of an aromatic that directly attached to phenyl amino moiety by inserting spacer atoms [(O=) C-N(H)-N(H)-C (=O or S)-N ]as in compounds **(5)** and **6** suggested that a lower activity towards MCF-7. Thus compound **(5b)** did not make any hydrogen bond with EGFR active site and this was in coincidence with its inactivity towards MCF-7. Introducing of a 2-substituted thiadizole cyclic structure para to phenyl amino moiety as in compounds **(7)**, resulted in a new hydrogen bond of thiadiazole NH with Lys 692 in a bond length of 2.51 Å and this modification maintain the cytotoxic activity hence compound **(7b)** showed the best inhibitory activity with $IC_{50}$ = 10.39 μM.

When the five membered ring was oxadiazole that was coplanner with other two aryl moieties represented by compounds **(9)**, this modification resulted more posing of the copmpound in hydrophobic pocket and in additional hydrogen with Glu780 in a length of 1.3 Å beside the usual interaction reported to the lead erlotinib and enhance the cytotoxic activity, that **(9b)** showed $IC_{50}$ = 15.45 μM. Also alteration of the five membered ring with thiazolidinione through amidic bridge to phenyl amino moiety giving a series of compounds **(11a-c)** those showed the same lead interaction features specially compound **(11c)** that had $IC_{50}$ = 16.30 μM. Through reaction with some anhydrides dioxo pyrrole, dioxopyrrolidine and dioxoisoindole moieties in series **(12)** were introduced those modification resulted in bet-

ter enzyme interaction. Generally compounds **(12a)** and **(12b)** interacted with amino acid of ATP active site in EGFR with the two amino acids (Thr766, and Met769) by two H-bond interactions which is the same to erlotinib leading to great hydrophobic interactions and great binding to the receptor with high docking score (-20.95) and this correlated with higher reactivity of **(12a)** against MCF-7 with $IC_{50}$ = 10.94 μM (Figure 3).

Specially compound **(12c)** with a pthalyl amido moiety showed good inhibitory activity with $IC_{50}$ = 21.89 μM but it interact with the enzyme in a new hydrogen bond between C=O of pthalyl moiety and lys692 acid residue with a bond length of 2.42 Å

## CONCLUSIONS

For establishing SAR for these compounds, Several pharmacophoric moieties were introduced to 4-amido-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine cores such as aryloxadiazole, aryltriazole, amidothiazolidinone, dioxo pyrrole, dioxopyrrolidine and dioxoisoindoles and. These moieties resulted on some potent kinase inhibitors that showed an acceptable cytotoxic activity. Induction of another aryl moiety with spascer atoms to the amidic function was the unpromising modification that results in compound **(5b)** with negative inhibitory activity towards result in respect to its cytotoxic activity.

As recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted and IC50 [the concentration required for 50% inhibition of cell viability] was calculated for each compound The results of *in vitro* cytotoxic activity experiments are presented in (TABLE 2).

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