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Synthesis, Docking and Biological Evaluation of 2,4-Disubstituted Quinazolines With Multi-Target Activities as Anti-cancer and Antimicrobial Agents

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Abstract

A series of 2,4-disubstituted quinazoline derivatives was designed and synthesized as multi-target therapeutic agents that may act as anti-cancer and antimicrobial agents. The target compounds were evaluated for primary anti-cancer activity followed by EGFR inhibition assay for most potent compounds. Compounds 6 and 8c exhibited good EGFR inhibition activity with IC50 values of 0.201 and 0.405 µM, respectively, in comparison to lapatinib as a reference with IC50 value of 0.115 µM. Docking study of the synthesized compounds into the binding site of EGFR tyrosine kinase was performed to compare the binding mode of these compounds to the known EGFR inhibitor, lapatinib. Moreover, antimicrobial activity, cytotoxity and hemolytic analysis were estimated according to CO-ADD (The Community for Antimicrobial Drug Discovery) procedures. Compounds 4 and 5c possessed potent antifungal activity with minimum inhibitory concentration (MIC) values of 8 and 4 µg/mL against C. albicans and C. neoformance, respectively, compared to fluconazole as a reference drug with MIC values of 0.125 and 8 µg/mL against same fungi.

Key words: Quinazoline; antitumor activity; EGFR assay; antimicrobial activity.

Introduction

Cancer is a dangerous disease in which the structure and normal function of body tissues are disrupted leading to death. However, there is a hopeful view that in the coming years advances in prevention and treatment will see cancer considered not as a fatal but as a chronic disease.[1] Accordingly, there is an urgent need to discover new chemotherapeutic anti-cancer drugs.

Protein kinases can be defined as enzymes that play key regulatory roles in mostly every portion of cell biology as it contributes in signal transduction modules and transcription. These enzymes can be classified into two groups: receptor tyrosine kinase (RTK) including several types, one of them is epithelial growth factor receptor (EGFR) and non-receptor tyrosine kinases such as Src kinase. EGFR is well known to be involved in several human malignances by promoting growth, local invasion, angiogenesis and finally metastasis of tumor cells. Subsequently, tyrosine kinase EGFR is an essential target for cancer treatments and several quinazoline based inhibitors approved by the FDA for clinical use in EGFR overexpressing solid tumors.[2,3] Gefitinib **I** has been one of the first-generation EGFR drugs for the treatment of non-small cell lung cancer (NSCLC). Erlotinib **II** was approved for the first-line treatment

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of pancreatic cancer, while lapatinib **III** showed a good activity in breast cancer.[4]

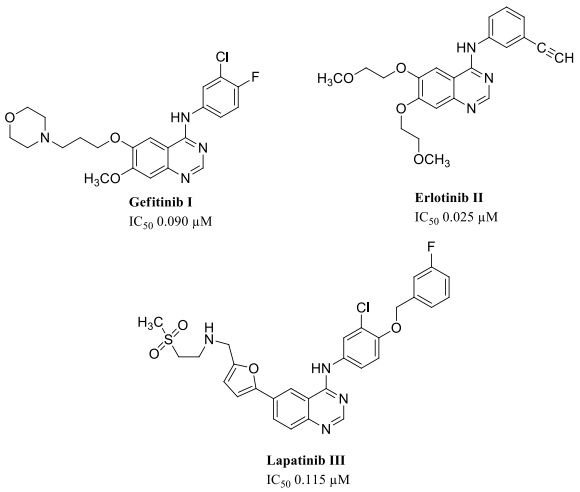


Fig.1. Chemical structures of some EGFR inhibitors approved by FDA.

On the other hand, the quinazoline scaffold is a core nucleus for designing of many potential bioactive agents as antitumor,[5-7] antifungal [8] and antibacterial activities.[9,10] Furthermore, the development of multi target therapeutic drugs is of growing interest nowadays to produce a single compound that may interact with multiple pathogenic pathways. Thus the present research has dealt with synthesizing multi target quinazoline derivatives with anti-cancer and antimicrobial activity. Compounds **I**, **II** and **III** are EGFR antagonists that bear a quinazoline scaffold. Moreover, 4-aminoquinazolines are considered to be a potent tyrosine kinase inhibitors.[11]

Diaryl urea is an essential pharmacophore in the design and synthesis of targeted anti-cancer drugs due to its pretty binding with tyrosine kinase receptors such as vascular endothelial growth factor receptor 2 (VEGFR-2)[12] and platelet derived growth factor receptor (PDGF).[13] EGFR and VEGFR-2 are closely linked through sharing general downstream signal transduction pathways.[14] Targeting EGFR strategy was explained as inhibition of tumor growth and decrease in the production of VEGFR thus increase the antitumoral effect of EGFR inhibitors.[15] VEGFR-2 inhibition increases the efficacy of EGFR TKIs by exerting a synergistic effect. 4-Anilino urea quinazoline derivative **IV** showed dual inhibitory activity against EGFR and VEGFR. [16]Accordingly, in an attempt to study the effect of 4-anilino aryl urea substitution, compounds **5a-c** were designed and synthesized aiming to enhance the antitumor activity and their *meta* substituted aniline analogues, **8a-c** have been also constructed in order to study the effect of substitution on the activity. (Figure 2)

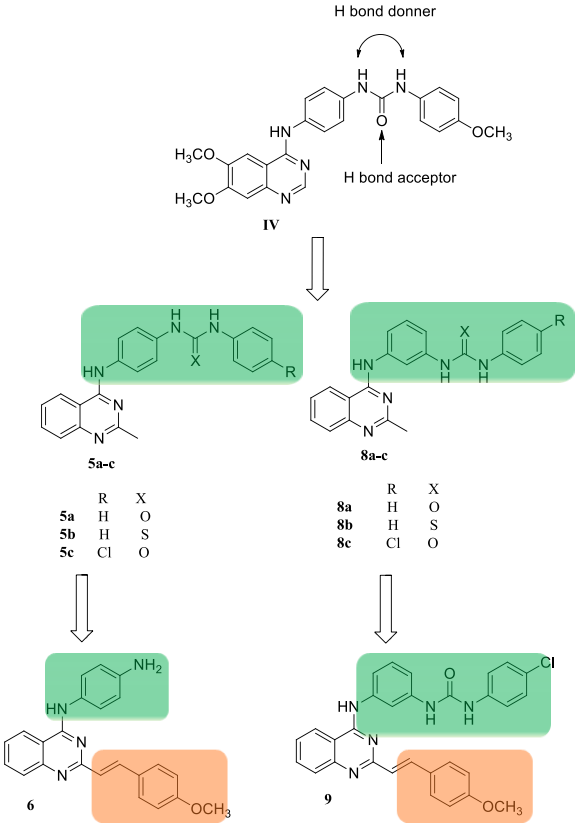


Fig.2. Design of 2,4-disubstituted quinazolines derived from reported EGFR inhibitors

In an attempt to synthesize potential anti-tumor agents, we selected the most active *para* diaryl urea derivatives **4** and the *meta* substituted one **8c** that showed broad antitumor activity in NCI screening for synthesis of compounds **6** and **9**, respectively, aiming to enhance the antitumor activity of the isolated products through the hybridization of *p*-methoxy phenyl styryl moiety at position two with urea derivative at position four. [11] (Figure 2)

Enteric bacterial infection is the first popular cause of morbidity and mortality all over the world. [17] A continuous increase in bacterial resistance to one or multiple class of antibiotics lead to many complications, so there is an essential need to solve this therapeutic problem. [18] Furthermore, quinazoline derivatives with antimicrobial activity are already reported. [19] From this literatures, it was revealed that 4-substituted quinazolines especially

with substituted/amine can improve their antimicrobial activities.[20,21] Modh *et al*. revealed that the introduction of one substituent at phenyl urea or phenyl thiourea resulted in an increase of anti-bacterial activity especially at para position. [22] Thus, we synthesized either unsubstituted or mono substituted urea and thiourea derivatives of quinazoline scaffold at position four but with extended phenyl spacer in order to construct new series of antimicrobial agents. Furthermore, we tried to potentiate the antimicrobial activity by styryl substitution at position two as compounds **Va-d** and **VIa-d**. (Figure 3)

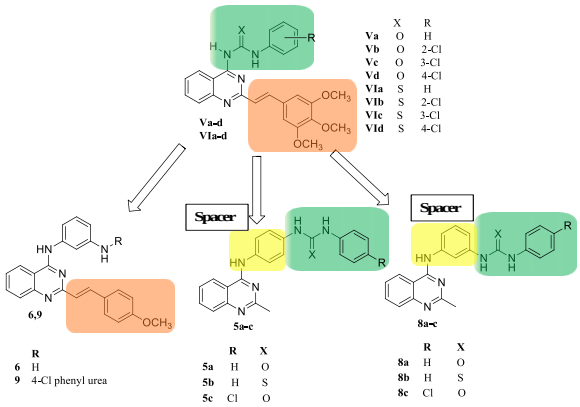


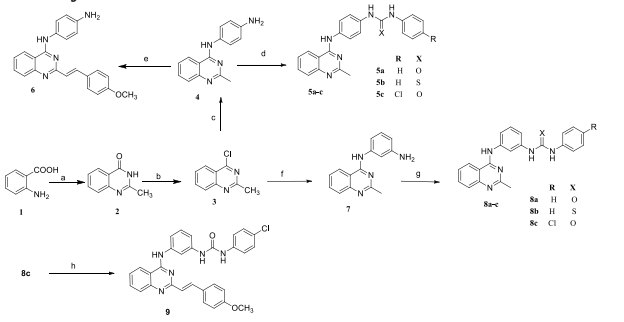
Fig.3. Design of newly synthesized compounds derived from known antimicrobial agents

2. Results and Discussion

2.1 Chemistry

The target compounds were synthesized according to **Scheme 1**. Quinazoline nucleus **2** was constructed from anthranilic acid **1** according to the previously reported method.[23] The key intrmediate **3** was prepared conferring to reported literatures.[24] In the present work, the target compound **4** was prepared by reflux of 4-chloro-2-methylquinazoline **(3)** with *p*-phenylene diamine in isopropanol. The structure was confirmed by IR spectra which showed vibrating bands at 3317 cm^{-1} related to amino group. In addition to ^{1}H-NMR spectra that displayed peaks at δ 4.0 and 6.06 ppm corresponding to amino protons. Consequently, reflux of compound **4** with different isocyanate or isothiocyanate in DMF in presence of TEA afforded diarylurea or thiourea derivatives **5a-c** in a good yield. The suggested structure was confirmed by IR spectra which showed peaks at 1670 and 1697 cm^{-1} corresponding to carbonyl group of compound **5a,c**, respectively. Furthermore, ^{1}H-NMR spectra revealed singlet peaks at δ 8.88-12.22 ppm corresponding to NH protons of urea. Also ^{13}C-NMR confirmed the presence of carbonyl group peaks at 162.22 and 162.24. Compound **4** which selected as a most active *para* substituted phenylene diamine derivative subjected to further styryl derivatization at position two by reaction with 4-methoxy benzaldehyde in glacial acetic acid in presence of sodium acetate to construct the target compound **6.** ^{1}H-NMR confirmed the introduction of styryl moeity because of appearance of doublet peaks of CH=CH protons at δ 6.84-7.89 ppm and singlet peak at δ 3.82 corresponding to methoxy group. In addition, ^{13}C-NMR exhibited peaks of olefinic carbons at 121.41, 134.94 and methoxy carbon at 55.79.

As previously explained in synthesis of compound **4**, the target compound **7** prepared by reflux of 4-chloro-2-methylquinazoline **(3)** with *m*-phenylene diamine in isopropanol. The structure was confirmed by IR spectra which showed vibrating bands at 3417 cm^{-1} related to amino group. In addition to ^{1}H-NMR spectra that displayed peaks at δ 9.10 ppm corresponding to amino proton. As discussed under synthesis of compounds **5a-c**, compounds **8a-c** also prepared by reaction of amino derivative **7** with isocyanate or isothiocyanate in DMF in presence of TEA. The predicted structure was confirmed by IR spectra that showed peaks at 1651 and 1635 cm^{-1} corresponding to carbonyl group of compound **8a,c**, respectively. Moreover, ^{1}H-NMR spectra revealed singlet peaks at δ 3.76-10.30 ppm corresponding to NH protons of urea. Also ^{13}C-NMR confirmed the presence of carbonyl group peaks at 162.24 and 162.86. On other hand, Compound **8c** which selected as a most active *meta* substituted phenylene diamine derivative subjected to further styryl derivatization at position two by reaction with 4-methoxy benzaldehyde in glacial acetic acid in presence of sodium acetate as previously mentioned to synthesize compound **9**. The structure of target compound was confirmed by spectral analysis. Where, ^{1}H-NMR showed doublet peaks of CH=CH protons at δ 6.58-7.94 ppm in addition to singlet peak at δ 3.86 corresponding to methoxy group. Otherwise, ^{13}C-NMR exhibited peaks at 122.54, 134.89 related to olefinic carbons and peak at 55.81 revealed to methoxy carbon.



Scheme 1. Reagents and conditions: (a) thioacetamide, reflux, 2 h; (b) $POCl_3$, reflux, 2 h; (c) *p*- phenylene diamine, isopropanol, reflux, 24 h; (d) isocyanate or isothiocyanate, DMF/TEA, reflux, 24 h; (e) *p*-methoxy benzaldehyde, glacial acetic acid, Na acetate, reflux, 24 h; (f) *m*-phenylene diamine, isopropanol, reflux, 24 h; (g) isocyanate or isothiocyanate, DMF/TEA, reflux, 24 h; (h) *p*-methoxy benzaldehyde, glacial acetic acid, Na acetate, reflux 24 h.

2.2. Biological evaluation

2.2.1. Preliminary anti-tumor screening

All newly synthesized compounds were selected by the U.S. National Cancer Institute for *in vitro* antitumor screening assay. Compounds were subjected to full NCI 60 cell line panel assay which included nine subpanels (non-small cell lung cancer, leukemia, CNS, colon, melanoma, ovarian, prostate, breast and renal cancer). The results obtained as mean graphs of the percent growth of treated cell and represented as percent of growth inhibition (GI%).[25-27] From these results, it was observed that only compounds **6** and **8c** showed a broad-spectrum antitumor activity toward various cell lines belonging to different tumor subpanels. Concerning selectivity towards certain cell lines, compounds **4** and **8c** exhibited good activity toward leukemia (SR cell line) with growth inhibition percent 43.16 and 77.94, respectively. Non-small cell lung cancer (NCI-H322M) was found to be remarkably sensitive to compound **6** with GI% 60.18. Compounds **6** and **8c** displayed selective activity against colon cancer (SW-620) with GI% 43.63 and 53.27, respectively. In addition, compound **6** showed GI% values of 50.45, 40.86 and 55.84 against melanoma (LOX IMVI, MDA-MB-435 and UACC-62, respectively). Regarding ovarian cancer OVCAR-4, compound **8c** represented best activity with GI% values of 52.23. (S.table 1 in supplementary data)

2.2.2. Cytotoxicity study results

From primary screening of the synthesized compounds at NCI, we selected the most broadly spectrum active compounds (**4**, **6** and **8c**) for further cytotoxicity study using non-small cell lung cancer (A549) obtained from the American Type Culture Collection (Rockville, MD, USA), using MTT test.[28] As shown in Table 1, compounds **8c**, **6** and **4** exhibited anticancer activity against A549 cell line with IC_{50} values of 8.44, 13.70 and 10.25 µg\mL, respectively. These results were consistent with screening ones at NCI, which also showed the potency of anticancer activity in descending manner starting from **8c**, **6** and finally **4.** This arrangement in activity could be attributed to higher lipid solubility of **8c** and **6** due to the presence of chloride and phenyl ring, respectively rather than low lipid solubility of compound **4**. (Table 1)

2.2.3. In vitro EGFR kinase assay

Upon primary antiprofilative screening against 60 cell lines and cytotoxicity study, compounds **4**, **6** and **8c** showed best activity. So these compounds were subjected for further EGFR enzyme assay to determine the mechanism of their activity. As shown in Table 1, compound **6** exhibited higher inhibition percent with value of 67.24 and lower IC_{50} (0.201 µM), attributed to the high lipophilicity of the phenyl substitution of amino group at position 4 and p-methoxy phenyl styryl derivative at position 2, which enhanced the EGFR activity as reported.[29] Furthermore, compound **8c** also showed moderate EGFR activity as compared to reference drug lapatinib with IC_{50} value of 0.405 versus 0.115 of lapatinib. Compound **4** represented lowest EGFR inhibition activity with IC_{50} value of 0.780, which could be attributed to the unsubstitution of amino group at position 4 and small methyl substitution at position 2.

2.2.4. In vitro anti-proliferative activity against EGFR-expressing in A549 cell line

We further evaluated the anti-proliferative activity of the representative compounds **4**, **6** and **8c** to inhibit EGFR that's known to be overexpressed in A549 cells. So NSCLC (A549) was treated with the desired compounds with their IC_{50} concentrations for accurate assay then lysed and subjected to enzyme-linked-immunosorbent assay (ELISA) to determine their activity against EGFR in this cell line. The results were presented as percentage inhibition. (Table 1) The results demonstrated that 2,4 disubstituted quinazoline **6** possessed potent inhibition of EGFR as compared with lapatinib with %inhibition values 80.92 and 83.33, respectively. This proved the importance of styryl derivative as EGFR inhibitor as previous literatures elucidated.[29] Furthermore, compound **8c** exhibited better activity than compound **4** with percentage inhibition values of 60.95 and 46.99, respectively. This could be explained by more lipophilic properties of *p*-chlorophenyl urea derivative **8c** instead of unsubstituted aminophenyl group in compound **4**, in addition to the great importance of diaryl urea nucleus as EGFR inhibitors as previously reported.[16]

Table 1: Cytotoxicity, cell free EGFR assay and EGFR assay expressed in A549 cells of target compounds **4**, **6** and **8c**.

Compound ID	**A549**		**EGFR**		**EGFR/ A549**		**CLog P**
	IC_{50} µg\ml (Mean±SEM)	IC_{50} µM	%inhibition at 1µg/ml	IC_{50} (Mean±SEM) (µM)	Conc.±SEM (ng/ml)	% inhibition	
4	10.25±0.37	40.95	49.30	0.780±0.022	0.942±75	46.99	3.13
6	13.70±0.53	37.19	67.24	0.201±0.005	0.339±7.29	80.92	5.24
8c	8.44±0.18	20.09	63.07	0.405±0.011	0.694±42.9	60.95	6.24
lapatinib	6.96±0.31	11.98	75.09	0.115±0.003	0.296±3.06	83.33	-
Control	-	-	-	-	78.4±1.777	0	-

IC_{50}: Compound concentration required to inhibit the growth by 50%, SEM = Standard error mean.

2.2.5. Molecular modeling

In order to illustrate the kinase activity of the most active compounds into the active binding site of EGFR enzyme, docking study was performed using 'Molecular Operating Environment 2019.0101' software. The crystal structure of the enzyme with the reference drug (lapatinib) was obtained from the protein data bank; PDB code: 1XKK.[30] Validation of docking was achieved by redocking lapatinib into the active site of EGFR (1XKK). Re-docking of lapatinib revealed the binding mode of lead compound into the active site of EGFR enzyme through interaction with two amino acid: Leu718 and Met793. The target compounds **4**, **6** and **8c** were docked by positioning them in the lapatinib binding site. The result of compound **6** showed two π bond one of them with key amino acid Leu718 similar to lapatinib and the other with Val726 in the active site of EGFR enzyme. Moreover, compound **8c** also exhibited π bond of 4-chlorophenyl moiety with Leu718 (bond length 4.10 Å) in addition to a hydrogen bond (H-donner; bond length 3.25 Å) with Asp855. The enhanced inhibitory activity of compound **6** over compound **8c** may be attributed to its similar binding mode with lapatinib. On other hand, a pi bond was observed between quinazoline ring and Lys745 in addition to a hydrogen bond (H-donner, bond length 2.79 Å) of amino group (NH_2) with Phe856, thus explaining the lower activity of this target compound **4** as it couldn`t interact with the key amino acids. (Figure 4)

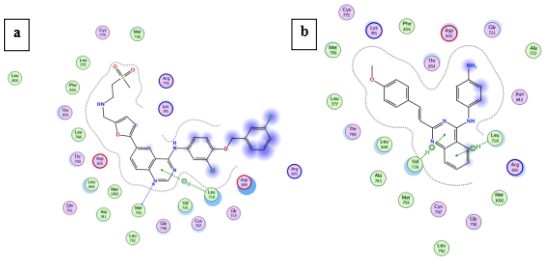


Fig.4. 2D Docking of (a) lapatinib and (b) compound **6** in binding site of EGFR (using PDB code: 1XKK)

2.2.6. Antimicrobial screening

The antimicrobial study was performed by CO-ADD (The Community for Antimicrobial Drug Discovery) at concentration 32 µg/mL, that funded by The University of Queensland (Australia) and Wellcome Trust (UK).[31] All synthesized compounds were selected for their antimicrobial activity against five pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (ATCC 43300) as Gram-positive bacteria, *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606) and *Pseudomonas aeruginosa* (ATCC 27853) as Gram-negative bacteria and antifungal activity against two pathogenic fungal strains *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (H99; ATCC 208821). (Table 2)

From these results, it was found that all the tested compounds displayed weak anti-bacterial activity except compound **4** that showed moderate activity against Gram positive bacteria MRSA (ATCC 43300) with percent of inhibition 33.76%.

Concerning the anti-fungal activity of the tested compounds, it was observed that compound **4** and **5c** exhibited potent inhibition against both *candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (H99; ATCC 208821). Furthermore, compound **4** possessed high anti-fungal activity against *C. albicans* and *C. neoformans* with inhibition percent 88.06% and 98.94%, respectively, while compound **5c** also represented strong anti-fungal activity against *C. albicans* and *C. neoformans* with inhibition percent 82.84% and 95.13%, respectively. So both of them were selected by CO-ADD for further confirmatory assays. In addition, *C.albicans* showed weak response to both compounds **7** and **9** with GI% 22.21% and 21.85%, respectively. Unfortunately, other synthesized compounds processed weak anti-microbial activity.

2.2.7. MIC determination, cytotoxicity and hemolytic analysis

Compounds (**4** and **5c**) with best antifungal activity were selected by CO-ADD organization for determination of minimum inhibitory concentration (MIC), the lowest concentration that inhibits visible growth of microorganisms and is usually reported as an 80% MIC value. For reference drugs, they used vancomycin and colistin - sulfate for antibacterial activity and fluconazole for antifungal activity.[31] The results in Table 3 revealed a moderate antimycotic activity of compound **4** against *C. albicans* with MIC value of 8 µg/mL. At the same time, introduction of *p*-chloro phenyl urea moiety **5c** resulted in an increase in the antimycotic activity against *C. neoformans* with MIC value of 4 µg/mL which is superior to that of reference drug fluconazole (MIC= 8 µg/mL). Unfortunately, there is no effective activity against neither Gram positive nor Gram negative bacteria.

An extremely important feature of antimicrobial agents is their toxicity. Therefore, compounds **4** and **5c** were tested for cytotoxicity and hemolytic activity, which were also examined by CO-ADD. Cytotoxicity was determined on HEK293 cells as CC_{50} (concentration at 50% cytotoxicity), both of the tested compounds exceeded 32 µg/ ml. Moreover, both compounds had no hemolytic activity against red blood cells at a dose of 32 µg /ml. It was concluded that compounds **4** and **5c** possessed the combination of high activity and low cytotoxicity and hemolytic activity.

Structure-Activity Relationship

Concerning anti-neoplastic activity, it was found that substitution of quinazoline nucleus at position 4 with *p*-chlorophenyl urea derivative **8c** showed broad spectrum cytotoxic activity with selective activity toward certain cell lines (leukemia, non-small lung cancer, colon cancer and ovarian cancer), while disubstituted quinazolines at position 2,4 as shown in compound **6** lead to a broad spectrum activity (non-small lung cancer, melanoma and colon cancer). Otherwise substitution of p-phenylene diamine moiety resulted in a sharp decrease in the activity. Thess results were confirmed by cytotoxicity study against NSLC (A549), non-cell EGFR assay and finally with EGFR assay expressed in A549 cell line.

Regarding anti-microbial activity, it was observed that unsubstituted *p*-aminophenyl derivative **4** showed strong anti-fungal activity, while either unsubstituted *m*-amino phenyl **7** or the substituted derivatives **8a-c** exhibited loss of the activity. Furthermore, the p-chlorophenyl urea derivative **5c** maintained the strong antifungal activity as compound **4.**

Table 2: Anti-microbial screening results of all synthesized compounds

Compound number	C. neoformans	C. albicans	A. baumannii	p. aeruginosa	K. pneumonia	E. coli	MRSA
4	98.84	88.06	-22.38	-8.59	-11.41	-28.27	33.76
5a	-1.03	17.75	-29.96	-13.65	-45.47	-33.94	-26.39
5b	-8.73	4.04	-0.17	8.23	-53.63	-16.70	8.71
5c	95.13	82.84	16.29	8.57	2.57	-4.90	13.05
6	14.89	6.61	-22.74	-0.29	-44.83	-23.51	-8.39
7	-8.99	22.21	11.51	9.69	13.61	-3.50	11.42
8a	-0.77	5.99	13.50	10.00	6.31	-3.77	7.91
8b	-33.96	7.03	4.83	9.78	6.29	-1.55	2.32
8c	-1.8	15.59	25.49	11.10	8.62	-4.72	4.70
9	3.59	21.85	1.62	7.59	-24.00	-22.94	-1.83

Table 3: MIC, cytotoxicity and hemolytic activity of active compounds **4**and **5c**

Compound no.	MIC (µg/mL)							Hk CC_{50} (µg/mL)	Hm Hc_{10} (µg/mL)
	MRSA	***E. coli***	***K. pneumonia***	***p. aeruginosa***	***A. baumannii***	***C. albicans***	***C. neoformans***		
4	>32	>32	>32	>32	>32	8	>32	>32	>32
5c	>32	>32	>32	>32	>32	>32	4	>32	>32
Vancomycin	1	-	-	-	-	-	-		
Colistin-sulfate	-	0.125	0.25	0.25	0.25	-	-		
Fluconazole	-	-	-	-	-	0.125	8		

MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%.
CC_{50} is concentration at 50% cytotoxicity.
Hc_{10} is concentration at 10% haemolytic activity.

Structure-Activity Relationship

Concerning anti-neoplastic activity, it was found that substitution of quinazoline nucleus at position 4 with *p*-chlorophenyl urea derivative **8c** showed broad spectrum cytotoxic activity with selective activity toward certain cell lines (leukemia, non-small lung cancer, colon cancer and ovarian cancer), while disubstituted quinazolines at position 2,4 as shown in compound **6** lead to a broad spectrum activity (non-small lung cancer, melanoma and colon cancer). Otherwise substitution of p-phenylene diamine moiety resulted in a sharp decrease in the activity. Thess results were confirmed by cytotoxicity study against NSLC (A549), non-cell EGFR assay and finally with EGFR assay expressed in A549 cell line.
Regarding anti-microbial activity, it was observed that unsubstituted *p*-aminophenyl derivative **4** showed strong anti-fungal activity, while either unsubstituted *m*-amino phenyl **7** or the substituted derivatives **8a-c** exhibited loss of the activity. Furthermore, the p-chlorophenyl urea derivative **5c** maintained the strong antifungal activity as compound **4.**

2.2.8. Results of the absorption and distribution prediction

The pharmacokinetic parameters, absorption and distribution were considered important parameter for selection of compounds as drug candidates. The online PreADMET program was used to predict ADME of most potent compounds **4**, **6** and **8c**. Prediction of absorption through determination of HIA (Human Intestinal Absorption), Caco-2 cell permeability and Madin-Darby canine kidney cell (MDCK). The Caco-2 cell model is a reliable *in vitro* model for prediction of oral drug absorption, while HIA is the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile and feces. The results showed that both compounds **6** and **8c** possessed good absorption and bioavailability after oral administration, while compound **4** was poorly absorbed. Concerning distribution properties, plasma protein binding was calculated from the same program. It was used because the degree of plasma protein binding of a drug has an important role on efficacy of the drug. Compound **8c** was very strongly bound however moderate binding showed by compound **6**. On the other hand, BBB (Blood-Brain Barrier) penetration values were calculated to know if the compounds are able to pass across the blood-brain barrier or not to avoid CNS side effects. All tested

compounds exhibited very moderate absorption from CNS. From the previous results we can conclude that compounds **6** and **8c** could be developed for more efficient anti-tumor candidates as they possessed good activity with high absorption and well distribution in addition to low BBB permeability. (Table 4)

Table 4: ADME properties obtained from PreADMET program

Compound ID	BBB	Caco2 (nm/s)	HIA (%)	MDCK (nm/s)	Plasma Protein binding (%)
4	0.52	3.80	94.03	41.08	79.00
6	0.58	39.07	95.87	0.16	88.88
8c	2.04	24.05	94.44	0.09	91.96

3. Material and Methods

3.1 Chemistry

Melting points were carried out by open capillary tube method using IA 9100 MK-Digital Melting Point Griffin Apparatus and are uncorrected. Elemental Microanalyses were carried out using Vario El III, CHNSO analyzer (Germany) at Faculty of Pharmacy, Al-azhar University. Infrared Spectra were done on Bruker FT-IR spectrophotometer Vector 22, Schimadzu 435, Perkin-Elmer 457 and Jasco FT.IR plus 460 Japan, and expressed in wave number (cm^{-1}), using KBr discs. ^{1}H-NMR Spectra were carried out using Bruker Avance III at 400 MHz (Bruker AG, Switzerland) using DMSO-d_6 or otherwise stated as a solvent. The chemical shifts were expressed in δ ppm units using trimethylsilane as the internal standard. ^{13}C-NMR spectra were carried out using Bruker Avance III at 100 MHz (Bruker AG, Switzerland) using DMSO-d_6 as a solvent. Mass Spectra was done on Shimadzu Qp-2010 plus. All the reactions were monitored by thin layer chromatography. Silica gel/TLC-cards DC-Alufolien-Kieselgel with fluorescent indicator 254 nm; layer thickness 0.2mm. Petroleum ether: ethyl acetate (1:1) or (1:2) was the adopted solvent system. Compounds **2** [23] and **3** [24] were synthesized acorrding to the reported procedures.

Synthesis of N1-(2-methylquinazolin-4-yl)benzene-1,4-diamine (4)

4-Chloro-2-methylquinazoline 3 (2.24 g, 10 mmole) was refluxed with *p*-phenylene diamine (2.24 g, 10 mmole) in isopropanol (10 mL) for 24 h. The reaction mixture was poured onto ice/water, filtered off, dried and crystallized from ethanol to give the desired compound. Yield 66%; mp 320-322 °C. IR (ν_{max}/cm^{-1}): 3317 (NH, NH_2), 3186, 2916 (CH aromatic, CH aliphatic). ^{1}H-NMR δ ppm: 2.35 (s, 3H, CH_3), 4.00 (s, 1H, NH, exch. D_2O), 6.06 (s, 2H, NH_2, exch. D_2O), 6.49 (d, 4H, CH aromatic, J = 7.2 Hz), 7.45 (t, 1H, CH aromatic, J = 16.0 Hz), 7.56 (d, 1H, CH aromatic, J = 8.0 Hz), 7.77 (t, 1H, CH aromatic, J = 16.0 Hz), 8.06 (d, 1H, CH aromatic, J = 7.6 Hz). ^{13}C-NMR δ ppm: 21.80 (CH_3), 118.54, 121.04, 123.99, 124.08, 126.14, 126.34, 126.96, 134.76, 135.87, 149.32, 154.81, 162.28 (aromatic carbons). Mass (m/z): 250 (M^+, 4.5), 107 (100). Anal. Calcd for $C_{15}H_{14}N_4$ (250.30): C, 71.98; H, 5.64; N, 22.38. Found: C, 72.17; H, 5.80; N, 22.16%

General procedure for the synthesis of compounds (5a-c)

A mixture of N1-(2-methylquinazolin-4-yl)benzene-1,4-diamine 4 (2.24 g, 10 mmole) and the appropriate isocyanate or isothiocyanate derivative (10 mmole) was refluxed in DMF (10 mL) for 24 h in presence of TEA (1 mL). The reaction mixture was poured onto ice/water, filtered and dried to give desired compounds.

1-(4-((2-methylquinazolin-4-yl)amino)phenyl)-3-phenylurea (5a)

Yield 62%; mp 280-282 °C. IR (ν_{max}/cm^{-1}): 3465, 3302 (NHs), 3047, 2867 (CH aromatic, CH aliphatic), 1643 (CO). ^{1}H-NMR δ ppm: 2.35 (s, 3H, CH_3), 6.96 (t, 1H, CH aromatic, J = 13.6 Hz), 7.08 (d, 2H, CH aromatic, J = 8.0 Hz), 7.26-7.37 (m, 4H, CH aromatic), 7.45-7.52 (m, 3H, CH aromatic), 7.56 (d, 1H, CH aromatic, J = 8.0 Hz), 7.75 (t, 1H, CH aromatic, J = 14.0 Hz), 8.06 (d, 1H, CH aromatic, J = 7.6 Hz), 8.88 (s, 1H, NH, exch. D_2O), 9.60 (s, 1H, NH, exch. D_2O), 12.22 (s, 1H, NH, exch. D_2O). ^{13}C-NMR δ ppm: 21.87 (CH_3), 118.58, 121.04, 121.33, 122.15, 122.76, 126.15, 126.36, 126.83, 129.18, 134.78, 139.65, 140.18, 149.33, 154.03, 154.84, 154.03 (aromatic carbons), 162.24 (C=O). Mass (m/z): 369 (M^+, 19.8), 52 (100). Anal. Calcd for $C_{22}H_{19}N_5O$ (369.42): C, 71.53; H, 5.18; N, 18.96. Found: C, 71.69; H, 5.29; N, 18.61%

1-(4-((2-methylquinazolin-4-yl)amino)phenyl)-3-phenylthiourea (5b)

Yield 56%; mp 170-172 °C. IR (vmax/cm-1): 3209 (NHs), 3032, 2846 (CH aromatic, CH aliphatic). 1H-NMR δ ppm: 2.35 (s, 3H, CH3), 6.88 (t, 1H, CH aromatic, J = 13.2 Hz), 7.12 (d, 2H, CH aromatic, J = 7.6 Hz), 7.25-7.35 (m, 4H, CH aromatic), 7.42-7.54 (m, 3H, CH aromatic), 7.56 (d, 1H, CH aromatic, J = 8.0 Hz), 7.75 (t, 1H, CH aromatic, J = 14.0 Hz), 8.06 (d, 1H, CH aromatic, J =

7.6 Hz), 9.84 (s, 1H, NH, exch. D2O), 10.11 (s, 1H, NH, exch. D2O), 11.09 (s, 1H, NH, exch. D2O). 13C-NMR δ ppm: 21.81 (CH3), 118.39, 120.04, 121.37, 122.23, 122.76, 126.15, 126.36, 126.92, 129.18, 129.77, 134.77, 139.69, 149.33, 153.07, 154.06, 154.78 (aromatic carbons), 162.24 (C=S). Mass (m/z): 385 (M+, 21.3), 93 (100). Anal. Calcd for C22H19N5S (385.48): C, 68.55; H, 4.97; N, 18.17. Found: C, 68.28; H, 5.21; N, 18.43%

1-(4-chlorophenyl)-3-(4-((2-methylquinazolin-4-yl)amino)phenyl)urea (5c)

Yield 60%; mp 273-275 °C. IR (ν_{max}/cm^{-1}): 3309 (NHs), 3032, 2985 (CH aromatic, CH aliphatic), 1697 (CO). ^{1}H-NMR δ ppm: 2.35 (s, 3H, CH_3), 6.57 (d, 2H, CH aromatic, J = 7.6 Hz), 7.29 (m, 4H, CH aromatic), 7.39-7.49 (m, 3H, CH aromatic), 7.55 (d, 1H, CH aromatic, J = 8.4 Hz), 7.73 (t, 1H, CH aromatic, J = 14.8 Hz), 8.06 (d, 1H, CH aromatic, J = 8.0 Hz), 9.05 (s, 1H, NH, exch. D_2O), 9.74 (s, 1H, NH, exch. D_2O), 12.22 (s, 1H, NH, exch. D_2O). ^{13}C-NMR δ ppm: 21.90 (CH_3), 118.35, 120.06, 121.09, 126.14, 126.30, 126.35, 126.97, 129.07, 129.12, 134.71, 134.75, 138.72, 140.79, 149.28, 153.93, 154.77 (aromatic carbons), 162.22 (C=O). Mass (m/z): 420 (M$^+$, 32.2), 127 (100). Anal. Calcd for $C_{22}H_{18}ClN_5S$ (419.93): C, 62.92; H, 4.32; N, 16.68. Found: C, 63.30; H, 4.60; N, 16.83%

***(E)-N1-(2-(4-methoxystyryl)quinazolin-4-yl)benzene-1,4-diamine* (6)**

This compound synthesized by heating of solution of 4 (2.5 g, 10 mmole) in glacial acetic acid (10 mL), 4-methoxybenzaldeyde (2.7 g, 20 mmole) was added in presence of sodium acetate (1.6 g, 20 mmole) and refluxed for 24 h. The product was poured onto ice/water, filtered off, dried and crystallized from ethanol to give the desired compound. Yield 68%; mp 140-142 °C. IR (ν_{max}/cm^{-1}): 3456, 3356 (NH, NH_2), 3194, 2947 (CH aromatic, CH aliphatic).^{1}H-NMR δ ppm: 3.82 (s, 3H, OCH_3), 6.84 (d, 1H, CH=CH, J = 16.4 Hz), 7.02 (d, 4H, CH aromatic, J = 8.0 Hz), 7.44 (t, 1H, CH aromatic, J = 16.0 Hz), 7.61 (d, 4H, CH aromatic, J = 8.0 Hz), 7.64 (d, 1H, CH aromatic, J = 8.0 Hz), 7.77 (t, 1H, CH aromatic, J = 16.0 Hz), 7.89 (d, 1H, CH=CH, J = 16.4 Hz), 8.10 (d, 1H, CH aromatic, J = 8.0 Hz), 12.18 (s, 2H, NH_2, exch. D_2O. ^{13}C-NMR δ ppm: 55.79 (OCH_3), 115.03, 118.96, 124.48, 126.33, 126.41, 127.26, 127.40, 128.07, 129.78, 138.52, 140.28, 141.57, 149.57, 152.31, 161.10, 162.37 (aromatic carbons), 121.41, 134.94 (olefinic Cs). Mass (m/z): 368 (M$^+$, 40.13), 281 (100). Anal. Calcd for $C_{23}H_{20}N_4O$ (368.34): C, 74.98; H, 5.47; N, 15.21. Found: C, 74.81; H, 5.63; N, 15.40%

Synthesis of N1-(2-methylquinazolin-4-yl)benzene-1,3-diamine (7)

4-Chloro-2-methylquinazoline 3 (1.78 g, 10 mmole) was refluxed with *m*-phenylene diamine (1.08 g, 10 mmole) in isopropanole (10 mL) for 24 h. The reaction mixture was poured onto ice/water, filtered off, dried and crystallized from ethanol to give the desired compound. Yield 62%; mp 273-275 °C. IR (ν_{max}/cm^{-1}): 3417 (NH, NH_2), 3106, 2816 (CH aromatic, CH aliphatic). ^{1}H-NMR δ ppm: 2.65 (s, 3H, CH_3), 6.89 (s, 2H, NH_2, exch. D_2O), 7.25 (d, 1H, CH aromatic, J = 7.2 Hz), 7.28 (t, 1H,CH aromatic, J = 16.0 Hz), 7.38 (d, 2H, CH aromatic, J = 8.0 Hz), 7.51 (s, 1H, CH aromatic), 7.66 (t, 1H, CH aromatic, J = 16.0 Hz), 7.89 (d, 1H, CH aromatic, J = 8.0 Hz), 7.98 (t, 1H, CH aromatic, J = 16.0 Hz), 8.16 (d, 1H, CH aromatic, J = 8.0 Hz), 9.10 (s, 1H, NH, exch. D_2O). ^{13}C-NMR δ ppm: 21.64 (CH_3), 118.43, 119.45, 120.96, 123.76, 124.68, 126.23, 126.52, 128.80, 129.10, 134.87, 140.04, 148.49, 155.29, 162.10 (aromatic carbons). Mass (m/z): 250 (M$^+$, 100), 250 (100). Anal. Calcd for $C_{15}H_{14}N_4$ (250.30): C, 71.98; H, 5.64; N, 22.38. Found: C, 72.06; H, 5.83; N, 22.01%

General procedure for the synthesis of compounds (8a-c)

A mixture of N1-(2-methylquinazolin-4-yl)benzene-1,3-diamine **7** (2.24 g, 10 mmole) and the appropriate isocyanate or isothiocyanate derivative (10 mmole) was refluxed in DMF (10 mL) for 24 h in presence of TEA (1 mL). The reaction mixture was poured onto ice/water, filtered and dried to give desired compounds.

1-(3-((2-methylquinazolin-4-yl)oxy)phenyl)-3-phenylurea (8a)

Yield 62%; mp 238-240 °C. IR (ν_{max}/cm^{-1}): 3317, 3201 (NHs), 3055, 2878 (CH aromatic, CH aliphatic), 1651(CO). ^{1}H-NMR δ ppm: 2.36 (s, 3H, CH_3), 3.76 (s, 1H, NH, exch. D_2O), 6.95-6.98 (m, 2H, CH aromatic), 7.26-7.30 (m, 4H, CH aromatic), 7.39-7.48 (m, 4H, CH aromatic), 7.57 (d, 1H, CH aromatic, J = 8.0 Hz), 7.74 (t, 1H, CH aromatic, J = 15.2 Hz), 8.08 (d, 1H, CH aromatic, J = 7.6 Hz), 8.80 (s, 2H, NH_2, exch. D_2O). ^{13}C-NMR δ ppm: 21.8 (CH_3), 118.35, 118.66, 118.92, 121.06, 122.29, 126.16, 126.36, 127.00, 128.11, 128.95, 129.24, 129.52, 134.78, 140.17, 149.28, 153.09, 154.79, 162.22 (aromatic carbons), 162.86 (C=O). Mass (m/z): 369 (M$^+$, 18.5), 91 (100). Anal. Calcd for $C_{22}H_{19}N_5O$ (369.42): C, 71.53; H, 5.18; N, 18.96. Found: C, 71.29; H, 5.34; N, 18.73%

1-(3-((2-methylquinazolin-4-yl)oxy)phenyl)-3-phenylthiourea (8b)

Yield 52%; mp 179-181 °C. IR (ν_{max}/cm^{-1}): 3309, 3176 (NHs), 3039, 2931 (CH aromatic, CH aliphatic). ^{1}H-NMR δ ppm: 2.38 (s, 3H, CH_3), 6.95 (t, 1H, CH aromatic, J = 13.2 Hz), 7.09-7.13 (m, 2H, CH aromatic), 7.25 (m, 2H, CH aromatic), 7.31 (t,

2H, CH aromatic, , *J* = 15.6 Hz), 7.44 (t, 1H, CH aromatic, *J* = 15.2 Hz), 7.57-7.61(m, 3H, CH aromatic), 7.76 (t, 1H, CH aromatic, *J* = 15.2 Hz), 8.08 (d, 1H, CH aromatic, *J* = 7.6 Hz), 9.15 (s, 1H, NH, exch. D_2O), 10.08 (s, 1H, NH, exch. D_2O), 10.30 (s, 1H, NH, exch. D_2O). ^{13}C-NMR *δ* ppm: 21.64 (CH_3), 118.43, 119.45, 120.47, 120.96, 122.01, 122.82, 123.76, 124.68, 126.23, 126.36, 126.52, 128.81, 129.25, 134.94, 140.10, 148.49, 155.29, 162.02 (aromatic carbons), 179.89 (C=S). Mass (m/z): 385 (M^+, 12.2), 43 (100). Anal. Calcd for $C_{22}H_{19}N_5S$ (385.48): C, 68.55; H, 4.97; N, 18.17. Found: C, 68.81; H, 5.14; N, 18.50%

1-(4-chlorophenyl)-3-(3-((2-methylquinazolin-4-yl)oxy)phenyl)urea (8c)

Yield 58%; mp 305-307 °C. IR (ν_{max}/cm^{-1}): 3302 (NHs), 3070, 2878 (CH aromatic, CH aliphatic), 1635 (C=O). ^{1}H-NMR *δ* ppm: 2.37 (s, 3H, CH_3), 7.09 (d, 1H, CH aromatic, *J* = 7.6 Hz), 7.11 (s, 1H, CH aromatic), 7.15 (t, 1H, CH aromatic, *J* = 16.0 Hz), 7.23 (d, 1H, CH aromatic, *J* = 8.0 Hz), 7.28-7.31 (m, 3H, CH aromatic), 7.42 (d, 1H, CH aromatic, *J* = 8.0 Hz), 7.50 (d, 2H, CH aromatic, *J* = 8.4 Hz), 7.76 (t, 1H, CH aromatic, *J* = 15.2 Hz), 8.07 (d, 1H, CH aromatic, *J* = 7.6 Hz), 8.99 (s, 1H, NH, exch. D_2O), 9.06 (s, 1H, NH, exch. D_2O), 9.19 (s, 1H, NH, exch. D_2O). ^{13}C-NMR *δ* ppm: 21.49 (CH_3), 115.39, 115.91, 115.95, 116.21, 121.09, 123.34, 124.69, 124.88, 126.14, 126.26, 127.04, 130.56, 134.73, 142.60, 149.40, 152.90, 154.58, 155.65 (aromatic carbons), 162.24 (C=O). Mass (m/z): 420 (M^+, 21.5), 126 (100). Anal. Calcd for $C_{22}H_{18}ClN_5S$ (419.93): C, 62.92; H, 4.32; N, 16.68. Found: C, 62.70; H, 4.56; N, 16.52%

(E)-1-(4-chlorophenyl)-3-(3-((2-(4-methoxystyryl)quinazolin-4-yl)amino)phenyl)urea (9)

As previously discussed, the target compound prepared by reflux of solution of 8c (1.6 g, 10 mmole) in glacial acetic acid (10 mL), 4-methoxybenzaldeyde (2.7 g, 20 mmole) was added in presence of sodium acetate (1.6 g, 20 mmole) and refluxed for 24 h. The product was poured onto ice/water, filtered off, dried and crystallized from ethanol to give the desired compound. Yield 60%; mp 192-194 °C. IR (ν_{max}/cm^{-1}): 3417, 3302 (NHs), 3078, 2862 (CH aromatic, CH aliphatic), 1643 (CO). ^{1}HNMR *δ* ppm: 3.86 (s, 3H, OCH_3), 6.55 (d, 1H, CH aromatic, *J* = 8.4 Hz), 6.58 (d, 1H, CH=CH, *J* = 13.6 Hz), 6.70 (t, 1H, CH aromatic, *J* = 16.0 Hz), 6.79 (s, 1H, CH aromatic), 7.01 (d, 1H, CH aromatic, *J* = 8.0 Hz), 7.09 (d, 2H, CH aromatic, *J* = 8.0Hz), 7.15 (t, 1H, CH aromatic, *J* = 16.0 Hz), 7.23 (d, 2H, CH aromatic, *J* = 8.8 Hz), 7.30 (d, 2H, CH aromatic, *J* = 8.8 Hz), 7.42 (d, 2H, CH aromatic, *J* = 8.4 Hz), 7.56 (d, 1H, CH aromatic, *J* = 8.4 Hz), 7.74 (t, 1H, CH aromatic, *J* = 15.2 Hz), 7.94 (d, 1H, CH=CH, *J* = 16.0 Hz), 8.07 (d, 1H, CH aromatic, *J* = 8.0 Hz), 8.98 (s, 1H, NH, exch. D_2O), 9.07 (s, 1H, NH, exch. D_2O), 9.18 (s, 1H, NH, exch. D_2O).^{13}C-NMR *δ* ppm: 55.81 (OCH_3), 108.48, 111.92, 115.01, 117.76, 118.40, 120.81, 124.28, 126.01, 126.98, 127.74, 129.37, 129.71, 130.51, 133.92, 138.72, 141.46, 144.50, 152.43, 154.84, 155.22, 161.01, 162.08 (aromatic carbons), 122.54, 134.89 (olefinic Cs), 178.25 (C=O). Mass (m/z): 522 (M^+, 20.74), 55 (100). Anal. Calcd for $C_{30}H_{24}ClN_5O_2$ (522.0): C, 69.03; H, 4.63; N, 13.42. Found: C, 68.79; H, 4.80; N, 13.68%

3.2. Bioassay

3.2.1. In vitro anti-proliferative activity assay

All ten derivatives were chosen by the U.S. National Cancer Institute for antitumor evaluation at single concentration of 10 µM. The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov). Primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases (lung, blood, CNS, colon, skin, kidney, ovary, prostate and breast). Detailed methods are described in a reference articles.[25-27]

3.2.2. Cytotoxicity study

Cytotoxicity study for the most active compounds (**4**, **6** and **8c**) was performed on non-small lung cancer A549 cell line obtained from the American Type Culture Collection (Rockville, MD, USA) at confirmatory diagnostic unit in VACSERA-Egypt. Cells survival was evaluated with MTT colorimetric assay according to Mosmann methodology.[28] The detailed method was explained in supplementary data.

3.2.3. In vitro EGFR assay

The in vitro cell free EGFR enzyme inhibition determination for compounds **4, 6 and 8c** were carried out in confirmatory diagnostic unit, Vacsera, Egypt. The evaluation performed using EnzyChromTM Kinase Assay Kit (EKIN-400) according to manufacturer's instructions by ELISA assay method using lapatinib as a reference according to the previously reported methods.[29,32] IC_{50} was determined using five different concentrations for each compound.

3.2.4. In vitro anti-proliferative assay of target compounds against EGFR-expressing in A549 cell line

The screening of tyrosine kinase inhibitors was based on enzyme-linked-immunosorbent assay (ELISA) using Human Bax ELISA (EIA-4487) kits. A549 cell line was treated with each compound with their IC_{50} concentration for 2 h and the concentration of EGFR in the samples is then determined by comparing the O.D. of the samples to the standard curve. Lapatinib also was used as a reference drug, the exact methodology was mentioned in supplementary data.

3.2.5. Docking study

Molecular modeling studies were performed by using 'Molecular Operating Environment 2019.0101' software (MOE of Chemical Computing Group Inc., on a Core i5 2.2 GHz workstation) running on a Windows 10 PC. Structures of **4**, **6** and **8c** were built in MOE. The X-ray crystallographic structure of EGFR kinase enzyme (PDB ID: 1XKK) was downloaded from the protein data bank (http://www.rcsb.org/) complexed with reference drug lapatinib.[30] Preparation of the enzyme for docking by removing the Co-crystallized ligand, sodium ion and water molecules then the enzyme was prepared using quick preparation protocol in MOE with default options. To ensure the validity of the docking protocol, re-docking of the co-crystallized native ligand into the active site was performed. The docked compounds were prepared for docking by drawing 2D structures using Marvin Sketch, 3D protonation of the structure, running conformational analysis using systemic search and finally selecting the least energetic conformer. Poses for compounds were scored by initial rescoring methodology (GBVI/WSA dg) and the final rescoring methodology (GBVI/WSA dg) after docking by placement using Triangle Matcher protocol and post-placement refinement was Rigid Receptor. The best scoring pose of the docked compounds was recognized. Receptor-ligand interactions of the complexes were examined in 2D and 3D styles.

3.2.6. Antimicrobial and antifungal screening

The antimicrobial and antifungal screening was performed according to CO-ADD (The Community for Antimicrobial Drug Discovery) procedures. Also, MIC determination, cytotoxicity and hemolytic activity were examined with CO-ADD procedure and analysis methods.[31]

3.2.7. Predicting the absorption and distribution properties

The PreADMET program was carried out at http://preadmet.bmdrc.org/. The most active target compounds **4**, **6** and **8c** were used in this study. The structure of all compounds were converted into molfile. The program automatically calculated the predictive absorption for Caco-2 cell permeability (nm/s), in vitro MDCK cell permeability (nm/s), HIA (human intestinal absorption (%), *in vivo* blood brain barrier penetration (c.blood/c.brain). and *in vitro* plasma protein binding (%).[33]

4. Conclusion

Two series of 2,4-disubstituted quinazoline derivatives bearing either *para* (**4, 5a-c, 6**) or *meta* (**7, 8a-c, 9**) substituted phenyl urea and thiourea derivatives were synthesized. All the synthesized compounds were evaluated for their anti-cancer and anti-microbial activity. It was found that compounds **6** and **8c** possessed broad spectrum anti-cancer activity. While compounds **4** and **5c** represented the best anti-fungal activity against *C. albicans* and *C. neoformans.*

The molecular modeling study ensured EGFR inhibition assay results. Compounds **6** fitted well to EGFR enzyme with two bond interactions in a similar manner to lapatimib. We suggest different derivatization of most active compounds **6** and **8c** for more potent anti-tumor activity with high EGFR selectivity and effective antimycotic activity.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Appendix A. supplementary material

Supplementary data of this article available.

References

[1] Sikora K, Timbs O. Cancer 2025: Introduction. *Expert Rev. Anticancer Ther.* 2004;4:11–12. DOI: 10.1586/14737140.4.5.s11

[2] Tabernero J. The Role of VEGF and EGFR Inhibition: Implications for Combining Anti–VEGF and Anti–EGFR Agents. *Mol. Cancer Res.* 2007;5:203e-220. DOI: 10.1158/1541-7786.MCR-06-0404

[3] Chilin A, Conconi MT, Marzaro G, Guiotto A, Urbani, L, Tonus F, Parnigotto P. Exploring epidermal growth factor receptor (EGFR) inhibitor features: the role of fused dioxygenated rings on the quinazoline scaffold. *J. Med. Chem.* 2010;53:1862-1866. DOI: 10.1021/jm901338g

[4] Rusnak DW, Affleck K, Cockerill SG, Stubberfield C, Harris R, Page M, Smith KJ, Guntrip SB, Carter MC, Shaw RJ, Jowett A, Stables J, Topley P, Wood ER, Brignola PS, Kadwell SH, Reep BR, Mullin RJ, Alligood KJ, Keith BR, Crosby RM, Murray DM, Knight WB, Gilmer TM, Lackey K. The characterization of novel, dual ErbB-2/EGFR, tyrosine kinase inhibitors: potential therapy for cancer. *Cancer Res.* 2001;61:7196–203.

[5] Abbas SE, Barsoum FF, Georgey HH, Mohammed ER. Synthesis and antitumor activity of certain 2,3,6-trisubstituted quinazolin-4(3H)-one derivatives. *Bulletin of Faculty of Pharmacy, Cairo University.* 2013;51:273-282. DOI: 10.1016/j.bfopcu.2013.08.003

[6] Abdel Gawad NM, Georgey HH, Youssef RM, El-Sayed NA. Synthesis and antitumor activity of some 2, 3-disubstituted quinazolin-4(3H)-ones and 4, 6- disubstituted- 1, 2, 3, 4-tetrahydroquinazolin-2H-ones. *Eur. J. Med. Chem.* 2010;45(12):6058–6067. DOI: 10.1016/j.ejmech.2010.10.008

[7] Khan I, Ibrar A, Ahmed, W, Saeed A. Synthetic approaches, functionalization and therapeutic potential of quinazoline and quinazolinone skeletons: the advances continue. *Eur. J. Med. Chem.* 2015;90:124-169. DOI: 10.1016/j.ejmech.2014.10.084

[8] Patel NB, Patel JC. Synthesis and antimicrobial activity of Schiff bases and 2-azetidinones derived from quinazolin-4(3H)-one. *Arab. J. Chem.* 2011;4(4):403–411. DOI: 10.1016/j.arabjc.2010.07.005

[9] Dahiya R, Mourya R. Synthetic studies on novel nitroquinazolinone analogs with antimicrobial potential. *Bull. Pharm. Res.* 2013;3;7-51.

[10] Rajput R, Mishra AP. A review on biological activity of quinazolinones. *Int. J. Pharm. Pharm. Sci.* 2012;4:0975-1491.

[11] El-Azab AS, Al-Omar MA, Abdel-Aziz AMM, Abdel-Aziz NI, El-Sayed MAA, Aleisa AM, Abdel-Hamide SG. Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: molecular docking study. *Eur. J. Med. Chem.* 2010;45(9);4188–98. DOI: 10.1016/j.ejmech.2010.06.013

[12] Wang C, Gao H, Dong J, Zhang Y, Su P, Shi Y, Zhang J. Biphenyl derivatives incorporating urea unit as novel VEGFR-2 inhibitors: design, synthesis and biological evaluation. *Bioorg. Med. Chem.* 2014;22;277-284. DOI: 10.1016/j.bmc.2013.11.027

[13] Ravez S, Barczyk A, Six P, Cagnon A, Garofalo A, Goossens L, Depreux P. Inhibition of tumor cell growth and angiogenesis by 7-Aminoalkoxy-4-aryloxy-quinazoline ureas, a novel series of multi-tyrosine kinase inhibitors. *Eur. J. Med. Chem.* 2014;79;369-381. DOI: 10.1016/j.ejmech.2014.04.007

[14] Qiao JX, Wang TC, Ruel R, Thibeault C, L'Heureux A, Schumacher WA, Spronk SA, Hiebert S, Bouthillier G, Lloyd J, Pi Z, Schnur DM, Abell LM, Hua J, Price LA, Liu E, Wu Q, Steinbacher TE, Bostwick JS, Chang M, Zheng J, Gao Q, Ma B, McDonnell PA, Huang CS, Rehfuss R, Wexler RR, Lam PYS. Conformationally Constrained ortho-Anilino Diaryl Ureas: Discovery of 1-(2-(1′-Neopentylspiro[indoline-3,4′-piperidine]-1-yl)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea, a Potent, Selective, and Bioavailable P2Y1

Antagonist. *J. Med. Chem.* 2013;56;9275-9295. DOI: 10.1021/jm4013906

[15] Semino CE, Kamm RD, Lauffenburger DA. Autocrine EGF receptor activation mediates endothelial cell migration and vascular morphogenesis induced by VEGF under interstitial flow. *Exp. Cell Res.* 2006;312;289–98. DOI: 10.1016/j.yexcr.2005.10.029

[16] Garofalo A, Goossens L, Lemoine A, Ravez S, Six P, Howsam M, Farce A, Depreux P. [4-(6,7-Disubstituted quinazolin-4-ylamino)phenyl] carbamic acid esters: a novel series of dual EGFR/VEGFR-2 tyrosine kinase inhibitors. *Med. Chem. Comm.* 2011;2(1);65-72. DOI: 10.1039/c0md00183j

[17] Devasia RA, Jones TF, Ward J, Stafford L, Hardin H, Bopp C, Beatty M, Mintz E, Schaffner W. Endemically Acquired Foodborne Outbreak of Enterotoxin-producing Escherichia coli Serotype O169:H41. *Am. J. Med.* 2006;119(2):168, e7–10. DOI: 10.1016/j.amjmed.2005.07.063

[18] Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2008;46(2):155–164. DOI: 10.1086/524891

[19] Mohamed MA, Ghanem HM, Abd El-Ghaffar NF, Mohamed SS. Biological Evaluation and Molecular Docking of Substituted Quinazolinones as Antimicrobial Agents. *Aust. J. Basic Appl. Sci.* 2013;7(2):263-274.

[20] Zayed MF, Hassan MH. Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents. *Saudi Pharm. J.* 2014;22:157-162. DOI: 10.1016/j.jsps.2013.03.004

[21] Ghorab MM, Ismail Z, Radwan AA, Abdalla M. Synthesis and pharmacophore modeling of novel quinazolines bearing a biologically active sulfonamide moiety. *Acta. Pharm.* 2013:63:1–18. DOI: 10.2478/acph-2013-0006

[22] Modh RP, Patel AC, Mahajan DH, Pannecouque C, De Clercq E, Chikhalia K.H. Synthesis and evaluation of novel 4-substituted styryl quinazolines as potential antimicrobial agents. *Arch. Pharm. (Weinheim).* 2012;345(12):964–972. DOI: 10.1002/ardp.201200291

[23] Neiementowski V. Neiementowski Quinazoline Synthesis. *Journal Für Praktische Chemie.* 1895;51:564.

[24] Hand ES, Baker DC. A definitive synthesis of 2-[(5-methyl-s-triazolo[4,3-c]quinazolin-3-yl)thio]-N-benzylacetanilide and other reactions of its precursors. *Can. J. Chem.* 1984;62:2570–2577. DOI: 10.1139/v84-439

[25] Boyd MR, Padl KD. Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* 1995;34:91-109. DOI: 10.1002/ddr.430340203

[26] Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-wolff A, Gray-goodrich M, Campbell H, Boyd M. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* 1991;83:757-766. DOI: 10.1093/jnci/83.11.757

[27] Skehan P, Storeng R, Scudiero D, Monks A, Vistica D, Warren JT, Bokesch H, Boyd MR. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* 1990;82:1107-112. DOI: 10.1093/jnci/82.13.1107

[28] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 1983;65:55–63. DOI: 10.1016/0022-1759(83)90303-4

[29] El-Sayed MA, El-Husseiny WM, Abdel-Aziz NI, El-Azab AS, Abuelizz HA, Abdel-Aziz AA. Synthesis and biological evaluation of 2-styrylquinolines as antitumour agents and EGFR kinase inhibitors: molecular docking study. *J. Enzym. Inhib. Med. Chem.* 2018;33(1):199-209. DOI: 10.1080/14756366.2017.1407926

[30] Verma N, Rai AK, Kaushik V, Brünnert D, Chahar KR, Pandey J, Goyal P. Identification of gefitinib off-targets using a structure-based systems biology approach; their validation with reverse docking and retrospective data mining. *Sci. Rep*. 2016;6:33949. DOI: 10.1038/srep33949

[31] Berger M, Roller A, Maulide N. Synthesis and antimicrobial evaluation of novel analogues of dehydroabietic acid prepared by CH-Activation. *Eur. J. Med. Chem.* 2017;126:937–943. DOI: 10.1016/j.ejmech.2016.12.012

[32] Mouineer AA, Zaher AF, El-Malah AA, Sobh E. *Mediterr.* Design, synthesis, antitumor activity, cell cycle analysis and elisa assay for cyclin dependant kinase-2 of a new (4-aryl-6-flouro-4h-benzo[4, 5] thieno[3, 2-b] pyran) derivatives. *J. Chem.* 2017;6(5):165–179. DOI: 10.13171/mjc65/01709262240-zaher

[33] Lee SK, Lee IH, Kim HJ, Chang GS, Chung JE, The PreADME Approach: Web-based program for rapid prediction of physico-chemical, drug absorption and drug-like properties, EuroQSAR. 2002 Designing Drugs and Crop Protectants: processes, problems and solutions, 2003;pp. 418-420. *Blackwell Publishing*, Massachusetts, USA.