ejpmr, 2020,7(8), 663-670

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS AND MOLECULAR MODELLING STUDY OF SOME CHROMENO [4', 3':4, 5] THIENO [2, 3-D] PYRIMIDINE DERIVATIVES AS ANTICANCER AGENTS

Tawfeek A. Yahya* and Jalal H. Abdullah

Medicinal, Pharmaceutical Organic and Analytical Chemistry Department, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

***Corresponding Author: Tawfeek A. Yahya**

Medicinal, Pharmaceutical Organic and Analytical Chemistry Department, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

Article Received on 20/06/2020 **Article Revised on 10/07/2020** **Article Accepted on 30/07/2020**

ABSTRACT

A series of chromeno[4',3':4,5]thieno[2,3-d]pyrimidine derivatives was designed and synthesized as erlotinib analogues. The new compounds were screened for their cytotoxic activity against breast cancer cell line MCF-7. Compounds **5b**, **5d** and **5f** showed good cytotoxic activity with IC_{50} values of 1.22, 1.42 and 1.67 µM respectively.

KEYWORDS: Molecular Modeling, EGFR, Benzopyran, thieno[2,3-d]pyrimidine, MCF-7, Anticancer.

INTRODUCTION

Cancer is a lethal disease especially in developed countries. In 2030, it is expected that, the mortality rate will be increased to be 13.1 million deaths.[1] The risk of cancer disease may affect people at all ages and tends to be increased with age. Such a killer disease is caused by abnormalities in the genetic material of cells. Cancer cells are characterized by three main properties, uncontrolled proliferation, lake of differentiation and a capability to invade many tissues in other locations in body (metastasis).[2] There is always a challenge for chemists and oncologists with cancer chemotherapy and antitumor agents. This is due to the non-selectivity, acute toxicity and cellular drug resistance of many anticancer agents. So, there is a continuing need for designing and developing new chemotherapeutic agents for cancer treatment.[3] Receptor tyrosine kinases (RTKs)-cell surface receptors bind to polypeptide growth factors such as cytokines and hormones – play a critical role in the development and progression of many types of cancer.[4] The millstone in the control of cellular proliferation is protein tyrosine kinases. Many of transforming oncogenes (e.g.: Src and Abl) possess tyrosine kinase activity. Moreover, the response of many cells to growth factors is initiated by RTKs activation.[5] Overexpression of certain RTKs (e.g.: the epidermal growth factor (EGF) receptor tyrosine kinases) shows an inverse correlation with survival especially in breast, colon and bladder cancers. Thus, inactivation of specific TKs in certain cancers, represent a potential strategy for designing new antiproliferative drugs.[6] Many research articles reported that 4-anilinoquinazolines (erlotinib **1** and gefitinib **2**) were the first inhibitor of EGFR and compounds containing thienopyrimidine or benzo[4,5]thieno[2,3-d]pyrimidine core could also act as EGFR-TK inhibitors.[6,8] EGFR inhibitors differ in their structure but have common pharmacophoric features, the first pharmacophore is a flat central aromatic heterocyclic fused system that acts as hydrogen bond acceptor (HBA) to reside in adenine binding pocket (hinge segment).[9] Moreover, EGFR inhibitors should have a hydrophobic moiety such as phenyl ring (hydrophobic head) linked to aromatic heterocyclic fused system with NH spacer to occupy the hydrophobic region I of the enzyme.[10,11] Finally, another hydrophobic moiety that is attached or fused to the aromatic flat, (hydrophobic tail) to occupy hydrophobic region II Fig. 1.[12,14] In addition to the ribose and phosphate binding site which are not exploited by the majority of EGFR-TKIs.[13,15] Motivated by these facts, herein, we designed and synthesized novel chromeno[4',3':4,5]-thieno[2,3-d]pyrimidine derivatives as EGFR inhibitors Fig. 1. The structural modifications involved replacement of 4-anilinoquinazoline scaffold with is their bioisostere thieno[2,3-d]pyrimidine core fused to large lipophilic benzopyran moiety guided by their reported potent anticancer activity[7,8,16,17] to occupy the hydrophobic region II of ATP binding site. In addition, we explored the substitution of the phenyl ring of 4-anilino with electron donating groups (methyl or methoxy) or electron withdrawing groups (fluro, chloro or bromo) to substantiate the effect of such variations on the EGFR inhibitory activity as well as their anticancer activity against breast cancer cell line (MCF-7).

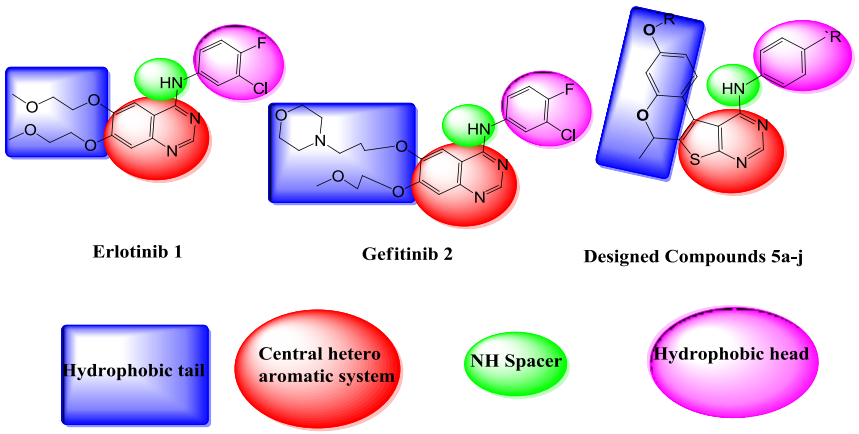


Fig.1 The basic pharmacophoric features of EGFR-TK inhibitors and designed compounds

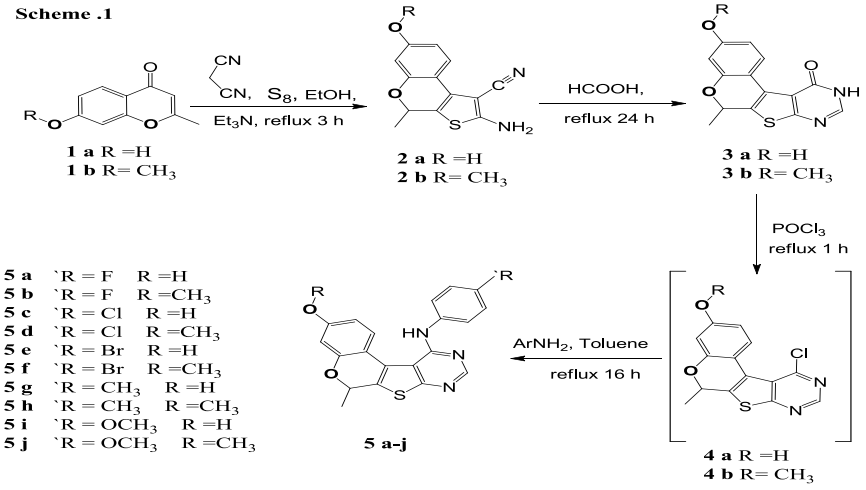
RESULTS AND DISCUSSION

Chemistry

The synthetic pathway for the targeted compounds are described synthesized via the routes illustrated in **scheme 1.** Treatment of starting compounds **(1a)** and **(1b)** with sulphur (S_8) in the presence of malononitril under basic condition to afford compounds **(2a)** and **(2b).**[18] IR spectra of compounds **(2a)** and **(2b)** revealed two bands around 2222, 3255 cm^{-1} due to CN and NH_2 respectively. In the same time, ^1H NMR showed singlet at 8.11 ppm for NH_2. Reaction of compounds **(2a)** and **(2b)** with refluxing formic acid underwent cyclization into the corresponding chromeno[4',3':4,5]thieno[2,3-d]pyrimidinone derivatives **(3a)** and **(3b)**. IR spectra of **(3a)** and **(3b)** revealed band around 1663 cm^{-1} indicating the appearance of C=O alongside with the absence of band of CN. Moreover, ^1H NMR displayed two singlets around 8.22 and 11.27 ppm attributed to CH=N and NH of pyrimidine respectively.

On the other hands, the final compounds 11-((4-substituted-phenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol **(5a, 5c, 5e, 5g, 5i)** and N-(4- substituted-phenyl)-3-methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11-amine **(5b, 5d, 5f, 5h, 5j)** were obtained from the chromeno[4',3':4,5]thieno[2,3-d]pyrimidinone derivatives **(3a)** and **(3b)** in two in situ consecutive steps involving reaction with phosphorous oxychloride to produce chloropyridopyrimidine derivatives as intermediates that upon treatment with aniline derivatives afforded the target compounds **(5a, 5c, 5e, 5g, 5i)** and **(5b, 5d, 5f, 5h, 5j)** in good yields.

The structures of the final compounds **(5a, 5c, 5e, 5g, 5i)** and **(5b, 5d, 5f, 5h, 5j)** were confirmed by microanalytical and spectral data. The IR spectra showed disappearance the band of the carbonyl group. The ^1H NMR confirmed the presence of NH and the additional aromatic protons by the existence of bands in aromatic region. Mass spectra proved parent peaks of the all synthesized compounds confirming the molecular weight.



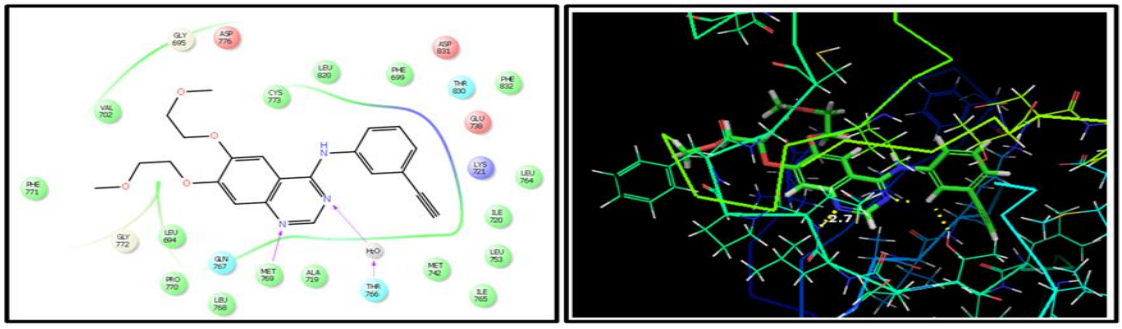
Docking studies

The results of docking studies against the EGFR revealed that the synthesized compounds have similar orientations inside the ATP binding site. The designed compounds **(5a-j)** gave good binding energies glide emodel ranging from -61.922 to -78.707 and docking score in between -6.694 and -9.087 kcal/mol (Table 1).

Table 1: The Glide emodel, Docking score and amino acid involved in interaction of the synthesized compounds 5a-j.

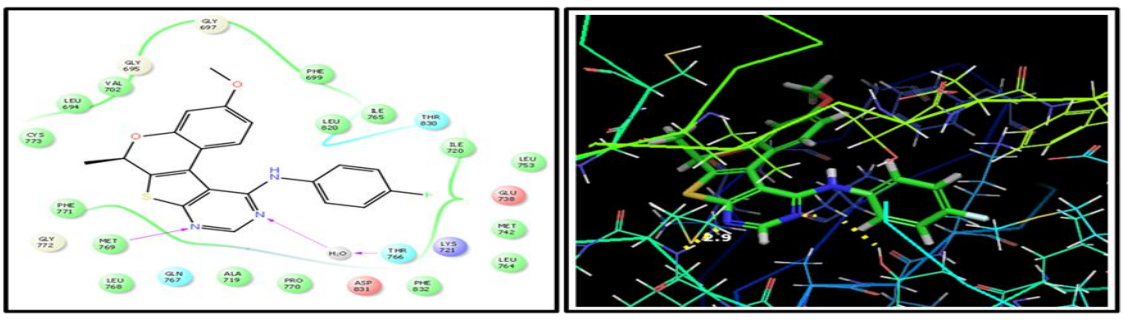
Compd No	Glide emodel, kcal/mol	Docking score kcal/mol	Interacting groups	Amino acids (length in A°)
Erlotinib	-80.588	-8.538	N1 N3	Met769 (2.7) Thr769 via H_2O
5a	-64.118	-7.311	N1 N3	Met769 (2.9) Thr769 via H_2O
5b	-78.707	-9.006	N1 N3	Met769 (2. 9) Thr769 via H_2O
5c	-63.844	-6.694	N1 N3	Met769(2.8) Thr769 via H_2O)
5d	-76.424	-8.542	N1 N3	Met769 (2.9) Thr769 via H_2O
5e	-65.124	-7.075	N1 N3	Met769 (3.0) Thr769 via H_2O
5f	-75.019	-8.323	N1 N3	Met769 (2.8) Thr769 via H_2O
5g	-59.987	-7.042	N1	Met769 (3.0)
5h	-60.077	-7.187	N1	Met769 (2.9)
5i	-58.519	-7.081	N1	Met769 (3.1)
5j	-61.922	-7.161	N1	Met769 (3.0)

The binding mode of the cocrystallized ligand, erlotinib, showed glide emodel of -80.588 and docking score of -8.538 kcal/mol. Quinazoline nucleus was oriented in the adenine pocket of the receptor, The N1 atom of pyrimidine ring formed a hydrogen bond with Met769 with a distance of 2.7 A° and the N3 atom of pyrimidine ring interact with the side chain of Thr766 through a water bridge. The cyanophenyl moiety occupied the hydrophobic pocket I forming hydrophobic interaction with Thr766, Thr830, Ala719, Lue764, Ile720, Lys721 and Ile765 residues. Besides, the bis (2-methoxyethoxy) groups occupied the hydrophobic region II forming hydrophobic interaction with Leu694, Val702 and Gly772 residues (Fig. 2).



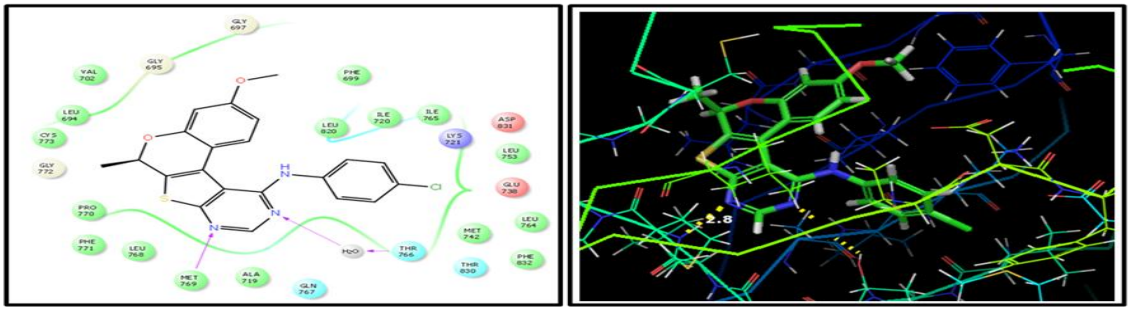
(A) (B)

Fig. 2 2D interaction (A) and 3D interaction (B) of **erlotinib** , inside EGFR binding pocket.



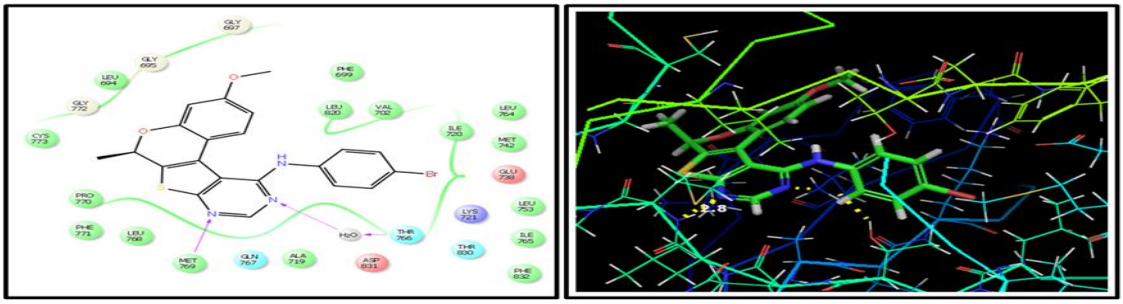
(A) (B)

Fig. 3 2D interaction (A) and 3D interaction (B) of compound **5b**, inside EGFR binding pocket.



(A) (B)

Fig. 4 2D interaction (A) and 3D interaction (B) of compound **5d**, inside EGFR binding pocket.



(A) (B)

Fig. 5 2D interaction (A) and 3D interaction (B) of compound **5f**, inside EGFR binding pocket.

Analysis of the molecular docking results showed that, compounds **5b**, **5d** and **5f** showed lowest energy bindings. Compound **5b** could fit into the binding site with glide emodel value of -78.707 and docking score of -9.006 kcal/mol (Fig. 3). The thieno[2,3-d]pyrimidine ring occupied the same position as quanazoline of erlotinib. The thieno[2,3-d]pyrimidine-N1 involved in a key H-bond interaction with the main chain carbonyl of Met769 with a distance of 2.9 A°. In addition, the N-3 of thieno[2,3-d]pyrimidine formed a water mediated H-bond with Thr766. The fluorophenyl moiety occupied the hydrophobic pocket I forming hydrophobic interactions with Thr766, Thr830, Ala719, Lys721, Lue753, Lue764, Ile720 and Ile765 residues. Moreover, the methoxybenzopyran ring occupied the hydrophobic region II forming hydrophobic interaction with Leu694 and Gly695 residues.

Compound **5d** showed a binding mode as like as that of erlotinib, with glide emodel value of -76.424 kcal/mol and docking score of -8.542 kcal/mol. The thieno[2,3-d]pyrimidine moiety was oriented in the adenine pocket of the receptor. The two nitrogen atoms of pyrimidine moiety formed hydrogen bond with Met769 (distance of 2.8 A°) and hydrogen bond with Thr766 via water. The chlorophenyl moiety occupied the hydrophobic pocket I forming hydrophobic interactions with Thr766, Thr830, Ala719, Ile720, Ile765, Lue753, Leu764, Lue820 and Lys 721 residues, while the methoxybenzopyran ring occupied the hydrophobic region II forming hydrophobic interaction with Leu694, Val702 and Gly772 residues (Fig. 4).

Finally, compound **5f** could fit into the binding site as like as that of erlotinib, with glide emodel value of -75.019 and docking score of -8.323 kcal/mol. The thieno[2,3-d]pyrimidine occupied the adenine binding site forming hydrogen bond with Met769 (distance of 2.8 A°) and hydrogen bond with Thr766 through water. The bromophenyl moiety occupied the hydrophobic pocket I forming hydrophobic interactions with Thr766, Thr830, Ala719, Lys721, Ile720, Lue820, and Lue764 residues. In addition, the methoxybenzopyran ring occupied the hydrophobic region II forming hydrophobic interaction with Leu694 and Gly772 residues (Fig. 5).

***In vitro* Cytotoxic Activity**

All the synthesized were evaluated to their cytotoxic activity against human breast cancer cell line MCF7. From the cytotoxic activity results mentioned in table 2, it can be observed that, compounds **5a-j** exhibited moderate to good cytotoxic activity with IC_{50} ranges IC_{50} 1.22-6.74 µM when compared to the reference standard doxorubicin ($IC_{50,}$ 0.82 µM), while the compound **5b, 5d** and **5f** showed the best activity among this series with IC_{50} value of 1.22, 1.42 and 1.67 µM respectively.

Moreover, it could be indicated that the lipophilic and electronic characters of substitutions on the aniline and benzopyran rings might play an important role in the cytotoxic activity.

Compounds with methoxy substituents on benzopyran ring **5b, 5d, 5f, 5h** and **5j** showed higher activities than the hydroxyl derivatives **5a, 5c, 5e, 5g** and **5i**. On the other hand, compounds with electron withdrawing groups (such as F, Cl, Br) on the aniline ring **5b, 5d, 5f**

exhibited more potent anticancer activities than those **5h** and **5i** have electron donating substituents (such as CH_3, OCH_3).

Finally, compound **5b** having fluro and methoxy groups on aniline and benzopyran rings respectively showed the highest cytotoxic activity with IC_{50} value of 1.22 µM

Table 2: *In vitro* cytotoxic activity of the synthesized compounds against MCF7 **cell line.**

Compound No	[a]IC_{50} (µM)	Compound No	[a]IC_{50} (µM)
5a	2.43	**5f**	1.67
5b	1.22	**5g**	4.32
5c	2.76	**5h**	6.32
5d	1.42	**5i**	4.02
5e	2.91	**5j**	6.74
Doxorubicin	0.82	**Doxorubicin**	0.82

[a] **IC_{50}** is a concentration that cause 50% growth inhibition.

Experimental

Chemistry

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). The FT-IR spectra (KBr) were recorded on Shimadzu FT-IR 110 spectrophotometer (Shimadzu, Koyoto, Japan) by using 1% potassium bromide discs. ^{1}H-NMR spectra were recorded on a Bruker proton 300 MHz (Bruker, Munuch, Germany) spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as internal standard. Chemical shift values are listed in δ scale. Mass spectra were determined using a GC/MS Mat 112 S at 70ev spectrometer. Elemental analysis (C, H, N) were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the microanalytical laboratories of the Faculty of Science, Cairo University. Completion of the reaction was monitored by thin layer chromatography (TLC) using precoated Aluminium sheets silica gel (Merck, 60 F254). Visualization was accomplished with ultraviolet UV lamp (Merck, Darmstadt, Germany). Synthesized compounds were purified by the re-crystallization process. The purity of the compounds was checked by a single spot in TLC and solvent system for TLC was determined on a trial and error basis. All the chemicals and solvents used were of commercial grade.

General procedure for synthesis of 2-Amino-7-methoxy-4-methyl-4H-thieno[2,3-c]-chromene-1-carbonitrile (2b) and 2-Amino-7-hydroxy-4-methyl-4H-thieno[2,3-c]-chromene-1-carbonitrile (2a)

A mixture of either compound **(1a)** or **(1b)** (10 mmol), sulphur (S_8) (0.32 g, 10 mmol) and malononitrile (0.66 g, 10 mmol) in the presence of Et_3N (1 ml) in absolute ethanol (40 ml) was refluxed for 3 h and left to cool. The obtained precipitate was filtered off, dried and recrystallized from and ethyl acetate to afford compounds **(2a)** & **(2b)** respectively.

2-Amino-7-hydroxy-4-methyl-4H-thieno[2,3-c]chromene-1-carbonitrile (2a)

Yield 28%, mp 186-188° C. IR spectrum, ν, cm^{-1}: 1625, 2224, 3052, 3250, 3420. ^{1}H NMR (300 MHz, DMSO-d_6): 2.31 (s, 3H, CH_3), 5.85 (s, 1H, CH), 6.72-7.45 (m, 3H, ArH), 8.11 (s, 2H, NH_2, exch. with D_2O), 10.20 (s, 1H, OH, exch. with D_2O). MS: (m/z) 258 (M^+) observed for $C_{13}H_{10}N_2O_2S$, Anal calcd: C, 60.45; H, 3.90; N, 11.85; found: C, 60.71; H, 3.66; N, 11.07.

2-Amino-7-methoxy-4-methyl-4H-thieno[2,3-c]chromene-1-carbonitrile (2b)

Yield 30%, mp 144-146° C. IR spectrum, ν, cm^{-1}: 1625, 2221, 3052, 3258. ^{1}H NMR (300 MHz, DMSO-d_6): 2.30 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 5.85 (s, 1H, CH), 6.70-7.46 (m, 3H, ArH), 8.11 (s, 2H, NH_2, exch. with D_2O). MS: (m/z) 272 (M^+) observed for $C_{14}H_{12}N_2O_2S$, Anal calcd: C, 61.75; H, 4.44; N, 10.29; found: C, 61.98; H, 4.12; N, 10.54.

General procedure for synthesis of 3-Hydroxy-6-methyl-6H-chromeno[4',3':4,5]thieno-[2,3-d]pyrimidin-11(10H)-one (3a) and **3-methoxy-6-methyl-6H-chromeno[4',3':4,5]-thieno[2,3-d]pyrimidin-11(10H)-one (3b)**

A mixture of either compound **(2a)** or **(2b)** (1 mmol) and formic acid (20 ml) was heated at 120 °C for 24 h and left to cool. Ice water was added and stirred to produce solid precipitate which was filtered, washed with water, dried and recrystallized from ethanol to give the compounds **(3a)** & **(3b)** respectively.

3-Hydroxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11(10H)-one (3a)

Yield 73%, mp 190-192° C. IR spectrum, ν, cm^{-1}: 1663, 3061, 3258, 3429. ^{1}H NMR (300 MHz, DMSO-d_6): 2.39 (s, 3H, CH_3), 6.01 (s, 1H, CH), 7.12-7.62 (m, 3H, ArH), 8.21 (s, 1H, CH=N), 11.25 (s, 1H, NH, exch. with D_2O), 12.20 (s, 1H, OH, exch. with D_2O). MS: (m/z) 287 (M^++1) observed for $C_{14}H_{10}N_2O_3S$, Anal calcd: C, 58.73; H, 3.52; N, 9.78; found: C, 59.03; H, 3.41; N, 9.49.

3-Methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11(10H)-one (3b)

Yield 79%, mp 155-157° C. IR spectrum, ν, cm^{-1}: 1663, 3060, 3255. ^{1}H NMR (300 MHz, DMSO-d_6): 2.41 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 6.06 (s, 1H, CH), 7.10-7.61 (m, 3H, ArH), 8.23 (s, 1H, CH=N), 11.29 (s, 1H, NH, exch. with D_2O). MS: (m/z) 300 (M^+) observed for $C_{15}H_{12}N_2O_3S$, Anal calcd: C, 59.99; H, 4.04; N, 9.33; found: C, 60.22; H, 4.35; N, 9.52.

General procedure for synthesis of 11-((4-substituted-phenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5a, 5c, 5e, 5g, 5i) and N-(4- substituted-phenyl)-3-methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11-amine (5b, 5d, 5f, 5h, 5j)

To compound **(3a)** or **(3b)** (4 mmol) in phosphorus oxychloride (15 ml) was refluxed for 1 h. The reaction mixture was evaporated and dried in vacuo. The residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated in vacuo to produce intermediate compound **(4a)** or **(4b)**. Aniline derivative (15 mmol) was added in situ to the resulting residue of compound **(4a)** or **(4b)** in toluene (40 ml) and the reaction mixture was refluxed for 16 h. After evaporation, the residue was re-crystallized from chloroform/methanol to obtain compound **(5a, 5c, 5e, 5g, 5i)** or **(5b, 5d, 5f, 5h, 5j)** respectively.

11-((4-Fluorophenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5a)

Yield 75%, mp 198-200° C. IR spectrum, ν, cm^{-1}: 1625, 3068, 3221, 3424. ^1H NMR (300 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 6.12 (s, 1H, CH), 6.88-7.61 (m, 8H, 7H, ArH, 1H, NH), 8.56 (s, 1H, CH=N), 11.92 (s, 1H, OH, exch. with D_2O). MS: (m/z) 379 (M^+) observed for $C_{20}H_{14}FN_3O_2S$, Anal calcd: C, 63.31; H, 3.72; N, 11.08; found: C, 63.62; H, 3.93; N, 11.36.

N-(4-fluorophenyl)-3-methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11-amine (5b)

Yield 81%, mp 162-164° C. IR spectrum, ν, cm^{-1}: 1625, 3068, 3221. ^1H NMR (300 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3), 6.10 (s, 1H, CH), 6.88-7.61 (m, 8H, 7H, ArH, 1H, NH), 8.59 (s, 1H, CH=N). MS: (m/z) 393 (M^+) observed for $C_{21}H_{16}FN_3O_2S$, Anal calcd: C, 64.11; H, 4.10; N, 10.68; found: C, 64.42; H, 3.96; N, 10.94.

11-((4-Chlorophenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5c)

Yield 71%, mp 210-212° C. IR spectrum, ν, cm^{-1}: 1624, 3062, 3220, 3420. ^1H NMR (300 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 6.12 (s, 1H, CH), 6.88-7.61 (m, 8H, 7H, ArH, 1H, NH), 8.55 (s, 1H, CH=N), 11.90 (s, 1H, OH, exch. with D_2O). MS: (m/z) 395 (M^+) observed for $C_{20}H_{14}ClN_3O_2S$, Anal calcd: C, 60.68; H, 3.56; N, 10.61; found: C, 60.62; H, 3.73; N, 10.46.

N-(4-chlorophenyl)-3-methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11-amine (5d)

Yield 78%, mp 165-167° C. IR spectrum, ν, cm^{-1}: 1620, 3065, 3225. ^1H NMR (300 MHz, DMSO-d_6): 2.37 (s, 3H, CH_3), 4.07(s, 3H, OCH_3), 6.08 (s, 1H, CH), 6.94-7.65 (m, 8H, 7H, ArH, 1H, NH), 8.64 (s, 1H, CH=N). MS: (m/z) 409 (M^+) observed for $C_{21}H_{16}ClN_3O_2S$, Anal calcd: C, 61.53; H, 3.93; N, 10.25; found: C, 61.76; H, 3.74; N, 10.61.

11-((4-Bromophenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5e)

Yield 82%, mp 231-233° C. IR spectrum, ν, cm^{-1}: 1622, 3066, 3225, 3429. ^1H NMR (300 MHz, DMSO-d_6): 2.41 (s, 3H, CH_3), 6.11 (s, 1H, CH), 6.81-7.60 (m, 8H, 7H, ArH, 1H, NH), 8.62 (s, 1H, CH=N), 11.99 (s, 1H, OH, exch. with D_2O). MS: (m/z) 441 (M^++2), 439 (M^+) observed for $C_{20}H_{14}BrN_3O_2S$, Anal calcd: C, 54.56; H, 3.20; N, 9.54; found: C, 54.31; H, 3.53; N, 9.82.

N-(4-Bromophenyl)-3-methoxy-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyramidin -11-amine (5f)

Yield 82%, mp 201-203° C. IR spectrum, ν, cm^{-1}: 1622, 3072, 3226. ^1H NMR (300 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 6.04 (s, 1H, CH), 6.91-7.60 (m, 8H, 7H, ArH, 1H, NH), 8.61 (s, 1H, CH=N). MS: (m/z) 455 (M^++2), 453 (M^+) observed for $C_{21}H_{16}BrN_3O_2S$, Anal calcd: C, 55.51; H, 3.55; N, 9.25; found: C, 55.78; H, 3.29; N, 8.96.

6-Methyl-11-(p-tolylamino)-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5g)

Yield 72%, mp 195-197° C. IR spectrum, ν, cm^{-1}: 1625, 3066, 3225, 3427. ^1H NMR (300 MHz, DMSO-d_6): 2.40 (s, 3H, CH_3), 6.12 (s, 1H, CH), 6.88-7.64 (m, 8H, 7H, ArH, 1H, NH), 8.60 (s, 1H, CH=N), 11.95 (s, 1H, OH, exch. with D_2O). MS: (m/z) 375 (M^+) observed for $C_{21}H_{17}N_3O_2S$, Anal calcd: C, 67.18; H, 4.56; N, 11.19; found: C, 67.37; H, 4.23; N, 11.43.

3-Methoxy-6-methyl-N-(p-tolyl)-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-11-amine (5h)

Yield 78%, mp 168-170° C. IR spectrum, ν, cm^{-1}: 1623, 3072, 3228. ^1H NMR (300 MHz, DMSO-d_6): 2.40 (s, 3H, CH_3), 4.04 (s, 3H, OCH_3), 6.03 (s, 1H, CH), 6.93-7.64 (m, 8H, 7H, ArH, 1H, NH), 8.64 (s, 1H, CH=N). MS: (m/z) 390 (M^++1) observed for $C_{22}H_{19}N_3O_2S$, Anal calcd: C, 67.84; H, 4.92; N, 10.79; found: C, 67.95; H, 4.79; N, 11.07.

11-((4-Methoxyphenyl)amino)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrimidin-3-ol (5i)

Yield 79%, mp 206-208° C. IR spectrum, ν, cm^{-1}: 1625, 3066, 3225, 3428. ^1H NMR (300 MHz, DMSO-d_6): 2.36 (s, 3H, CH_3), 6.10 (s, 1H, CH), 6.84-7.61 (m, 8H, 7H, ArH, 1H, NH), 8.58 (s, 1H, CH=N), 11.93 (s, 1H, OH, exch. with D_2O). MS: (m/z) 390 (M^+-1) observed for $C_{21}H_{17}N_3O_3S$, Anal calcd: C, 64.43; H, 4.38; N, 10.73; found: C, 64.67; H, 4.20; N, 10.97.

3-Methoxy-N-(4-methoxyphenyl)-6-methyl-6H-chromeno[4',3':4,5]thieno[2,3-d]pyrim-idin-11-amine (5j)

Yield 83%, mp 171-173° C. IR spectrum, ν, cm^{-1}: 1623, 3070, 3228. ^1H NMR (300 MHz, DMSO-d_6): 2.40 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 6.03 (s, 1H, CH), 6.94-7.62 (m, 8H, 7H, ArH, 1H, NH), 8.64 (s, 1H, CH=N). MS: (m/z) 405 (M^+) observed for $C_{22}H_{19}N_3O_3S$, Anal

calcd: C, 65.17; H, 4.72; N, 10.36; found: C, 65.44; H, 4.45; N, 10.06.

Docking studies

Preparation of enzyme

The 3-dimensional (3D) crystallographic model of EGFR tyrosine kinase [Protein Data Bank (PDB) ID: 1M17] was retrieved from Protein Data Bank (www.rcsb.org)[19] using the Protein preparation wizard in Maestro. The protein was preprocessed by performing assign bond orders, removing original hydrogens, adding hydrogens, creating zero-order bonds to metals, creating disulfide bonds, deleting water molecules beyond 5 Å from hetero groups, and generating het states using Epik at pH: 7.0 ± 2.0. Then, the protein was refined by doing H-bond assignment by sample water orientations, using PROPKA pH: 7.0, and optimization was carried out. The restrained protein minimization was performed using OPLS3 force field.

Receptor grid generation

Receptor grid of size 20 Å was generated after preparing the protein and by selecting the co-crystallized ligand inside the cavity of EGFR tyrosine kinase as the centroid of the active site.

Preparation of ligand

All the molecules were built, 3D optimized, and energy minimized using ligprep and confgen function in Maestro. The library was saved in .maegz format.

Molecular docking

Subsequently, molecular docking was performed using Glide at the SP algorithm to evaluate the protein–ligand interaction and their binding affinities. For our analysis, we checked to add Epik state penalties to docking score and enhanced planarity of conjugated pi groups. The validation for molecular docking was done by re-docking the co-crystalized ligand synthesized compound in the grid. The resulting poses and interactions of co-crystalized ligand and library were compared. The results of binding energies, docking scores and the amino acid residues involved in interactions were determined **(Fig. 2-5** and **Table 1)**.

***In vitro* cytotoxic activity**

The cytotoxic activity of the synthesized compounds was measured against human breast cancer cell line MCF7 in the National Cancer Institute, Cairo University. The screening involves a calculation of the percentage growth or the surviving fraction of the drug treated cell lines compared with untreated control using Sulforhodamine B (SRB) colorimetric assay.[20] Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0.0, 1.0, 2.5, 5.0 and 10.0 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 ºC and in an atmosphere of 5% CO_2. After 48 h, cells were fixed and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of the tumor cell line after the specified compound. The results were described in the **table 2**.

CONCLUSION

In this study, [4',3':4,5]thieno[2,3-d]pyrimidine derivatives was designed and synthesized as erlotinib analogues. The structures of the compounds were characterized using spectroscopic and spectrometric techniques, confirming the integrity of these molecules. The new compounds were screened for their cytotoxic activity against breast cancer cell line MCF-7. The results of *in vitro* cytotoxic activity indicated that all of the tested compounds exhibited moderate to good activities. Compounds **5b**, **5d** and **5f** showed good cytotoxic activity with IC_{50} values of 1.22, 1.42 and 1.67 µM respectively.

Conflicts of interests

Authors declare no conflicts of interest.

REFERENCES

1. Dube PN, Waghmare MN, Mokale SN. Synthesis *in vitro* and *in vivo* biological evaluation and molecular docking analysis of novel 3-(3-oxo-substitutedphenyl-3-)4-(2-(piperidinyl)ethoxy)phenyl)propyl)-2H-chromen-2-one derivatives as anti-breast cancer agents. Chem Biol Drug Des, 2016; 87: 608-617.
2. Bhuvaa HA, Kinib SGJ. Synthesis, anticancer activity and docking of some substituted benzothiazoles as tyrosine kinase inhibitors. J Mol Graph Model, 2010; 29: 32-37.
3. Hussain MK, Ansari MI, Yadav N, Gupta PK, Gupta AK, Saxena R, *et al*. Design and synthesis of ERa/ERb selective coumarin and chromene derivatives as potential anti-breast cancer and anti-osteoporotic agents. RSC Adv, 2014; 4: 8828-8845.
4. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr Relat Cancer, 2001; 8: 161-173.
5. Gullick WJ. Prevalence of aberrant expression of the epidermal growth factor receptor in human cancer. Br Med Bull, 1991; 47: 87-98.
6. Noolvi MN, Patel HM, Kaur M. Benzothiazole: search for anticancer agents. Eur J Med Chem, 2012; 54: 447-462.
7. Pedeboscq S, Gravier D, Casadebaig F, Hou G, Gissot A, De Giorgi F, *et al*. Synthesis and study of antiproliferative activity of novel thienopyrimidines on glioblastoma cells. Eur J Med Chem, 2010; 45: 2473-2479.

8. Rheault TR, Caferro TR, Dickerson SH, Donaldson KH, Gaul MD, Goetz AS, *et al*. Thienopyrimidine-based dual EGFR/ ErbB-2 inhibitors. Bioorg Med Chem Lett, 2009; 19: 817-820.
9. Zhao Z, Wu H, Wang L, Liu Y, Knapp S, Liu Q, *et al*. Exploration of type II binding mode: a privileged approach for kinase inhibitor focused drug discovery?. ACS Chem Biol, 2014; 9: 1230-1241.
10. Mowafy S, Galanis A, Doctor ZM, Paranal RM, Lasheen DS, Farag NA, *et al*. Toward discovery of mutant EGFR inhibitors; Design, synthesis and in vitro biological evaluation of potent 4-arylamino-6-ureido and thioureido-quinazoline derivatives. Biorg Med Chem, 2016; 24: 3501-3512.
11. Furet P, Caravatti G, Lydon N, Priestle JP, Sowadski JM, Trinks U, Traxler P. Modelling study of protein kinase inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411. J Comput Aided Mol Des, 1995; 9: 465-472.
12. Gaber AA, Bayoumi AH, El-morsy AM, Sherbinya FF, Mehany ABM, Eissa IH. Design, synthesis and anticancer evaluation of 1H-pyrazolo[3,4-d]pyrimidine derivatives as potent EGFRWT and EGFRT790M inhibitors and apoptosis inducers. Bioorg Chem , 2018; 80: 375-395.
13. Gandin V, Ferrarese A, Dalla Via M, Marzano C, Chilin A, Marzaro G. Targeting kinases with anilinopyrimidines: discovery of N-phenyl-N'-[4-(pyrimidin-4-yla-mino) phenyl] urea derivatives as selective inhibitors of class III receptor tyrosine kinase subfamily. Sci Rep, 2015; 5: 16750. DOI: 10.1038/srep16750.
14. Liu Y, Gray NS. Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol, 2006; 2: 358-364.
15. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer, 2009; 9: 28-39.
16. Kassab AE, Gedawy EM. Synthesis and anticancer activity of novel 2-pyridyl hexahyrocyclooctathieno[2,3-d]pyrimidine derivatives. Eur J Med Chem, 2013; 63: 224-230.
17. Adly ME, Gedawy EM, El-Malah AA, El-Telbany FA. Synthesis of novel thieno[2,3-d]pyrimidine derivatives and evaluation of their cytotoxicity and EGFR inhibitory activity. Anticancer Agents Med Chem, 2018; 18: 747-756.
18. Elgazwy A-SSH, Edrees MM, Ismail NSM. Molecular modeling study bioactive natural product of khellin analogues as a novel potential pharmacophore of EGFR inhibitors. J Enzyme Inhibition Med Chem, 2012. DOI: 10.3109/14756366.2012.719504.
19. Park JH, Liu Y, Lemmon MA, Radhakrishnan R. Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain. Biochem J, 2012; 448: 417-423.
20. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al*. New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst, 1990; 82: 1107-1112.