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One-pot Synthesis, Cytotoxic Evaluation and Molecular Docking of 3,4,7,8-tetrahydroquinazoline-2,5-(1H,6H)-dione Derivatives on EGFR

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Abstract:

In this study, a proper multicomponent process was developed for synthesis of tetrahydroquinazolinone derivatives. Dimedone, urea, and various aryl aldehydes were applied to the evaluation of a one-pot reaction under solvent-free and solvent conditions in the presence of $CaCl_2.2H_2O$ and aniline as catalysts. It was optimized by employing at 110 °C and 10% mol for $CaCl_2.2H_2O$ and 60% mol in the presence of EtOH at room temperature for aniline. All chemical structures of tetrahydroquinazolinones were determined by FT-IR, ^{1}HNMR, ^{13}CNMR, mass spectroscopy, elemental analysis, and melting point. The synthesized compounds were evaluated for their cytotoxicity activity against MCF-7 cell line by MTT assay. All of the synthesized compounds showed moderate cytotoxicity activity against MCF-7 cell line. Especially, Compound **4h** was the most potent compound. Also, the potential EGFR inhibitory activity of these compounds was investigated *in silico* using molecular docking simulation method. Especially compound **4h** which showed the lowest ΔG_{bind} results (-7.37 Kcal/mol).

Keywords: One-pot synthesis; Quinazolinones; Solvent-free; Molecular docking; Cytotoxicity; MCF-7.

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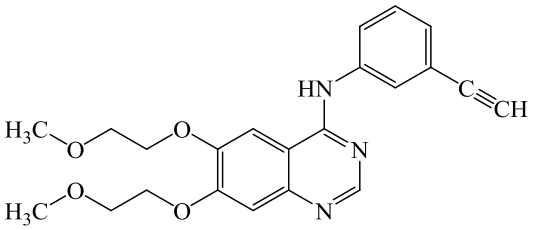
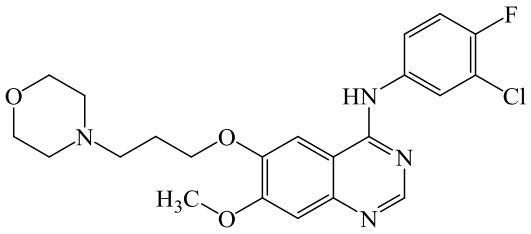
1. Introduction

Quinazolinones are very interesting heterocyclic components due to their wide range of pharmacological and therapeutic activity such as antibacterial, anticancer, antidiuretic, antifungal, and anti-inflammatory [1-4]. Likewise, quinazolinones are an important pharmacophore for a range of enzyme inhibitors such as tyrosine kinase, dihydrofolate reductase and thymidylate synthase [5-7]. EGFR tyrosine kinase is a cell-surface receptor which is over-expressed in numerous human tumors, including the ovarian, breast, prostate, bladder, lung, and colon [8]. Some structures of the important commercial drugs with the quinazolinone cores as important epidermal growth factor receptor inhibitors are shown in scheme 1 [9-11].

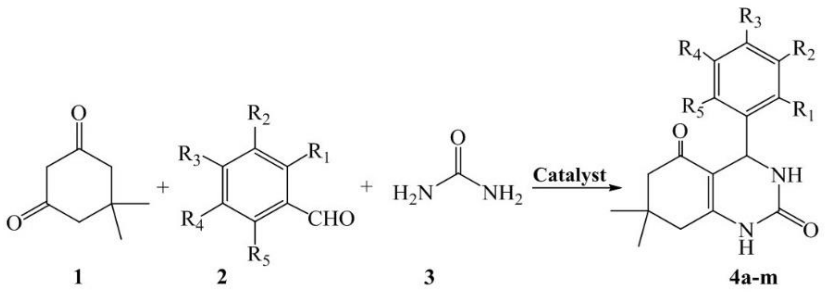


Erlotinib (Tarceva)



Gefitinib (Iressa)

Scheme1. Chemical structure of some epidermal growth factor receptor tyrosine kinase inhibitors[9-11].



Catalyst: **A)** $CaCl_2.2H_2O$, solvent-free, 110 °C **B)** Aniline, EtOH, r.t

	a	b	c	d	e	f	g	h	i	j	k	m
R_1	H	H	H	H	H	H	OH	Br	OMe	OMe	H	OMe
R_2	H	H	H	H	H	H	H	H	OMe	H	OMe	H
R_3	H	*i*-Pr	OMe	Me	NH_2	NMe_2	H	H	H	H	OMe	OMe
R_4	H	H	H	H	H	H	H	H	H	OMe	OMe	H
R_5	H	H	H	H	H	H	H	H	H	H	H	OMe

Scheme 2. Synthesis of 3,4,7,8-tetrahydroquinazoline-2,5-(1*H*,6*H*)-dione.

Some of the quinazolinone scaffold based effective anticancer agents were synthesized by Hassanzadeh et al [12, 26]. In recent years, multicomponent reactions (MCRs) have drawn special attention over conventional bimolecular reactions owing to their convergence, their atom economy, multiple bond forming efficiency, operational simplicity, and high selectivity and hence become current area of interest in organic, combinatorial chemistry research, and medicinal [13-20]. Several methods for the synthesis of this scaffold have been quoted in the literature. However, these methods have various disadvantages such as prolonged reaction time, harmful catalysts, low yields and using of toxic organic solvents. Therefore, the development of more efficient methods for the synthesis of these kinds of compounds is still in high demand. On the other hand, molecular docking is used to predict the interactions between a ligand and a receptor molecule in order to predict ligand conformation and orientation within a targeted binding site [21]. Moreover, to the best of our knowledge, no docking study of 3,4,7,8-tetrahydroquinazoline-2,5-(1H,6H)-dione with EGFR protein has been reported so far.

Herein we report a one-pot, three-component protocol for the synthesis of tetrahydroquinazolinones using $CaCl_2.2H_2O$ and aniline as catalysis via a multicomponent reaction starting from dimedone **1**, various aryl aldehydes, **2,** and urea **3** (Scheme 2). *In vitro* cytotoxicity activities of these compounds were evaluated against MCF-7(breast cancer) cell line. The synthesized compounds were docked into the binding pocket of EGFR protein and their binding energies were calculated.

2. Materials & Methods

2.1. Chemistry

All chemicals and solvents were purchased from Merck and were used as received without further purification. Infrared (IR) spectra was taken by Nicolet 4700 FT-IR spectrophotometer in wave number range 400-4000 cm^{-1}. ^1H and ^{13}C NMR spectra were measured by Bruker-Instrument DPX-400 Avance 2, operating at 400 and 100MHz for ^{1}HNMR and ^{13}CNMR, respectively. Mass spectra were recorded on the Shimadzu Mass Spectrometer. Melting points of all the synthesized compounds were determined by Electrothermal 9300 apparatus. The reactions were monitored by TLC (silica-gel 60 F254, hexane: AcOEt) for checking the purity and progress of reaction.

2.2. Cytotoxic Activity

MCF-7 cell line was purchased from Pasteur Institute of Iran (Tehran, I.R. Iran). Dimethyl sulfoxide (DMSO) and 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Merck, Germany. Erlotinib (Roche, Switzerland) was used as a reference for comparison. The cytotoxicity of the compounds was determined by MTT in a standard MTT assay against the breast cancer (MCF-7) cell line, cultured either in the RPMI-1640 medium containing 10% v/v fetal bovine serum (FBS) and 1% antibiotic (mixture of 50 unit/mL penicillin /50 µg/mL streptomycin). $NaHCO_3$ (1 g) and 1% of L glutamine (2 mM) for MCF-7. The standard drug, Erlotinib, was used as the positive control. Negative

Control cells contain cell suspension and DMSO (1%) without tested compounds [22]. The cytotoxic evaluations were carried out according to reported methods [12].

2.3. Molecular Docking

Hardware and Software: All computational activities of this work were performed on ASUS Laptop (Intel® Core™i7-7500T CPU @ 2.70 GHz, RAM 8 GB) running Windows 10 64-bit HomeBasic Operating System. AutoDock 4.2, Discovery studio 2.5, Lig Plot, and Hyper Chem 7.0 software packages were used, for *in silico* protein-ligand docking simulation.

The novel tetrahydroquinazolinone derivatives were subjected to dock in the active site of EGFR protein using Autodock 4.2 software. The crystal structure of the EGFR (PDB code 1M17) with resolution 2.6 Å was chosen as the protein model for the present study [21]. The co-crystallized Ligand and water molecules were removed from the protein file. Then, this file was imported in AutoDock. The two dimensional (2D) structures of the ligands were optimized using HyperChem 7.0 software as explained in previous studies [28]. We investigated the theoretical binding mode of 12 ligands at the erlotinib binding site using molecular docking modeling. The docking studies were carried out according to reported methods [21,28]. Also, to ensure the validity of docking, erlotinib was re-docked to the binding site. A grid box size of 60 × 60 × 60 (all in Å) points with a grid spacing of0.375 Å was considered. A Lamarckian genetic algorithm program was used to calculate 100 different conformers [21].

2.4. General procedure for the synthesis of tetrahydroquinazolinone derivatives (4a-m)

To a mixture of urea (1.5 mmol), aryl aldehyde (1 mmol), and dimedone (1 mmol), 0.1 mmol of $CaCl_2.2H_2O$ as catalyst was added under solvent-free conditions. After the homogenization of mixture by mechanical stirrer, it was heated in a preheated oil bath at 110 °C for an appropriate time until the reaction was complete. After completion of the reaction, which was confirmed by TLC, the reaction was adjusted to ambient temperature and added to 10 ml of ethanol. The mixture was stirred for 4 min. The catalyst was separated by filtration and the solvent was evaporated and precipitate filtered. After washing with water, the corresponding tetrahydroquinazolinone products were obtained in good yields. The same reaction was carried out in the presence of aniline as catalyst under r.t and solvent conditions.

2.4.1. Synthesis of 7,7-dimethyl-4-phenyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4a):

Light yellow crystal, (MS: m/z (%): 271.14 (M+, 100), M.W. 270.33; IR (KBr) *v* max / cm^{-1}: 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR ($CDCl_3$, 400 MHz) δ /ppm: 1.12 (3H, s, CH_3), 1.26 (3H, s, CH_3), 2.31-2.38 (2H, m, CH_2), 2.41-2.46 (2H, m, CH_2), 5.56 (1H, s, CH), 7.11-7.31(5H, m, Arom), 8.61 (1H, s, NH), 9.53 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ /ppm: 27.40 (CH_3), 31.43 (C), 32.75 (CH_2), 46.45(CH), 47.05(CH_2), 76.74(C=C), 77.37(C=C), 115.60(CH), 125.87(CH), 126.79(CH), 128.25(CH), 138.05(CH), 189.45(CO), 190.54(CO) ppm. Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36; O, 11.84. Found: C, 71.12; H, 6.73; N, 10.41; O, 11.87.

2.4.2. Synthesis of 4-(4-isopropylphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4b):

Yellow crystal, (MS: m/z (%): 313.30 (M+,100), M.W. 312.41, IR (KBr) *v* max / cm^{-1}: 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR (CDCl3, 400 MHz)δ /ppm: 1.13 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.25 (3H, s, CH_3), 2.32-2.50 (1H, m, CH), 2.85-2.87 (2H, m, CH_2), 2.88-2.90 (2H, m, CH_2), 5.53 (1H, s, CH), 7.03-7.31 (4H, m, Arom), 8.55 (1H, s, NH), 9.95 (1H, s, NH) ppm; ^{13}C NMR (CDCl3, 100 MHz) δ /ppm : 24.02 (CH_3), 27.35 (CH_3), 29.75 (CH_3), 32.40 (CH_3), 32.44 (C), 33.53 (C), 46.45 (CH2), 47.07 (CH), 77.07 (C=C), 77.38 (C=C), 115.71 (CH), 126.33 (CH), 126.72 (CH), 135.24 (CH), 146.25 (C), 189.34 (CO), 190.44 (CO) ppm. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97; O, 10.24. Found: C, 73.10; H, 7.79; N, 8.99; O, 10.29.

2.4.3. synthesis of 4-(4-methoxyphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4c):

Light yellow crystal, (MS: m/z (%): 301.12 (M+,100), M.W. 300.35, IR (KBr) *v* max / cm^{-1}: 3453 (s), 3201 (s), 1691 (s), 1675 (s), 1654 (s); ^{1}H NMR (CDCl3, 400 MHz)δ /ppm: 0.91 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.99 (1H, d, j=16Hz, CH_2), 2.18 (1H, d, j=16Hz, CH_2), 3.66 (3H, s, OCH_3), 4.76 (1H, s, CH), 7.07-6.86 (4H, m, Arom), 7.76 (1H, s, NH), 9.25 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ /ppm : 27.02 (CH_3), 29.35 (CH_3), 31.44 (C), 33.53 (CH_2), 50.45 (CH_2), 51.07 (CH), 55.20 (OCH_3), 77.07 (C=C), 77.38 (C=C), 115.71 (CH), 126.33 (CH), 126.72 (CH), 135.24 (CH), 153.60 (C), 157.54 (CO), 194.80 (CO)ppm. Anal. Calcd for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33; O, 15.98. Found: C, 68.22; H, 6.42; N, 9.23; O, 15.29.

2.4.4. Synthesis of 7,7-dimethyl-4-p-tolyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4d):

White crystal, (MS: m/z (%): 285.15(M+, 100), M.W. 284.35, IR (KBr) *v* max / cm^{-1}: 3285 (s), 3190 (s), 1646

(s),1605(s); ^{1}HNMR($CDCl_3$,400MHz)δ/ppm: 0.79 (3H, s, CH_3), 0.87 (3H, s, CH_3), 1.95 (2H, d, J=16Hz, CH_2), 2.02 (2H, d, J=16Hz ,CH_2) ,2.26 (3H, s, CH_3), 4.50 (1H, s, CH), 6.80-6.98 (4H, m, Arom), 7.06 (2H, br, NH) ppm; ^{13}C NMR (CDCl3, 100 MHz) δ /ppm: 24.02 (CH_3), 27.35 (CH_3), 29.75 (CH_3), 32.44 (C), 33.53 (CH_2), 46.45 (CH_2), 47.807 (CH), 79.07 (C=C), 80.38 (C=C), 118.71 (CH), 129.33 (CH), 130.72 (CH), 135.44 (CH), 146.25 (C), 189.34 (CO), 190.44 (CO) ppm. Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85; O, 11.25. Found: C, 71.79; H, 7.06; N, 9.89; O, 11.25.

2.4.5. Synthesis of 4-(4-aminophenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4e):

Light yellow crystal, (MS: m/z (%): 286.35(M+,100), M.W. 285.34, IR (KBr) ν max / cm^{-1}: 3285 (s), 3190 (s), 1646 (s), 1605 (s); ^{1}H NMR (CDCl3,400 MHz)δ/ppm: 0.79(3H, s, CH_3), 0.89 (3H, s, CH_3), 1.82 (2H, d, J=16Hz, CH_2), 2.12 (2H, d, J=16Hz, CH_2),4.01(2H, s, NH_2), 4.64 (1H, s, CH), 6.88-7.01(4H, m, Arom), 8.06 (2H, br, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ /ppm : 25.02 (CH_3), 26.35 (CH_3), 30.75 (CH_3), 31.40 (CH_3), 32.64 (C), 33.83 (C), 47.45 (CH_2), 49.07 (CH), 78.07 (C=C), 80.38 (C=C), 120.71 (CH), 124.33 (CH), 126.72 (CH), 137.24 (CH), 149.25 (C), 185.34 (CO), 191.44 (CO) ppm. Anal. Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73; O, 11.21. Found: C, 67.30; H, 6.76; N, 14.79; O, 11.25.

2.4.6. synthesis of 4-(4-(dimethylamino) phenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4f):

Yellow crystal, (MS: m/z (%): 314.39 (M+,100), M.W. 313.39, IR (KBr) ν max / cm^{-1}: 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)δ /ppm: 1.12 (3H, s, CH_3), 1.25 (3H, s, CH_3), 2.30-2.37 (2H, m, CH_2), 2.40-2.44 (2H, m, CH_2), 2.92(6H, s, 2CH_3), 5.50 (1H, s, CH), 6.67-6.98 (4H, m, Arom), 8.93 (1H, s, NH), 9.97 (1H, s, NH) ppm. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 68.98; H, 7.40; N, 13.41; O, 10.21. Found: C, 68.76; H, 7.80; N, 13.58; O, 10.39.

2.4.7. Synthesis of 4-(2-hydroxyphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4g):

Light yellow crystal, (MS: m/z (%): 287.33 (M+, 100), M.W. 286.33, IR (KBr) ν max / cm^{-1}: 3500 (br), 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)δ /ppm: 1.17 (3H, s, CH_3), 1.29 (3H, s, CH_3), 2.31-2.39 (2H, m, CH_2), 2.41-2.47 (2H, m, CH_2), 5.40 (1H, s, OH), 5.58 (1H, s, CH), 6.68-7.08 (4H, m, Arom), 8.83 (1H, s, NH), 9.67 (1H, s, NH) ppm. Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; O, 16.76. Found: C, 67.16; H, 6.30; N, 9.71; O, 16.70.

2.4.8. Synthesis of 4-(2-bromophenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4h):

White crystal, (MS: m/z (%): 350.24 (M+, 100), M.W. 349.22, IR (KBr) ν max / cm^{-1}: 3343 (s), 3292 (s), 1671 (s), 1655 (s), 1644 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)δ /ppm: 0.76 (3H, s, CH_3), 0.88 (3H, s, CH_3), 1.85-1.99 (2H, m, CH_2), 2.19-2.34 (2H, m, CH_2), 5.64 (1H, s, CH), 7.15-7.28 (4H, m, Arom), 8.10 (1H, s, NH), 9.43 (1H, s, NH)ppm ; ^{13}C NMR (DMSO-d6, 100 MHz) δ /ppm : 27.64 (CH3), 31.33 (CH3), 37.85 (CH2), 51.38(CH2), 109.41 (C), 129.99 (CH), 131.55 (CH), 132.01 (CH), 134.46 (CH), 140.79 (CH), 157.66 (C), 163.84 (CO), 196.24 (CO) ppm; ppm. Anal. Calcd for $C_{16}H_{17}BrN_2O_2$: C, 55.03; H, 4.91; Br, 22.88; N, 8.02; O, 9.16. Found: C, 55.10; H, 4.96; Br, 22.90 N, 8.11; O, 9.19.

2.4.9. Synthesis of 4-(2,3-dimethoxyphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4i):

Yellow crystal, (MS: m/z (%): 331.30 (M+, 100), M.W. 330.38, IR (KBr) ν max / cm^{-1}: 3543 (s), 3292 (s), 1671 (s), 1655 (s), 1634 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)δ /ppm: 1.15 (3H, s,CH_3), 1.20 (3H, s, CH_3), 2.28-2.42 (3H, m, CH_2), 2.42-2.47 (2H, m, CH_2), 3.78(3H, s, OCH_3), 3.80 (3H, s, OCH_3), 5.55 (1H, s, CH), 6.76-6.97 (3H, m, Arom), 8.98 (1H, s, NH), 9.27 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ /ppm: 27.79(CH_3), 31.10(CH_3), 33.13(C), 34.79 (CH_2), 44.33(CH), 47.09(CH_2), 49.86(OCH_3), 62.91(C=C), 78.74(C=C), 106.04(CH), 117.58(C), 135.77(C), 156.84(CH), 190.34(CO), 192.47(CO) ppm. Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48; O, 19.37. Found: C, 65.41; H, 6.82; N, 8.78; O, 19.35.

2.4.10. Synthesis of 4-(2,5-dimethoxyphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4j):

Light yellow crystal, (MS: m/z (%): 331.30 (M+, 100), M.W. 330.38, IR (KBr) ν max / cm^{-1}: 3543 (s), 3292 (s), 1671 (s), 1655 (s), 1634 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)δ /ppm: 1.15 (3H, s,CH_3), 1.20 (3H, s, CH_3), 2.28-2.42 (2H, m, CH_2), 2.42-2.47 (2H, m, CH_2), 3.78 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 5.55(1H, s, CH), 6.76-6.97 (3H, m, Arom), 8.98 (1H, s, NH), 9.27 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ /ppm: 27.79 (CH_3), 31.10 (CH_3), 33.13 (C), 34.79 (CH_2), 44.33 (CH), 47.09 (CH_2), 49.86

(OCH_3), 62.91 (C=C), 78.74 (C=C), 106.04 (CH), 117.58 (C), 135.77 (C), 156.84 (CH), 190.34 (CO), 192.47 (CO) ppm. Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48; O, 19.37. Found: C, 65.41; H, 6.82; N, 8.78; O, 19.35.

2.4.11. Synthesis of 7,7-dimethyl-4-(3,4,5-trimethoxyphenyl)-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4k):

Yellow crystal, (MS: m/z (%): 361.17 (M+, 100), M.W. 360.4, IR (KBr) *v* max / cm^{-1}: 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)*δ* /ppm: 1.14 (3H, s, CH_3), 1.26 (3H, s, CH_3), 2.38-2.40 (2H, m, CH_2), 2.43-2.45 (2H, m, CH_2), 3.77(3H, s, OCH_3), 3.83(3H, s, OCH_3), 3.93(3H, s, OCH_3), 5.52(1H, s, CH), 6.36-6.37 (2H, m, Arom), 8.93 (1H, s, NH), 9.06 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* /ppm: 26.79 (CH_3), 30.10 (CH_3), 31.13 (C), 32.79 (CH_2), 46.33 (CH), 47.09 (CH_2), 45.86 (OCH_3), 60.91 (C=C), 76.74 (C=C), 104.04 (CH), 115.58 (C), 133.77 (C), 152.84 (CH), 189.34 (CO), 190.47 (CO) ppm. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 63.32; H, 6.71; N, 7.77; O, 22.20. Found: C, 63.41; H, 6.82; N, 7.88; O, 22.31.

2.4.12. Synthesis of 4-(2,4,6-trimethoxyphenyl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (4m):

White crystal, (MS: m/z (%): 361.17 (M+, 100), M.W. 360.4, IR (KBr) *v* max / cm^{-1}: 3443 (s), 3192 (s), 1681 (s), 1665 (s), 1624 (s); ^{1}H NMR ($CDCl_3$, 400 MHz)*δ* /ppm: 1.14 (3H, s,CH_3), 1.26 (3H, s, CH_3), 2.38-2.40 (2H, m, CH_2), 2.43-2.45 (2H, m, CH_2), 3.77 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 5.52 (1H, s, CH), 6.36-6.37 (2H, m, Arom), 8.93 (1H, s, NH), 9.06 (1H, s, NH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* /ppm: 26.79 (CH_3), 30.10 (CH_3), 31.13 (C),32.79 (CH_2), 46.33 (CH), 47.09 (CH_2), 45.86 (OCH_3), 60.91 (C=C), 76.74 (C=C), 104.04 (CH), 115.58 (C), 133.77 (C), 152.84 (CH), 189.34 (CO), 190.47 (CO) ppm. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 63.32; H, 6.71; N, 7.77; O, 22.20. Found: C, 63.41; H, 6.82; N, 7.88; O, 22.31.

3. Results and Discussion

3.1. Chemistry

Significant attention has been attached to the synthesis of 3,4,7,8-tetrahydroquinazoline-2,5-(1*H*,6*H*)-dione derivatives by a three-component reaction of dimedone, aryl aldehydes, and urea (Scheme 2). This reaction was highly dependent on several parameters [14-15, 20]. Therefore, the main focus of the present study is the evaluation of the effects of the catalysts, temperature, nature of the substituents and solvents on the reaction. In order to optimize the reaction conditions, initially, synthesis of **4a** was opted as a model reaction.

At first, the results of different types of catalysts for the synthesis of **4a** are given in Table 1. Maximum yields of the products were obtained for $CaCl_2.2H_2O$ and aniline. It can be seen that $CaCl_2.2H_2O$ gives better yields in shorter reaction time than aniline (Table 1).

Table 1. Evaluation of various types of catalysts used for the synthesis of 4a (model reaction)

Entry	Catalyst	Time	Yield (%)[a]
1	$FeCl_3.6H_2O$	600	**Trace**
2	**$CaCl_2.2H_2O$**	**20 min**	92
3	$NiN_2O_6.6H2O$	60	**50**
4	$ZrCl_2$	560	**Trace**
5	Aniline	90	**56**

[a] Yields refer to isolated products

This highest yield may relate to the interaction of divalent calcium cations with the carbonyl groups, while this regularity is not seen in aniline structure [23-25]. However, the model reaction was examined by using two different catalysts ($CaCl_2.2H_2O$ and aniline) under solvent and solvent-free conditions in order to explore the substrate scope of this reaction. Thus, the effects of different amounts of the catalysts, reaction time and temperature on the model reaction were studied. The use of various mole percentages of the catalysts was revealed that the best quantities of catalysts for this reaction were found to be 10 mol % of $CaCl_2.2H_2O$ under solvent-free condition at 110 °C (Table 2). A further increase in the mole percentages of the catalyst up to 20 mol% did not show any effects on the product yield or reaction time. Of course, at high temperature of the reaction, higher yield of the products is encountered (Table 2).

Table 2. Evaluation of temperature (°C), time (min), Yield (%) (isolated) the amount (mol%) of the $CaCl_2.2H_2O$ as catalysts on the model reaction.

Entry	mol%	Temp	Time	Yield(%)[a]
1	-	110	600	Trace
2	**10**	**110**	**4**	**92**
3	20	110	4	93
4	10	80	35	57.14
5	10	90	30	78.57
6	10	100	20	85.71

[a] Yields refer to isolated products

Furthermore, the obtained results from the reaction in the presence of aniline as catalyst were gathered in Table 3. The amount of 60 mol % of aniline in EtOH as the solvent were found to be the optimum condition for the maximum yield of **4a** (Tables 3).

Table 3. Evaluation of time (min), Yield (%) (isolated) the amount (mol%) of the aniline as catalysts on the model reaction at room temperature.

Entry	mol%	Time	Yield(%)
1	-	600	Trace
3	20	147	16
4	30	105	19.5
5	40	84	32
6	50	75	55
7	**60**	**90**	**56**

The results showed satisfactory success to obtain the products with suitable conditions at ambient temperature. It was noticed that the reaction in the absence of the catalyst did not show any proceed even at 110 °C after 120 min. For examining the effects of solvents, the model reaction carried out in different solvents such as H_2O, CH_3CN, DMSO, EtOH, and DMF under the same conditions and following comparison with solvent-free condition. Results showed that the solvent-free condition provided an excellent condition for the effective synthesis of tetrahydroquinazolinones with respect to the solvent condition (Table 4). This protocol leads to obtain the tetrahydroquinazolinones with the highest yields in short reaction times under optimized reaction conditions. The scope of this MCR was examined using a variety of aromatic aldehydes (**2**) in the presence of $CaCl_2.2H_2O$ and aniline under solvent and solvent-free conditions (Table 5). It was effectively condensed to give 7,7-dimethyl-4-phenyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione derivatives (**4a-m**) (Scheme 2). In all cases, corresponding tetrahydroquinazolinones were isolated with good to excellent yields, which $CaCl_2.2H_2O$ was found to be the best catalyst for the reaction (Table 5). It is apparent from the Table 4 that the $CaCl_2.2H_2O$ proved to be effective for synthesis of tetrahydroquinazolines as compared to the aniline where longer reaction time was needed, however, the products were obtained in good yields. There was no significant effect of electron donating and electron withdrawing substituents in case of the compounds **4a-m**. The most general method for the preparation of tetrahydroquinazolinones derivatives involves the one-pot and solvent-free condensation reaction of dimedone, aromatic aldehydes, and urea in the presence of the $CaCl_2.2H_2O$ as a Lewis acid.

Table 4. Evaluation of solvent effect for the model reaction.

		$CaCl_2.2H_2O$			Aniline		
Entry	Solvent	Temp ˚C	Time	Yield (%)	Temp ˚C	Time	Yield (%)
1	CH_3CN	Reflux	24 h	48	r.t	24 h	Trace
2	EtOH 50%	Reflux	24 h	Trace	r.t	24 h	30
3	DMSO	Reflux	24 h	Trace	r.t	24 h	-
4	DMF	Reflux	24 h	Trace	r.t	24 h	-
5	H_2O	Reflux	24 h	Trace	r.t	24 h	Trace
6	-	**110**	**4 min**	**92**			

Table 5. Catalytic synthesis of 3,4,7,8-tetrahydroquinazoline-2,5-(1H,6H)-dione by the use of $CaCl_2.2H_2O$ and aniline under solvent and solvent-free conditions.

Entry	Aryl aldehyde	$CaCl_2.2H_2O$	Aniline	M.P. (°C)
		Time,Yield(%)[a]	Time,Yield(%)	
1	Benzaldehyde (**4a**)	20min-92%	110min-55%	170
2	Isopropylbenzaldehyde (**4b**)	25min-91%	380min-52%	159
3	4-methoxybenzaldehyde (**4c**)	45min-93%	> 5 days	155
4	4-methylbenzaldehyde (**4d**)	55min-96%	> 5 days	145
5	Para-aminobenzaldehyde (**4e**)	50min-99%	> 5 days	150
6	4-dimethylaminbenzaldehyde (**4f**)	35min-96%	4 days-76%	200
7	2-hydroxybenzaldehyde (**4g**)	35min-98%	> 5 days	164
8	2-bromobenzaldehyde (**4h**)	15min-98%	16min-72%	225
9	2,3-dimethoxybenzaldehyde (**4i**)	55min-98%	-	148
10	2,5-dimethoxybenzaldehyde (**4j**)	40min-98%	3min- 65%	168
11	3,4,5-trimethoxybenzaldehyde (**4k**)	60min-95%	6min-70%	180
12	2,4,6-trimethoxybenzaldehyde(**4m**)	50min-99%	-	188

[a] Yields refer to isolated products

3.2. Cytotoxic Activity

Quinazolinone derivatives exhibited diverse biological properties and particularly cytotoxic effects [22, 27]. All compounds were evaluated against MCF-7 cell line using MTT assay [12, 21]. Based on the results presented in Table 6, the most of the compounds were active against MCF-7 cell line. The cytotoxic evaluation of the compounds was comparable to erlotinib as a reference compound. Introduction of the electron-withdrawing Br group to the quinazoline ring led to compound **4h**, which showed lower cytotoxicity than first against MCF-7cell line (Table 6). Based on the MTT results, compound **4h** substituted with Br group may play an important role in growth inhibition of the MCF-7 cell line with IC50 value of 34.90 ± 3.24 µM compared to erlotinib. This can be attributed to the effects of electrons withdrawing on the heterocyclic ring.

Structure-activity relationship studies based on the observed results showed that in **4a-4m**, presence of electron withdrawing groups (bromine group) at the ortho positions of the phenyl ring could be responsible for better activities because of its inductive effect.

Table 6. The IC_{50} (µM) of final compounds against MCF-7 cell lines.

Final Compounds	MCF-7
4a	61.00 ± 2.67
4b	59.80 ± 1.87
4c	57.37 ± 2.73
4d	43.33 ± 2.30
4e	49.77 ± 2.66
4f	52.77 ± 2.66
4g	47.13 ± 2.60
4h	34.90 ± 3.24
4i	59.73 ± 2.06
4j	53.20 ± 3.49
4k	57.73 ± 1.12
4m	59.27 ± 2.15
Erlotinib	1.47 ± 0.47

3.3. Molecular Docking Studies

Table 7 summarizes the binding characterizations between synthesized compounds and EGFR. According to the data presented in this table, ligands interact with the EGFR binding site through hydrogen bonding. Amongst various conformations of the ligands obtained from the docking procedure, the conformation with the best scored pose, with the lowest binding energy (~ -5.90 – -7.37kcal/mol), and as the most populated cluster was selected. Figure 1 and 2 show a 2D schematic of compound **4h** as well as erlotinib while docked into the erlotinib binding site, supporting the idea that the compounds are well incorporated into the binding pocket. The hydrophobic sites of the ligands are conserved in the majority of the structures (Fig. 1).

As shown in Table 7, some compounds (**4a**,**4b** ,**4f, 4h,** and **4j**) can create a strong hydrogen bond with Asp831 at distance 2.75Å. It is interesting that other complex stabilization might result from the hydrogen bonds between these ligands and Thr830, Met769, Lys721, These results are consistent with the X-ray cocrystal structure and previous studies indicating the important roles of those residues [21]. No hydrogen bond interaction for **4c**, **4d**, **4g,** and **4i** was predicted (Table7).

Arg817, cys 773 via NH_2 and OCH_3 electron donating substitution on the phenyl ring (for **4e, 4j, 4k,** and **4m**).

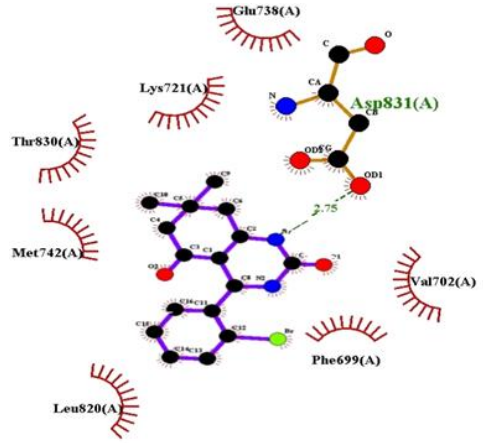


Figure 1. Docked conformation of compound **4h** in the binding site of EGFR. Hydrogen bonds are shown by green dashed line.

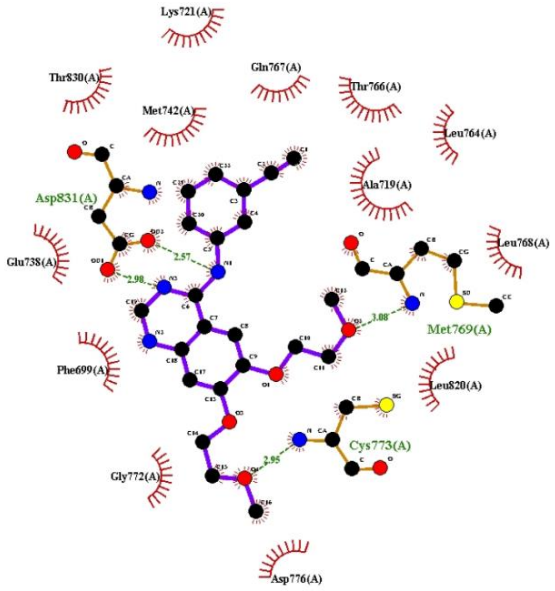


Figure 2. Docked conformation of erlotinib in the binding site of EGFR. Hydrogen bonds are shown by green dashed line.

Table7. Energy-based interactions for 3,4,7,8-tetrahydroquinazoline-2,5-(1*H*,6*H*)-dione derivatives docked into EGFR

]Compound	Estimated free energy of binding (kcal/mol)	Hydrogen bond
4a	-6.74	Asp831(2.75Å)
4b	-6.59	Asp831(2.74Å)
4c	-6.63	-
4d	-6.68	-
4e	-6.90	Thr830(2.90Å)
4f	-5.90	Asp831(2.75Å)
4g	-6.59	-
4h	-7.37	Asp831(2.75Å)
4i	-6.72	-
4j	-6.30	Met769(3.10Å) – Asp831(2.46Å)
4k	-6.15	Lys721(2.70Å) – Arg817(2.94Å)
4m	-6.76	Met769(3.08Å) - cys773(2.95Å)
Erlotinib	-6.92	Asp831 (2.98Å) – Met769(3.08Å) – cys773(2.95Å)

These compounds also significantly stabilize the EGFR through hydrophobic contacts with Lys721, Glu738, Phe699, Val702, Met742, and Leu820. The ligands were embedded in the hydrophobic pocket. In all the synthesized compounds, it is clear that the hydrophobic pocket of the inhibitor binding site is occupied by quinazolinone along with the substituent groups on these rings. The docking protocol used in this study was validated by docking of erlotinib as a known inhibitor to the energy minimized EGFR protein. The residues Asp831, Met769, and cys773 are important in making hydrogen bond [21]. Most of the compounds in this study also showed a strong hydrogen bond with Asp831. The highest dock score in docking protocol for these series was -7.37 kcal/mol for compound **4h**. Rest of the molecules showed a proper dock scores ranging from -5.90 to -7.37 kcal/mol. Thus, the binding model reported here, suggests that these tetrahydroquinazoline derivatives behave as EGFR inhibitors and show some key structural points to be considered in future optimization.

Conclusion

The work reported here involved an effective, easy, and rapid procedure which has been developed for the preparation of tetrahydroquinazolines using dimedone, aryl aldehyde, and urea in the presence of $CaCl_2.2H_2O$ under solvent- free conditions. This simple procedure, short reaction times and excellent yields of the products make this protocol as an attractive approach to other routes for the synthesis of new tetrahydroquinazolines. All of the synthesized compounds showed moderate cytotoxicity activity against MCF-7 cell line. Especially, Compound **4h** was the most potent compound. In the docking experiments, compound **4h** exhibited the docking $\Delta G_{binding}$ value lower than erlotinib. This study indicates that tetrahydroquinazoline derivatives could be a suitable scaffold for EGFR inhibitors.

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