Indian Journal of Chemistry
Vol. 60B, February 2021, pp. 267-272

Design, synthesis of 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-phenyl/methyl-3,4-dihydroquinolin-2(1*H*)-one derivatives and evaluation of their *in vitro* tyrosine kinase inhibitor activity

Viveka Fonseca[a], Sachin Chandavarkar*[b], Renuka Dabholkar[a], Prachita Gauns Dessai[a], Mangirish Deshpande[c] & Shivalingrao N Mamle Desai[a]

[a] Department of Pharmaceutical Chemistry, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda, Goa 403 401, India

[b] Department of Pharmacognosy, ASPM College of Pharmacy, Sangulwadi 416 810, India

[c] Department of Pharmacology, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda, Goa 403 401, India

E-mail: chandsachin@gmail.com; smamledesai@rediffmail.com

Received 14 March 2020; accepted (revised) 19 January 2021

The present investigation deals with molecular docking, synthesis, characterization, and evaluation of *in vitro* tyrosine kinase inhibitor activity of a series of 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-phenyl/methyl-3,4-dihydroquinolin-2(1*H*)-one derivatives {III-a(1-12)/III-b(1-12)}. Molecular docking studies of the title compounds were carried out using Molegro Virtual Docker (MVD-2013, 6.0) software. The MolDock scores of the derivatives ranged from (−66.508) to (−101.274); whereas the MolDock score of standard 4-anilinoquinazoline ligand was found to be (−105.219). Most of the synthesized qunolin-2-one derivatives showed better affinity towards EGFRK protein as compared to standard drug imatinib (−104.253). All the synthesized compounds were satisfactorily characterized by physical and spectral analysis (UV, IR, ^{1}H NMR and ^{13}C NMR and mass spectral data). Twelve derivatives were tested for their *in vitro* tyrosine kinase inhibitor activity using MDA-MB cell line. Compound 4-[2-(4-bromophenyl)hydrazono]-3-(1-hydroxyethyl)-1- methyl-3,4-dihydroquinolin-2(1*H*)-one (III-b4) was found to be the most cytotoxic compound as compared to other synthesized derivatives, with IC_{50} value of 0.0515 μM against MDA- MB cell line.

Keywords: Quinolin-2-one, anticancer, MDA-MB cell line, Molegro Virtual Docker, EGFRK protein

Cancer is the second leading cause of death after heart disease in men, but is the leading cause of death in women of every age group[1]. Cancer is responsible for an estimated 9.6 million deaths in 2018[2]. Globally, about one in six deaths is due to cancer[2]. Around 1.7 million new cases *i.e.* around 4600 cases per day were projected for the year 2019[1]. Although current use of chemotherapeutic agents has resulted in reduction of mortality and morbidity among the cancer patients; the major drawback of these anticancer drugs is its high toxicity and non-specific targeting[3]. These drawbacks along with drug resistance are a major challenge in the treatment of this disease thus; discovery of target selective anticancer agents is the need of the hour[3].

The quinolin-2-one structure is characteristic of a number of natural products and synthetic analogues. Clinically used quinolone derivatives have a wide therapeutic importance such as carteolol with β-blocker (used in ophthalmic preparations), camptothecin with antitumor, vesnarinone with cardiotonic, rebapimide with antiulcer and antioxidant, aripiprazole with antipshychotic, and brexpipriprazole with antidepressant activity[4-6]. Quinolones are amongst the most popular N-heteroaromatic compounds and are of considerable interest clinically since their discovery. Research on 2-quinolone, led to a discovery of 4-hydroxyquinolin-2(1*H*)-ones which was found to be a fundamental ring system of a large number of alkaloids of Rutaceae family[7]. 3-Substituted-4-hydroxy quinolin-2-one is an important moiety which was found in a number of compounds with interesting antitumor activity. This structural key feature encouraged and attracted the interest of several research groups.

One of the clinically important analogues of quinolin-2-one derivatives is Linomide[8,9]. Linomide, IUPAC name (4-hydroxy-N, 1-dimethyl-2-oxo-N-phenyl-1, 2-dihydroquinolin-3- carboxamide), a synthetic immunomodulator (withdrawn from III-stage of clinical trials), protects animals against a variety of experimental autoimmune diseases. The structure of linomide has served as the prototype for the synthesis of a variety of analogues in an effort to optimise this lead compound. In present study, the

carbonyl group present at the acetyl side chain of the 3rd position of 3-acetyl-4-hydroxy-1-phenyl/methyl quinolin-2(1*H*)-one was reduced to an (±)-4-hydroxy-3-(1-hydroxyethyl)-phenyel/methylquinolin-2(1*H*)-one. The purpose of reduction of carbonyl group to its hydroxyl derivative was to avoid interference of the former group in the reaction with the substituted phenylhydrazines.

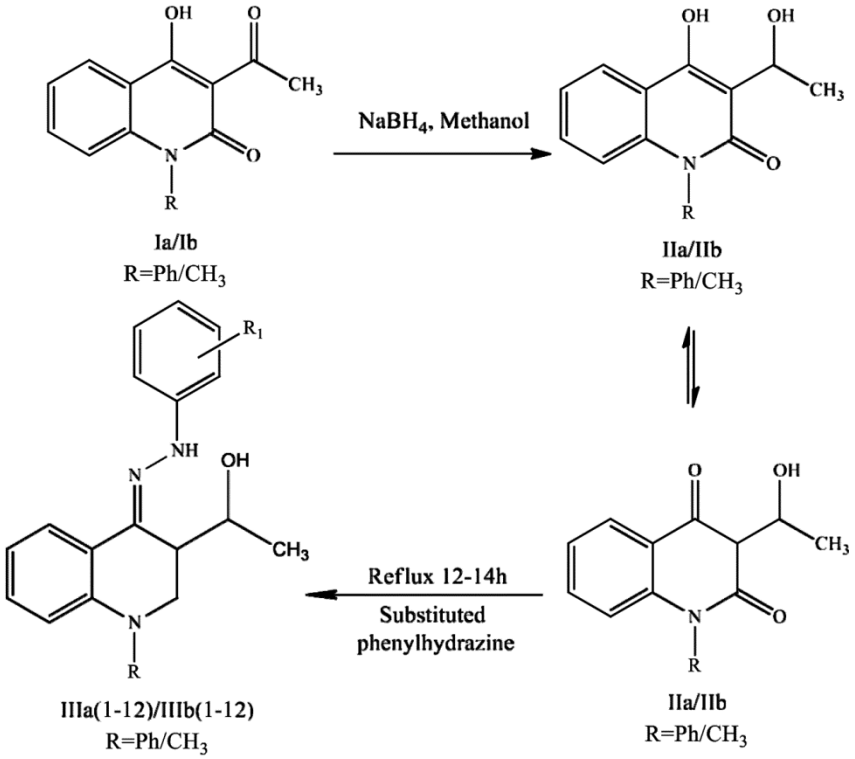
Results and Discussion

The starting material, 3-acetyl-4-hydroxy-1-phenyl/methylquinolin-2(1*H*)-one [Ia/Ib] was synthesized following the literature[10]. Reduction of compound [I-a/I-b] using sodium borohydrate gave 4-hydroxy-3-(1-hydroxyethyl)-1phenyl/methyl quinolin-2(1*H*)-one [II-a/II-b]. Further treatment on [II-a/II-b] with substituted aromatic phenylhydrazines with acetic anhydride gave 4-[2-(substituted phenyl)hydrazono]-3-(1-hydroxyethyl)-1-phenyl/methyl-3,4-dihydroquinolin-2(1*H*)-one[III-a(1-12)/III-b(1-12)] (Scheme I).

The physical data of all synthesized compounds is given in Table I and Table II. All the synthesized compounds were characterized by physical and spectral analysis. The *in vitro* tyrosine kinase inhibitor activity of twelve derivatives was performed by MTT assay on MDA- MB cell line. Compound 4-[2-(4-bromophenyl)hydrazono]-3-(1-hydroxyethyl)-1-methyl-3,4- dihydroquinolin-2(1*H*)-one (III-b4) was found to be the most cytotoxic as compared to other derivatives with IC_{50} value of 0.0515 μM. Compounds (III-a2), (III-a4) and (III-b2) had less potent activity with IC_{50} value of 0.0641 μM, 0.0555 μM, and 0.0764 μM respectively. Compounds (III-a3), (III-a5), (III-a6) and (III-b1) which had an IC_{50} value of 0.0756 μM, 0.0808 μM, 0.072 μM, and 0.0873 μM respectively also showed less potent activity. The least activity was seen in compounds (III-a1), (III-a7), (III-b3) and (III-b10) with IC_{50} value of 0.1232 μM, 0.1297 μM, 0.1495 μM, and 0.1322 μM respectively.

Molecular Docking studies

Epidermal Growth Factor Receptor tyrosine kinase (EGFRK) was selected as the target enzyme for the designed molecules. Protein kinase inhibitors represent an important and still emerging class of targeted therapeutic agents. Drug discovery and development strategies have explored numerous approaches to target the inhibition of protein kinase signaling[11]. Tyrosine kinases are an especially important target because they play an important role in the modulation of growth factor signaling[12]. Tyrosine kinase inhibitors (TKIs) compete with the ATP binding site of the catalytic domain of several oncogenic tyrosine kinases[12]. TKIs such as imatinib mesylate, gefitinib, erlotinib, iapatinib,



Scheme I — Synthesis of 4-[2-(substituted phenyl)hydrazono]-3-(1-hydroxyethyl)-1phenyl/methyl-3,4-dihydroquinolin-2(1*H*)-one

Table I — Physical data of compounds 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-phenyl-3,4-dihydroquinolin-2 (1*H*)-one {III-a(1-12)}

Compd	R	R_1	Mol. formula	Mol. Wt.	Yield (%)	m.p. (°C)	R_f value
III-a1	$-C_6H_5$	-3Cl	$C_{23}H_{20}ClN_3O_2$	405.88	88.51	72-4	0.78
III-a2	$-C_6H_5$	-3F	$C_{23}H_{20}FN_3O_2$	389.42	82.39	98-2	0.75
III-a3	$-C_6H_5$	-4CN	$C_{24}H_{20}N_4O_2$	396.44	80.31	76-9	0.78
III-a4	$-C_6H_5$	-4Br	$C_{23}H_{20}BrN_3O_2$	450.33	78.51	86-8	0.82
III-a5	$-C_6H_5$	-H	$C_{23}H_{21}N_3O_2$	371.43	90.21	110-2	0.81
III-a6	$-C_6H_5$	$-3NO_2$	$C_{23}H_{20}N_4O_4$	416.43	80.58	122-2	0.73
III-a7	$-C_6H_5$	$-2CH_3$	$C_{24}H_{23}N_3O_2$	385.46	71.09	161-3	0.69
III-a8	$-C_6H_5$	$-3CH_3$	$C_{24}H_{23}N_3O_2$	385.46	84.94	140-2	0.65
III-a9	$-C_6H_5$	$-4CH_3$	$C_{24}H_{23}N_3O_2$	385.46	91.78	180-4	0.90
III-a10	$-C_6H_5$	-2,4-Cl	$C_{23}H_{19}Cl_2N_3O_2$	440.32	77.96	145-7	0.50
III-a11	$-C_6H_5$	$-2,4-CH_3$	$C_{25}H_{25}N_3O_2$	399.48	87.50	175-7	0.88
III-a12	$-C_6H_5$	$-2,4-NO_2$	$C_{23}H_{19}N_5O_6$	461.43	92.48	135-6	0.78

Table II — Physical data of compounds 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-methyl-3,4-dihydroquinolin-2 (1*H*)-one {III-b(1-12)}

Compd	R	R_1	Mol. formula	Mol. Wt.	Yield (%)	m.p. (°C)	R_f value
III-b1	$-CH_3$	-3Cl	C18H18ClN3O2	343.81	95.45	138-9	0.79
III-b2	$-CH_3$	-3F	C18H18FN3O2	327.35	93.68	136-8	0.87
III-b3	$-CH_3$	-4CN	C19H18N4O2	334.37	88.52	140-4	0.77
III-b4	$-CH_3$	-4Br	C18H18BrN3O2	388.26	80.94	133-4	0.83
III-b5	$-CH_3$	-H	C18H19N3O2	309.36	75.47	132-5	0.77
III-b6	$-CH_3$	$-3NO_2$	C18H18N4O4	354.36	78.98	120-6	0.87
III-b7	$-CH_3$	$-2CH_3$	C19H21N3O2	323.39	70.32	110-8	0.79
III-b8	$-CH_3$	$-3CH_3$	C19H21N3O2	323.39	82.45	1424	0.75
III-b9	$-CH_3$	$-4CH_3$	C19H21N3O2	323.39	90.18	146-7	0.71
III-b10	$-CH_3$	-2,4-Cl	C18H17Cl2N3O2	378.25	76.29	174-8	0.50
III-b11	$-CH_3$	$-2,4-CH_3$	C20H23N3O2	337.18	93.15	196-8	0.90
III-b12	$-CH_3$	$-2,4-NO_2$	C18H17N5O6	399.36	82.53	182-4	0.88

etc. have a lot of clinical significance. First, in many tumor types they tend to stabilize tumor progression and may create a chronic disease state which is no longer immediately life threatening. Second, side effects are minimal when compared to conventional chemotherapeutic agents. Third, synergistic effects are seen *in vitro* when TKIs are combined with radiotherapy and/or conventional chemotherapeutic agents[13]. Molecular docking studies of the synthesized compounds were performed using Molegro Virtual Docker (MVD-2013, 6.0) software[14]. Following the literature, imatinib was used as the reference standard for docking[12]. Docking of the synthesized compounds on EGFR tyrosine kinase enzyme exhibited well-conserved hydrogen bonds with one or more amino acid residues at the active binding site. The highest MolDock score exhibited by compound (III-a12) was (-101.274) comparable to that of the standard ligand 4-anilinoquinazoline (-105.219) and imatinib (-104.259). Most of the synthesized novel analogues of quinolin-2-one exhibited better affinity towards EGFRK protein than imatinib[12]. The crystal structure of the target enzyme including forty amino acids from the carboxyl-terminal tail has been determined to 2.6-A resolution. Unlike any other kinase enzymes, the EGFR family members possess constitutive kinase activity without a phosphorylation event within their kinase domains. Despite its lack of phosphorylation, the EGFRK activation loop adopts a conformation similar to that of the phosphorylated active form of the kinase domain from the insulin receptor. It is observed that the key residues of a dimerised structure lying between the EGFRK domain and carboxyl-terminal substrate docking sites are found in close contact with the kinase domain[11].

The site at which the known 4-anilinoquinazoline inhibitor binds with the target protein was selected as the active site (Figure 1). It is lined with amino acid residues such as Met769, Thr830, Asp831, Lys721, Cys773, Pro770, Glu738, Gln767, *etc*. Hence to identify other residual interactions, a grid box (include residues within a 15.0 Å radius) large enough to

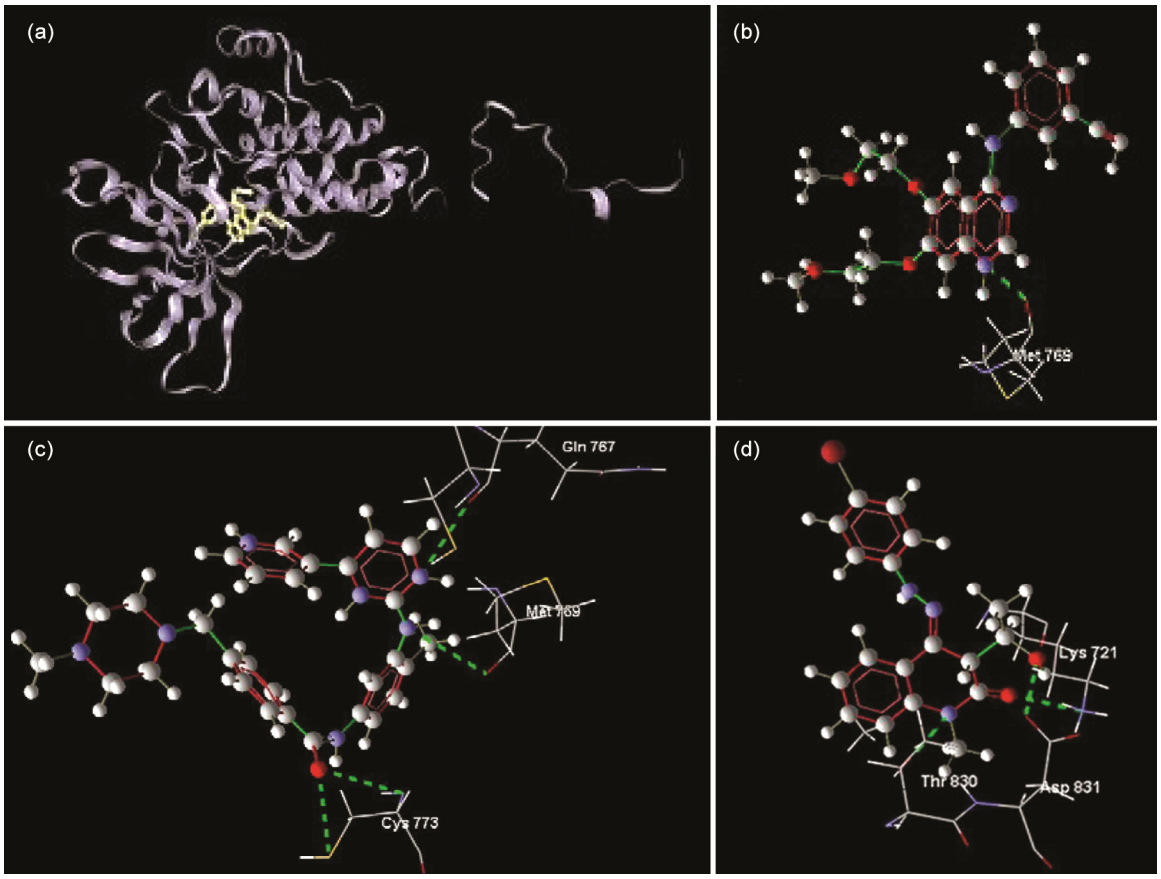


Figure 1 — (a) Structure of EGFR-tyrosine kinase domain complexed with 4- anilinoquinazoline inhibitor (PDB ID: 1m17); (b) Ligand 4-anilinoquinazoline docked in its best conformation into the binding site of 1m17. The –NH at 1st of the quinazoline moiety shows hydrogen bonding interaction with oxygen of Met 769; (c) Imatinib docked in best of its conformation into the binding site of 1m17. One of the –NH of the pyrimidine ring system forms one hydrogen bond with the oxygen of Gln 767. The –N at the amino linkage between pyrimidine and *o*-methyl substituted phenyl ring system forms hydrogen bond with oxygen of Met 769. The oxygen of the peptide linkage forms two hydrogen bonds with –SH and –NH of Cys 773; (d) Compound 4-[2-(4-bromo-phenyl)hydrazono]-3-(1-hydroxyethyl)-1-methyl-3,4- dihydroquinolin-2(1*H*)-one (III-b4) (III-b4): The –N at 1st position of the quinolone moiety forms hydrogen bond with –OH of Thr 830. The oxygen at 2nd position of the quinolone moiety forms hydrogen bond with –NH of Lys 721. The –OH at the side chain (at position 3) of the quiolin-2-one moiety forms hydrogen bond with –O of Asp 831.

accommodate the active site was constructed. Since 4-anilinoquinazoline is a known inhibitor, the centre of the binding site of this ligand was considered as the centre of search space for docking. The MolDock scores of the derivatives ranged from (−66.508) to (− 101.274); whereas the MolDock score of standard 4-anilinoquinazoline ligand was found to be (−105.219). Imatinib was used as the reference standard which exhibited MolDockK score of (−104.253). The docking results of the best poses of compounds are summarised in Table III.

Experimental Section

The reagents and chemicals were purchased from SD Fine-Chem Limited, Mumbai and Molychem, Mumbai. Melting points of synthesized compounds were determined by Thiel's melting point apparatus and are uncorrected. λ_{max} was recorded on UV-1800 Shimadzu UV Spectrophotometer. FT-IR spectra were recorded on Shimadzu IR AFFINITY-1 spectrophotometer by using KBr pellets. The ^{1}H and ^{13}C NMR was recorded on Bruker Advance II 400 NMR Spectrometer by using CDCl3 or DMSO-d_6 as solvent and TMS as internal standard, chemical shifts are expressed as δ values (ppm). The Mass spectra were recorded on Waters, Q-TOF Micromass (LC-MS).

General procedure for the synthesis of 3-(1-hydroxyethyl)-4-hydroxy-1-phenyl/methyl- quinolin-2(1*H*)-one {II-a/II-b}

The suspension of the 3-substituted-4-hydroxyquinolin-2-one Ia/Ib (1mmole) in methanol (5 mL) was cooled to 0°C, a solution of sodium borohydride (3.7 mmol) in ethanol was added dropwise

Table III — Docking results of of 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-phenyl/methyl-3,4-dihydroquinolin-2 (1*H*)-one derivatives

Compd	MolDock Score	Rerank Score	H-Bond	Compd	MolDock Score	Rerank Score	H-bond
III-a1	−88.7932	−71.2242	−2.5	III-b2	−85.2209	−17.0946	−5.98031
III-a2	−88.8587	−71.0985	−2.5	III-b3	−79.4644	−59.391	−2.86824
III-a3	−86.4336	−13.6673	−4.84744	III-b4	−78.2007	−68.6968	−4.29864
III-a4	−85.6434	−69.9135	−2.5	III-b5	−70.1892	−63.2473	−2.5
III-a5	−66.5082	6.05593	−2.5	III-b6	−73.8145	−10.4383	−7.69484
III-a6	−97.2011	−78.3445	−8.71635	III-b7	−75.4982	−66.4725	−4.48108
III-a7	−91.711	−74.3248	−2.5	III-b8	−67.133	−39.3542	−3.51184
III-a8	−88.8456	−71.9322	−2.5	III-b9	−81.1738	−70.3943	−4.30778
III-a9	−87.1244	−71.0905	−2.5	III-b10	−78.5617	−65.4074	−2.40001
III-a10	−97.0357	−75.4252	−2.5	III-b11	−84.9681	−72.7725	−4.0689
III-a11	−95.424	−76.4441	−2.5	III-b12	−92.7837	−73.0288	−8.56848
III-a12	−101.274	−78.1923	−9.7562	Imatinib	−104.253	−37.0559	−3.55828
III-b1	−77.536	−67.8361	−3.16219	4-Anilinoquinazoline (Active Ligand)	−105.219	−67.2192	−6.53953

till the suspension becomes a clear solution. To the clear solution, a mixture of *n*-hexane and diethyl ether (1:1, 15 mL) was added. The solvents were removed by using rota evaporator (IKA make) and the residue was dried and purified by column chromatography on silica gel eluting with hexane and ethyl acetate 5:1and recrystallized from methanol.

Spectral data of the compounds

4-Hydroxy-3-(1-hydroxyethyl)-1-phenylquinolin-2(1*H*)-one II-a: UV-Vis: λ_{max} = 234 nm; IR (KBr): 3469.94 (-OH); 3062.96 (aromatic -C-H); 2918.30 cm^{-1} (aliphatic-C-H str.); 1597.06 (-C=O amide); ^{1}H NMR (DMSO-d_6): δ 17.1 (s, 1H, -OH); 8.2-6.4 (m, 9H, ArH); 3.9 (t, 1H, -CHOH); 3.3 (s, 1H, -OH of CHOH); 2.6 (s, 3H, -CH_3).

4-Hydroxy-3-(1-hydroxyethyl)-1-methylquinolin-2(1*H*)-one II-b: UV-Vis: λ_{max} = 231.70 nm; IR (KBr): 3469.4 (-OH); 3062.96 (aromatic -C-H); 2613.5 (aliphatic -C-H str.); 1691.57 cm^{-1} (-C=O amide); ^{1}H NMR ($CDCl_3$): δ 16.79 (s, 1H, -OH); 8.1-7.0 (m, 4H, ArH); 3.75(t, 1H, -CHOH); 3.72(s, 3H, -N-CH_3); 3.5 (s, 1H, -OH of CHOH); 2.7 (s, 3H, CH_3).

General procedure for the synthesis of 4-[2-(substituted phenyl)hydrazono]-3-(1- hydroxyethyl)-1phenyl/methyl-3,4-dihydroquinolin-2(1*H*)-one { III-a(1-12)/III-b(1-12)}

To a solution of II-a/ II-b (3mmoles), substituted phenylhydrazine (3.6 mmoles) and acetic anhydride (0.5 mL) in methanol (25 mL) was refluxed for 12-14 hours. The solvent was removed under reduced pressure using rotaevaporator (IKA make). The resulting solid was washed with cold water and recrystallized using methanol. The purity of all the newly synthesized compounds was ascertained by TLC using ethyl acetate: benzene in the ratio 1:3 as the mobile phase, silica gel G as stationary phase and iodine vapours as visualizing agent.

Spectral data of synthesized compounds

4-[2-(3-Chlorophenyl)hydrazono]-3-(1-hydroxyethyl)-1-phenyl-3,4-dihydroquinolin-2(1*H*)- one (III-a1): UV-Vis: λ_{max} = 288.20 nm IR (KBr): 3460.3 (-OH); 3292.49 (-NH); 3064.89 (aromatic C-H); 3010.88 (aliphatic-C-H str.); 1658.78 (-C=N); 1595.13 (-C=O amide); 773.46 cm^{-1} (CCl); ^{1}H NMR (DMSO-d_6): δ 9.6 (s, 1H, N-H); 8.0-6.5 (m, 13H, ArH); 3.4 (quintet, 1H, - CHOH); 3.19 (s, 1H, OH of CHOH); 2.5 (d, 1H, -CH aliphatic); 1.9 (d, 3H, -CH_3); ^{13}C NMR ($CDCl_3$): δ 169.20 (1C, C=O amide); 150.73 (1C,C=N); 133.59-110.61(18C, aromatic carbon); 61.46 (1C, CHOH); 52.20 (1C, CH aliphatic); 20.46 (1C, CH_3). MS: *m/z* 407 [M^{+1}].

4-(2-(3-Chlorophenyl)hydrazono)-3-(1-hydroxyethyl)-1-methyl-3,4-dihydroquinolin-2(1*H*)- one (III-b1): UV-Vis: λ_{max} = 316 nm; IR (KBr): 3620.39 (-OH); 3290.56(-NH); 3010.88, 2926.01(aromatic -C-H); 2378.23, 2316.51 (aliphatic-C-H str.); 1658.78 (-C=N); 1595.13 (-C=O amide); 771.53 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 9.6 (s, 1H, N-H); 8.9- 6.6 (m, 8H, ArH); 3.7-3.6 (quintet, 1H, CHOH); 3.5(s, 1H, OH of CHOH); 3.2 (s, 3H, N-CH3); 2.5 (d, 1H, -CH aliphatic); 1.9 (d, 3H, - CH3); ^{13}C NMR ($CDCl_3$); δ 169.15 (1C, C=O amide); 150.75(1C, C=N); 133.58-110.61(12C, aromatic carbon); 60.0(1C, CHOH); 50.6 (1C, CH aliphatic); 36.1 (1C, N-CH_3); 20.50 (1C, CH_3); MS: *m/z* 345 [M^{+1}].

Biological activity[15]

The selected 4-[2-(substituted phenyl) hydrazono]-3-(1-hydroxyethyl)-1-phenyl/methyl-3,4-dihydroquin-

Table IV — Cell viability(%) of synthesized compounds on MDA-MB (Human mammary gland) cell line

Conc (μg/mL)	III-a1	III-a2	III-a3	III-a4	III-a5	III-a6	III-a7	III-b1	III-b2	III-b3	III-b4	III-b10
10	70.53	55.2	63.59	53.12	70.22	59.24	60	68.52	58.42	63.28	72.30	71.36
20	65.8	51.36	62.27	50.98	60.95	57.29	58.86	68.01	55.58	59.24	49.46	69.53
25	62.4	49.21	57.85	49.78	50.98	51.48	57.85	58.42	43.15	58.3	48.14	59.24
30	56.97	43.15	49.59	49.15	38.11	46.69	56.53	43.79	41.45	52.37	42.59	53.63
50	42.71	29.65	40.76	41.32	37.79	41.51	49.9	42.78	39.87	44.92	37.48	41.77
Control	100	100	100	100	100	100	100	100	100	100	100	100

Table V — IC50 values of all the tested compounds

Compd	III-a1	III-a2	III-a3	III-a4	III-a5	III-a6	III-a7	III-b1	III-b2	III-b3	III-b4	III-b10
IC50 (μM/mL)	0.123	0.064	0.076	0.056	0.081	0.072	0.13	0.087	0.076	0.15	0.052	0.132

olin-2(1*H*)-one derivatives were tested for their *in vitro* tyrosine kinase inhibitor activity against MDA-MB-Human mammary gland cell line by the following method.

(i) [3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) Assay: MTT solution preparation (stock solution): 5 mg in 1 mL of phosphate buffered saline (PBS). (*p*H- 7.4).

(ii) *In vitro* growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells.

50 μL of 1×10^5 cells/mL cell suspension was seeded into each well in a 96 well micro titer plate and final volume was made upto 150 μL by adding Dolbecco's Modified Eagle's Medium (DMEM) media. 100 μL of test compounds of different concentrations (10, 20, 25, 30 and 50 μg/mL) was added to the wells and incubated for 24 hours, in presence of 5% CO_2, at 37°C into CO_2 incubator. After 24 hours, 20 μL of 5 mg/mL MTT reagent was added to the wells. The plate was kept for 4 hours incubation in dark place at room temperature. The plate was covered with aluminium foil, since MTT reagent is photosensitive. The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100 μL of DMSO was added to dissolve the crystals formed. The optical density (OD) was measured at wavelength of 570 nm. The study was performed in triplets and the result represents the mean of three readings using the following formula:

Surviving cells (%) = (Mean OD of test compound/Mean OD at control) × 100 Inhibiting cells (%) = 100-Surviving cells

The cell viability of the title compounds are given in Table IV and IC_{50} values of the tested compounds are given in Table V.

All the compounds tested, showed potent tyrosine kinase inhibitor activity at different concentrations.

Conclusion

The *in vitro* tyrosine kinase inhibitor activity of twelve selected compounds was carried out against MDA-MB cell line based upon MTT assay method. From the results obtained, all the selected compounds showed activity compared to the control. The active compound III-b4 showed three interactions with amino acids Thr830, Lys 721, Asp 831 residues of the protein EGFR tyrosine kinase and the compound was most potent with an IC_{50} value of 0.0515μM. However, it did not show good MolDock score compared to the standard interaction of imatinib.

Reference

1 Siegel R L, Miller K D & Jemal A, *CA Cancer J Clin*, 69 (2019).
2 WHO, Cancer, Sep 2018.
3 Soares A, Mamle Desai S, Tiwari P, Palkar M & Shingade S, *Indian J Chem*, 58B (2019) 1167.
4 Afzal O, Kumar S, Haider R, Ali R, Kumar R & Jaggi M, *Eur J Med Chem*, 97 (2015).
5 Triandquand C, Romanet J P & Allaire C, *J Fr Opthalmol*, 26 (2003) 131.
6 De Fruyt J, Deschepper E Audenaert K, Constant E, Flori M & Pitchot W, *J Psychopharmacol*, 26 (2012) 603.
7 Thomas K, *Il Farmaco*, 54 (1999) 309.
8 Bianca d'Al, Claudia R B G & de Izabel C P, *Bioorg Med Chem Lett*, 16 (2006) 1010.
9 Jiandong S, Zili X, Michael A I, Chandrashekhar K, Bulbul P & Zhigen H, *Bioorg Med Chem Lett*, 13 (2003) 1187.
10 Peter R, Werner F & Wolfgang S, *J Heterocycl Chem*, 29 (1992) 225.
11 Grant S K, *Cell Mol Life Sci*, 66 (2009).
12 Arora A & Scholar E M, *J Pharmacol Exp Ther*, 315(3) (2005) 971.
13 Steeghs N, Nortier J W & Gelderblom H, *Ann Surg Oncol*, 15(2) (2007) 942.
14 Palkar M B, Singhai A S, Ronad P M, Vishwanathsamy A, Boreddy T S & Veerapur V P, *Bioorg Med Chem*, 29 (1992) 225.
15 Rashad F M, Abd Ei-Nasser N H, Dawoud I F & Motawe F H, *Res J Pharm Biol Chem Sci*, 6(2) (2015) 1919.