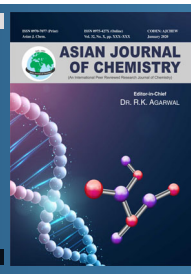
Asian Journal of Chemistry; Vol. 32, No. 12 (2020), 3067-3074

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22930



Design, Synthesis and Molecular Modelling Studies of 1-Methyl-3-(4-Substituted phenyl-1,3-thiazol-2-yl)-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-ones as Potent Anticancer Agents

NIZAMUDDIN NAGALADINNE[1,*], ABDUL AHAD HINDUSTAN[2] and DEVANNA NAYAKANTI[3]

[1]Department of Research and Development, Jawaharlal Nehru Technological University Anantapur, Anathapuramu-515001, India
[2]Department of Industrial Pharmacy, Raghavendra Institute of Pharmaceutical Education & Research, Ananthapuramu-515721, India
[3]Department of Chemistry, Oil Technological & Pharmaceutical Research Institute (OTPRI)-Jawaharlal Nehru Technological University Anantapur, Anathapuramu-515001, India

*Corresponding author: E-mail: nnizamuddin1988@gmail.com

Received: 5 August 2020; *Accepted*: 16 September 2020; *Published online*: 7 December 2020; AJC-20141

The present study involves the design, synthesis, characterization and molecular docking studies of biologically active quinazolin-4-ones, which were synthesized by condensing 2-amino-4-substituted phenylthiazole with *N*-methylbenzoxazin-4-one. The *N*-methylbenzoxazin-4-one and 2-amino-4-substituted phenylthiazole were synthesized from *N*-methylanthranilic acid and substituted ketones, respectively. The ADME properties determined the synthetic accessibility of quinazolin-4-ones by *in silico* Swiss ADME. The colorectal anticancer screening was done by using cell HT-29 human colorectal adenocarcinoma based on molecular docking studies on 3GC7-the structure of p38alpha in complex with dihydroquinazolinone. Finally, compounds **5Dh8**, **5DF6**, **5Db2** and **5Di9** exhibited better activity at a concentration < 10 µg/mL when compared to 5-fluorouracil. The ADME properties revealed that all the compounds were within the range and docking studies showed the highest binding with glide score -7.19 and -7.027 Kcal/mol compared to the target protein -10.67 Kcal/mol.

Keywords: Quinazolin-4-ones, 2-Amino-1,3-phenylthiazole, Molecular docking, Colorectal anticancer activity.

INTRODUCTION

In the synthesis of quinazolin-4-one derivatives, several ameliorations were made, which lead to the synthesis of several new derivatives and claimed for Intellectual Property Rights. Febrifugine (known as Chan-San′s alkaloid) was the first compound isolated, which possessed a quinazolin-4-one skeleton [1]. Most of these compounds have been tested for their pharmacological, herbicidal, biocidal and other properties. At present, quinazolin-4-one derivatives exhibit wide variety of activities like antitubercular [2], antimalarial [3], antimicrobial [4], antioxidant [5] and analgesic [6]. Besides, triazolo-quinazoline system possesses multiple biological activities such as antioxidant & antifungal [7], anticonvulsant [8], antimicrobial [9], and anticancer [10] activities. This prompted us to synthesize, using conventional synthesis, a new series of 3-(4-substituted phenyl-1,3-thiazol-2-yl)-2-(pyridin-3-yl)-2,3-dihydroquinazoline-4(1*H*)-one derivatives by incorporating the phenyl thiazole moiety at the third position of the quinazolinone nucleus.

The findings of new structures with potential chemotherapeutic activities, a new series of 1-methyl-3-(4-substituted phenyl-1,3-thiazol-2-yl)-2-(pyridin-3-yl)-2,3-dihydroquinazoline-4(1*H*)-one have been synthesized and screened for antitumor activity. These sequences comprise derived 2,3-disubstituted quinazolinone pharmacophores which are structurally related to ispinesib and nolatrexed. The efforts in the derivatization of such types of compounds focused on the aryl moiety of the 4-substituted quinazoline.

In this study, the substitution pattern at 2,3-disubstituted quinazolin-4-one pharmacophore was selected to confer different structure environments that would affect the lipotropic and activity of the target molecules. The purpose of forming these hybrids is an attempt to reach a vigorous antitumor agent with enhancing activity and selectivity toward cancerous cells. Drug-likeness and molecular docking (MD) methodology were used to identify the structural mark required for antitumor activity of these new series. The results of molecular docking could support the postulation that the present compounds may

act on the same enzyme target where mitogen-activated protein kinase (MAPK) inhibitors work confirming the molecular design of the reported class of antitumor agents [11].

EXPERIMENTAL

The chemicals and reagents used in this work were of L.R. grade, procured from AVRA chemicals, Hyderabad, India. Melting points were determined in open capillary tubes and are uncorrected. Compounds were checked for their purity by TLC on silica gel G plates and spots identified by iodine vapours. The infrared spectra of synthesized compounds were recorded in the range of 4000-400 cm^{-1} on FTIR Bruker 8400, Shimadzu. The ^1H & ^{13}C NMR spectra were recorded on Bruker-Avance III 500 MHz NMR spectrophotometer (BIOSPIN) in $CDCl_3$ solvent and tetramethylsilane as an internal standard. Mass spectra were recorded on the Jeol GCMATE II GC-MS instrument at 70 eV.

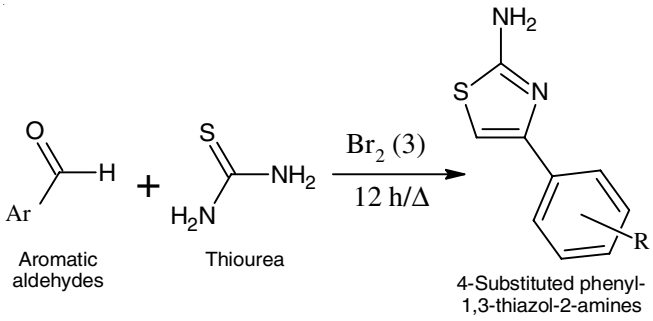
Synthesis of 4-substituted phenyl-1,3-thiazol-2-amines (4a1-4k11): To a mixture of substituted aromatic aldehydes (0.1 mol) and thiourea (15 g, 0.2 mol), in 100 mL of ethanol, bromine (15.98 g, 0.2 mol) was added dropwise. Addition of bromine resulted in a reaction mixture hot, so about 50 mL water was added and the heated reaction mixture until all the solid dissolved. The reaction mixture was filtered while hot and allowed to cool the filtrate. It was made alkaline with conc. NH_4OH to separate 2-amino-4-substituted phenyl-1,3-thiazole [12]. The product was purified, sponged with alcohol and dried over P_2O_5. It was recrystallized from absolute ethanol as colourless needled shaped crystals (**Scheme-I**). Yield 84.2%, m.p.: 120-122 ºC. IR (KBr, ν_{max}, cm^{-1}): 3455 (NH_2), 3118 (CH of thiazole), 1630 (Ar C=C), 1333 (C=N), 700 (C-S).

Synthesis of 1-methyl-2-(pyridin-3-yl)-1,2-dihydro-4*H*-3,1-benzoxazin-4-one (3D): In a 250 mL round bottom flask, *N*-methyl anthranilic acid (9.06 g, 0.06 mol) dissolved in 100 mL of benzene containing 2-3 drops of pyridine and pyridine-3-carboxylic acid (7.38 g, 0.06 mol) was added in dry benzene under the cold conditions and then refluxed for 3 h, The solution was then allowed to cool, filtered and washed with petroleum ether, dried and recrystallized with a ethanol-acetone mixture (1:1) to get 1-methyl-2-(pyridin-3-yl)-1,2-dihydro-4*H*-3,1-benzoxazin-4-one (**Scheme-II**). The reaction was determined by TLC using cyclohexane:ethyl acetate (2:1) as mobile phase. Yield: 88%; m.p.: 162-164 ºC; IR (KBr, ν_{max}, cm^{-1}): 2991 (C-H *str.*), 1663 (C=O *str.*), 1328 (C-N *str.*), 1328 (C-O *str.* ether); MS: *m/z* 161.0 (M^+). Anal: calcd. (found) % for $_{C14}H_{12}N_2O_2$: C, 69.99 (69.77); H, 5.03 (5.23); N, 11.66 (13.88); O, 13.32 (11.32).

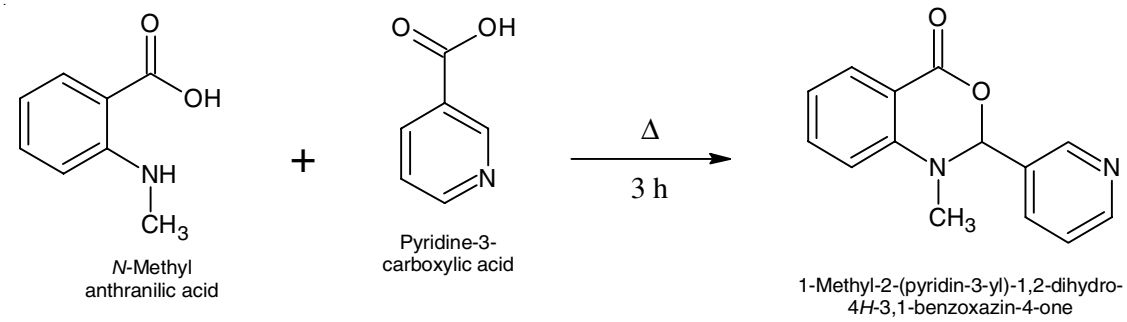
Synthesis of 1-methyl-3-(4-substituted phenyl-1,3-thiazol-2-yl)-2-(pyridin-3-yl)-2,3-dihydroquinazoline-4(1*H*)-one (5Da1-5Dk11): Equimolar portion of 1,2-dimethyl-1,2-dihydro-4*H*-3,1-benzoxazine-4-one (0.01 mol) and 4-substituted phenyl-1,3-thiazol-2-amine (**4a1-4k11**, 0.01 mol) was refluxed in presence of K_2CO_3 in 100 mL of dry ethanol for 4-5 h. The reaction mixture was filtered and excess ethanol was allowed to evaporate. The residue washed thoroughly with hot water and recrystallized from acetone:ethanol mixture (1:1) to yield title compounds (**5Da1-5Dk11**) (**Scheme-III**).

Compound 5Da1: Yield: 85%; m.p.: 210-215 ºC; R_f: 0.52; IR (KBr, ν_{max}, cm^{-1}): 2923 (C-H *str.*), 3072 (C-H arom.), 1650 (C=O *str.* in ring), 1588 (C=N *str.*), 1321 (C-N *str.*), 690 (C-S *str.* in ring); ^1H NMR (DMSO-d_6) δ ppm: 2.85 (s, 3H, CH_3), 6.01 (s, 1H, methine), 7.15-7.50 (m, 5H, Ar-H), 6.6 (s, 1H, thiazole), 6.55-7.99 (m, 4H, Ar-H), 7.12-8.95 (m. 4H, pyridine); ^{13}C NMR (DMSO-d_6) δ ppm: 32.9, 77.4, 100.0, 114.4, 118.4, 123.0, 127.5, 127.5, 128.8, 129.3, 129.3, 131.6, 133.1, 134.4, 147.4, 148.2, 149.1, 150.2, 160.4, 161.2: GC-MS: *m/z* 398 (M^+). Anal. calcd. (found) % for $C_{23}H_{18}N_4OS$ (m.w.: 398.48); C, 69.32 (69.32); H, 4.55 (4.45); N, 14.06 (14.06); O, 4.02 (4.04); S, 8.05 (7.90).

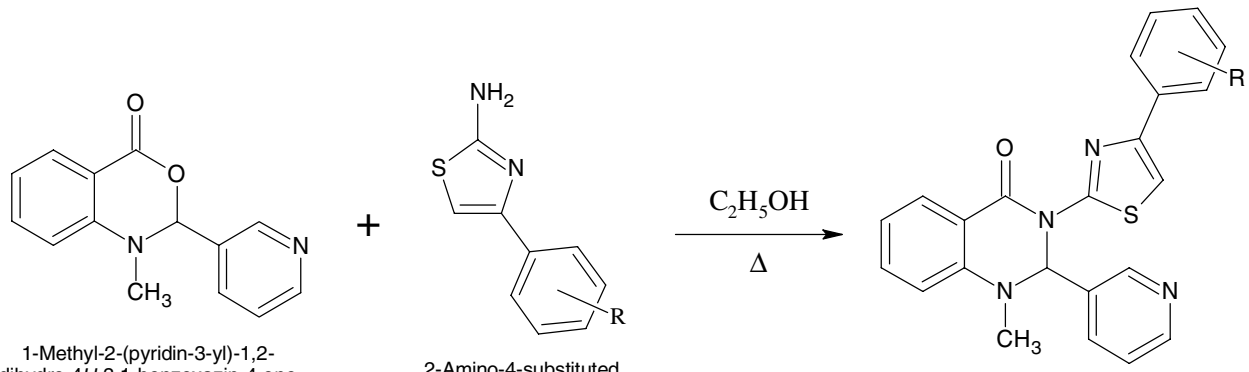
3-[4-(3-Aminophenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Db2): Yield: 80%; m.p.: 210-214 ºC; R_f: 0.36; IR (KBr, ν_{max}, cm^{-1}): 2991 (C-H arom.), 2911 (C-H *str.*), 1663 (C=O *str.* in ring), 1593 (C=N *str.*), 1326 (C-N *str.*), 692 (C-S *str.* in ring); ^1H NMR (DMSO-d_6) δ ppm: 2.95 (s, 3H, CH_3), 6.01 (s, 1H, methine), 6.32-7.17 (m, 4H, Ar-H), 6.4 (s, 1H, thiazole), 6.65-7.99 (m, 4H, Ar-H), 7.32-8.95 (m, 4H, pyridine), 4.2 (s, 2H, NH_2): ^{13}C NMR (DMSO-d_6) δ ppm: 32.7, 77.2, 99.8, 114.2, 114.4, 116.3, 117.5, 122.9, 127.5, 128.4, 130.1, 133.9, 134.2, 147.2, 148.0, 148.9, 149.1, 150.0, 159.9, 160.2: GC-MS: *m/z*



Scheme-I: Synthesis of 4-substituted phenyl-1,3-thiazol-2-amines



Scheme-II: Synthesis of 1-methyl-2-(pyridin-3-yl)-1,2-dihydro-4*H*-3,1-benzoxazin-4-one



Scheme-III: Synthesis of 1-methyl-3-(4-substituted phenyl-1,3-thiazol-2-yl)-2-(pyridin-3-yl)-2,3-dihydro quinazolin-4(1*H*)-one analogues

413 (M^+), Anal. calcd. (found) % for $C_{23}H_{19}N_5OS$ (m.w.: 413.49); C, 66.81 (66.71); H, 4.63 (4.63); N, 16.94 (16.84); O, 3.87 (3.97); S, 7.73 (7.73).

3-[4-(4-Aminophenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dc3): Yield: 79%; m.p.: 215-219 ºC; R_f: 0.36; IR (KBr, ν_{max}, cm^{-1}): 3074 (C-H arom.), 2967 (C-H *str*.), 1650 (C=O *str*. in ring), 1590 (C=N *str*.), 1180 (C-N *str*.), 690 (C-S *str*. in ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.65 (s, 3H, CH_3), 6.11 (s, 1H, methine), 6.32-7.43 (m, 4H, Ar-H), 6.8 (s, 1H, thiazole), 6.77-7.99 (m, 4H, Ar-H), 7.32-8.85 (m. 4H, pyridine), 4.4 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ ppm: 32.7, 77.6, 99.6, 114.6, 116.0, 116.8, 118.4, 123.0, 123.3, 128.3, 128.3, 131.5, 133.3, 134.6, 147.6, 148.2, 148.4, 149.3, 150.4, 160.4, 161.2: GC-MS: *m/z* 414 (M^+). Anal. calcd. (found) % for $C_{23}H_{19}N_5OS$ (m.w.: 413.49): C, 66.81 (66.81); H, 4.63 (4.63); N, 16.94 (16.84), O, 3.87 (3.97), S, 7.73 (7.73).

3-[4-(2-Hydroxyphenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dd4): Yield: 50%; m.p.: 220-224 ºC; R_f: 0.23; IR (KBr, ν_{max}, cm^{-1}): 2819 (C-H *str*.), 3071 (C-H arom.), 1588 (C=O *str*. in ring), 1588 (C=N *str*.), 689 (C-S *str*. in ring), 1180 (C-N *str*.); ^{1}H NMR (DMSO-d_6) δ ppm: 2.85 (s, 3H, CH_3), 6.10 (s, 1H, methine), 6.68-7.31 (m, 4H, Ar-H), 6.9 (s, 1H, thiazole), 6.55-7.99 (m, 4H, Ar-H), 7.12-8.95 (m. 4H, pyridine), 5.2 (s, 1H, OH): ^{13}C NMR (DMSO-d_6) δ ppm: 32.9, 77.4, 100.2, 114.6, 116.6, 118.6, 120.6, 121.9, 123.3, 128.4, 128.9, 130.2, 131.6, 133.3, 131.8, 147.6, 148.4, 149.3, 150.4, 155.4, 160.6, 161.4: GC-MS: *m/z* (M^+). 414, Anal. calcd. (found) % for $C_{23}H_{18}N_4OS$ (m.w.: 414.47): C, 66.65 (66.55), H, 4.38 (4.28), N, 13.52 (11.52), O, 7.72 (7.62), S, 7.74 (7.72).

3-[4-(4-Hydroxyphenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5De5): Yield: 49%; m.p.: 224-228 ºC; R_f: 0.50; IR (KBr, ν_{max}, cm^{-1}): 3073 (C-H arom.), 2820 (C-H *str*.), 1589 (C=O *str*. in ring), 1589 (C=N *str*.), 1180 (C-N *str*.), 690 (C-S *str*. in ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.25 (s, 3H, CH_3), 6.21 (s, 1H, methine), 6.65-7.45 (m, 4H, Ar-H), 6.6 (s, 1H, thiazole), 6.55-7.79 (m, 4H, Ar-H), 7.40-8.90 (m, 4H, pyridine), 5.2 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ ppm: 33.1, 76.4, 99.2, 113.6, 116.0, 116.4, 117.6, 123.0, 125.5, 128.9, 128.9, 131.6, 133.9, 147.8, 148.4, 149.7, 150.8, 158.9, 160.9, 161.9: GC-MS: *m/z* 415 (M^+). Anal. calcd. (found) % for $C_{23}H_{18}N_4OS$ (m.w.: 414.47): C, 66.65 (66.25); H, 4.38 (4.28); N 13.52 (11.52); O 7.72 (7.32); S 7.74 (7.22).

3-[4-(2,4-Dihydroxyphenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Df6): Yield: 55%; m.p.: 230-235 ºC; R_f: 0.49; IR (KBr, ν_{max}, cm^{-1}): 3074 (C-H arom.), 2822 (C-H *str*.), 1634 (C=O *str*. in ring), 1590 (C=N str.), 689 (C-S *str*. in ring), 1322 (C-N str.); ^{1}H NMR (DMSO-d_6) δ ppm: 2.85 (s, 3H, CH_3), 6.25 (s, 1H, methine), 6.20-7.20 (t, 3H, Ar-H), 6.4 (s, 1H, thiazole), 6.70-7.79 (m, 4H, Ar-H), 7.12-8.95 (m. 4H, pyridine), 5.2, 5.0 (s, 1H, 1H, OH): ^{13}C NMR (DMSO-d_6) δ ppm: 33.6, 78.4, 101.4, 104.1, 109.1, 113.2, 114.4, 116.0, 118.4, 128.4, 130.6, 131.9, 133.1, 131.6, 147.1, 148.2, 149.1, 150.2, 156.9, 159.9, 160.9, 161.6: GC-MS: *m/z* 430 (M^+). Anal. calcd. (found) % for $C_{23}H_{18}N_4O_3S$ (m.w.: 430.47); C, 64.17 (64.27); H, 4.21 (4.11); N, 13.01 (15.01); O, 11.15 (9.15); S, 7.45 (7.75).

3-[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dg7): Yield: 59%; m.p.: 230-235 ºC; R_f: 0.43; IR (KBr, ν_{max}, cm^{-1}): 3074 (C-H arom.), 2850 (C-H *str*.), 1634 (C=O *str*. in ring), 1590 (C=N *str*.), 1322 (C-N *str*.), 690 (C-S *str*. in ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.65 (s, 3H, CH_3), 6.21 (s, 1H, methine), 6.4 (s, 1H, thiazole), 7.25-7.50 (m, 4H, Ar-H), 6.55-7.99 (m, 4H, Ar-H), 7.22-8.95 (m. 4H, pyridine): ^{13}C NMR (DMSO-d_6) δ ppm: 32.6, 79.4, 100.0, 114.4, 116.0, 118.9, 123.4, 128.4, 129.9, 131.2, 131.6, 133.6, 134.6, 147.4, 148.9, 149.7, 150.6, 160.4: GC-MS: *m/z* 432 (M^+). Anal. calcd. (found) % for $C_{23}H_{17}N_4OSCl$ (m.w.: 432.92): C, 63.81 (63.61); H, 3.96 (3.96); N, 12.94 (12.14); O, 3.70 (3.10); S, 7.41 (7.11); Cl, 8.19 (8.09).

3-[4-(2,4-Dichlorophenyl)-1,3-thiazol-2-yl]-1-methyl-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dh8): Yield: 63%; m.p.: 235-240 ºC; R_f: 0.36; IR (KBr, ν_{max}, cm^{-1}): 3072 (C-H arom.), 2819 (C-H *str*.), 1588 (C=O *str*. in ring), 1588 (C=N *str*.), 1086 (C-N *str*.), 689 (C-S *str*. in ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.80 (s, 3H, CH_3), 6.21 (s, 1H, methine), 6.4 (s, 1H, thiazole), 7.10-7.36 (m, 3H, Ar-H), 6.59-7.99 (m, 4H, Ar-H), 7.42-8.85 (m. 4H, pyridine): ^{13}C NMR (DMSO-d_6) δ ppm: 32.9, 77.4, 100.4, 114.4, 116.4, 118.1, 123.9, 127.4,

128.1, 128.9, 130.9, 131.9, 133.6, 133.9, 135.6, 147.6, 148.2, 149.1, 150.6, 160.4, 161.2: GC-MS: *m/z* 467(M^+). Anal. calcd. (found) % for $C_{23}H_{16}N_4OSCl_2$ (m.w.: 467.37); C, 59.11 (59.51); H, 3.45 (3.05); N, 11.99 (11.89); O, 3.42 (3.40); S, 6.86 (6.83); Cl, 15.17 (15.19).

1-Methyl-3-[4-(4-methylphenyl)-1,3-thiazol-2-yl]-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Di9): Yield: 49%; m.p.: 210-215 ºC; R_f: 0.32; IR (KBr, ν_{max}, cm^{-1}): 3072 (C-H arom.), 2923 (C-H *str.*), 1650 (C=O *str*. in ring), 1588 (C=N *str*.), 1321 (C-N *str*.), 690 (C-S *str*. in ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.80 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.20 (s, 1H, methine), 6.3 (s, 1H, thiazole), 7.10-7.36 (m, 4H, Ar-H), 6.59-7.99 (m, 4H, Ar-H), 7.42-8.85 (m. 4H, pyridine); ^{13}C NMR (DMSO-d_6) δ ppm: 24.3, 33.9, 77.4, 100.0, 114.8, 116.0, 118.4, 123.0, 127.4, 127.5, 128.9, 129.9, 129.9, 130.9, 131.9, 133.1, 131.2, 138.6, 147.6, 148.6, 149.8, 150.9, 160.9, 161.9: GC-MS: *m/z*: (M^+). 412, Anal. calcd. (found) % for $C_{24}H_{20}N_4OS$ (m.w.: 412.50); C, 69.88 (67.88); H, 4.89 (4.69); N, 13.58 (13.18); O, 3.88 (3.04); S, 7.77 (7.17).

1-Methyl-3-[4-(-4-methoxyphenyl)-1,3-thiazol-2-yl]-2-(pyridine-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dj10): Yield: 52%; m.p.: 180-185 ºC; R_f: 0.29; IR (KBr, ν_{max}, cm^{-1}): 2932 (C-H *str*.), 3119 (C-H arom.), 1608 (C=O *str*. in ring), 1608 (C=N *str*.), 695 (C-S *str*. in ring), 1249 (C-N *str*.); ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 5.80 (s, 1H, methine), 6.1 (s, 1H, thiazole), 6.70-7.52 (m, 4H, Ar-H), 6.77-7.99 (m, 4H, Ar-H), 7.42-8.85 (m, 4H, pyridine): ^{13}C NMR (DMSO-d_6) δ ppm: 33.9, 55.9, 78.4, 100.9, 114.0, 114.8, 114.8, 116.2, 118.8, 123.9, 125.9, 128.5, 128.5, 131.3, 133.9, 134.4, 147.8, 149.9, 150.3, 160.9, 162.5: GC-MS: *m/z* 428 (M^+). Anal. calcd. (found) % for $C_{24}H_{20}N_4O_2S$ (m.w.: 428.50); C, 67.27 (67.32); H, 4.70 (4.55); N, 13.07 (13.16); O, 7.47 (7.04); S, 7.48 (7.17).

1-Methyl-3-[4-(3-nitrophenyl)-1,3-thiazol-2-yl]-2-(pyridin-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (5Dk11): Yield: 69%; m.p.: 195-197 ºC; R_f: 0.59; IR (KBr, ν_{max}, cm^{-1}): 2928 (C-H *str*.), 3074 (C-H arom.), 1619 (C=O *str*. in ring), 1619 (C=N *str*.), 691 (C-S *str*. in ring), 1240 (C-N *str*.); ^{1}H NMR (DMSO-d_6) δ ppm: 2.89 (s, 3H, CH_3), 6.29 (s, 1H, methine), 6.9 (s, 1H, thiazole), 7.50-8.55 (m, 4H, Ar-H), 6.77-7.89 (m, 4H, Ar-H), 7.32-8.95 (m, 4H, pyridine): ^{13}C NMR (DMSO-d_6) δ ppm: 31.9, 76.4, 99.9, 113.4, 116.2, 118.2, 121.9, 121.9, 128.1, 128.1, 131.6, 133.1, 134.8, 148.1, 147.9, 148.4, 148.4, 150.5, 160.1, 161.5: GC-MS: *m/z* 443 (M^+). Anal. calcd. (found) % for $C_{23}H_{17}N_5O_3S$ (m.w.: 443.47): C, 62.29 (62.39), H, 3.86 (3.76), N, 15.79 (15.89), O, 10.82 (11.82), S, 7.23 (7.13).

Colorectal anticancer activity: All the synthesized compounds were examined for their colorectal anticancer activity by employing the HT-29 HCAC cancer cell line. A cell lines were maintained in 96 wells microtiter plate containing MEM media augment with 10% heat and deactivate fetal calf serum (FCS), containing 5% of a mixture of gentamycin, penicillin (100 Units/mL) and streptomycin (100 µg/mL) in presence of 5% CO_2 at 37 ºC for 3-4 days. After 3-4 days the supernatant was removed. The MEM media was replaced with MTT solution supplemented with gentamycin, penicillin and streptomycin and incubated overnight.

Cytotoxicity assay: *in-vitro* growth reserve effect of the test, the compound was evaluated by colorimetric or spectrophotometric determination of conversion of MTT into formazan blue solution by living cells. The supernatant was separated from the plate, added fresh MTT solution and then treated with different concentrations of test compounds properly diluted with DMSO. In the current study, 10, 20, 25, 30, and 50 µL of the stock solution (10 mg/mL prepared in DMSO) were added to respective wells containing 100 µL of the medium. Hence, the final concentrations were 10, 20, 25, 30, and 50 µg/mL. The control group contains only DMSO.

After 24 h incubation at 37ºC in a moisturise atmosphere of 5% CO_2, the medium was restored with MTT solution (100 µL, 1 mg per mL in sterile Hank's balanced salt solution) for further 4 h incubation. Then the supernatant was carefully consonant and the precipitated crystals of formazan blue were solubilized by adding DMSO (200 µL). The optical density (OD) was measured at a wavelength of 492 nm.

Molecular docking studies

Preparation of ligand: The 2D structures of the designed quinazolinone derivatives were drawn by using ChemDraw professional 16.0 and stored in a library of sdf format. All the 2D sdf structures are converted to a 3D structure by 3D optimization tool, *i.e*. Ligprep of Schrodinger suit. The drawned ligands were optimized for geometry by using OPLS-2005 (Optimized Potentials for Liquid Simulations) force field steepest descent method. All the proposed structures with default settings were processed for chirality, low energy in 3D form. Furthermore, the extra precision (XP) was done for the processed ligands by using the Glide module of the Schrödinger suite [13,14].

Protein selection/preparation: In this study, the DNA target (PDB ID: 3GC7) was selected from the Protein Data Bank (PDB) [15,16]. Protein is selected based on the various factors, *i.e*. the resolution should be 1.5-2.5 Å, it should contain co-crystallized ligand, and the structure should be evaluated by X-ray diffraction. Selected protein should not contain breaks in their 3D structure.

Grid generation: In structure-based drug design, the identification of an active site is a crucial move. The receptor grid generation was used for grid generation in the Glide software. After recognition of the active site region, the grid box was prepared in such a way that it circles the whole active site. In this process, other options were kept as default [17]. Subsequently, a multivariate investigation was performed on the compounds according to their similarity to reference molecules. It was observed that the majority of the studied ligands show a closeness towards the 5-fluorouracil (5-FU) molecule [18].

Molecular docking: After energy minimization was performed using Schrödinger Glide for possible conformation of Ligand in the active-site region of a receptor, using a set of filters [19]. Using the XP glide methodology, the candidate ligands were semi-quantitatively ranked according to their capability to bind a particular conformation of the protein receptor. By using default settings, the designed ligand was docked with the 3GC7 protein using XP mode in Schrödinger's glide software [20].

Molecular mechanics/generalized born surface area (MM/GBSA): The MM/GBSA is a force-field based method that enumerates free energy of binding based on the generalized Born continuum solvent model [21,22] as opposed to also modeling free energies of single entities [23]. The ligand strain energies and ligand binding to docked receptor-ligand complexes were calculated according to MM/GBSA, using the prime module of Schrödinger software. The binding free energy (ΔG_{bind}) was calculated using the following eqn. 1 [24]:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{sol} + \Delta G_{SA} \quad (1)$$

where, ΔE_{MM} is the difference in energies between protein-ligand complex and sum of the energies of unliganded protein and free ligand, using the OPLS force field [25,26]. ΔG_{sol} is the difference in GBSA solvation energy of protein-inhibitor complex and the sum of the solvation energies for the unliganded protein and ligand. ΔG_{SA} is a difference in surface area energies for complex structure and the sum of the surface area energies for non-liganded protein and ligand.

Docking studies: Docking software Schrödinger was used to dock the protein with the drug molecule. The docking program was executed to forecast the binding pocket of 3GC7. All the designed ligand docked using the standard accuracy. The analysis of molecular docking of synthetic compounds and 5-fluorouracil (5-FU) molecule was executed using Schrödinger software [19].

***in-silico* ADMET prediction by Swiss ADME tool:** The ADME study of the selected docked library was carried out using the QikProp [20]. The present study, properties like molecular weight predicted central nervous system activity, octanol/water partition coefficient, aqueous solubility, IC_{50} value for the blockage of MAPK channels, cell permeability, binding of a drug to human serum albumin, number of hydrogen bond donors and acceptors was calculated for each compound. The drug-likeness was evaluated [27]. Moreover, the properties of ligand to noxious and carcinogenicity were analyzed online using the ADMET SAR and Swiss ADME [28]. QSAR studies and drug-likeness are also predicted to know the octanol/water partition coefficient (log P_o/w), topological polar surface area (TPSA), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), Lipinski rule and synthetic accessibility.

Lipinski′s rule of five: The Lipinski′s rule of five is based on certain criteria to estimate drug-likeness of a molecule having a pharmacological activity [29]. These criteria are log 5C lower than 5, number of HBD < 5, HBA < 10, and m.w. not exceeding 500 Da. The rule is used in drug design to preselect molecules presenting good absorption, distribution, metabolism and excretion (ADME) properties that must have a medicament in the organism. The Molinspiration property calculator (http://www.SwissADME.com) was used to calculate the four parameters of Lipinski′s rule in addition to the number of rotatable bonds that have to be inferior to 10 to have a good oral bioavailability [30].

RESULTS AND DISCUSSION

Anticancer activity: The synthesized compounds (**5Da1-5Dk11**) were evaluated as HT-29 human colorectal adenocarcinoma (HT-29 HCAC) cancer cell lines using 5-fluorouracil as a positive control. The compounds **5Dh8**, **5DF6**, **5Db2** and **5Di9** exhibited maximum activity at a concentration < 10 µg, while compounds **5Da1**, **5Cc3**, **5Dd4**, **5DJ10** and **5Dk11** exhibited moderate activity at a concentration < 20 µg. The rest of the compounds **5De5** and **5Dg7** exhibited no activity at the concentration >30 µg (Table-1).

TABLE-1
MEDIAN GROWTH INHIBITORY CONCENTRATION OF HT-29 HCAC

Code	Concentration (µg)	O.D. at 492 nm	% of cell lysis	IC_{50} (µg)
5Da1	10	0.389	>50%	< 20
	20	0.467	>75%	
	30	0.488	100%	
5Db2	10	0.789	>50%	< 10
	20	1.232	>75%	
	30	1.865	100%	
5Dc3	10	0.893	>50%	< 20
	20	1.362	>75%	
	30	1.956	100%	
5Dd4	10	0.834	>50%	< 20
	20	1.254	>75%	
	30	1.885	100%	
5De5	10	0.389	No lysis	> 30
	20	0.467	No lysis	
	30	0.488	No lysis	
5Df6	10	0.676	>50%	< 10
	20	1.121	>75%	
	30	1.631	100%	
5Dg7	10	0.389	No lysis	> 30
	20	0.467	No lysis	
	30	0.488	No lysis	
5Dh8	10	0.768	>50%	< 10
	20	1.231	>75%	
	30	1.845	100%	
5Di9	10	0.723	>50%	< 10
	20	1.115	>75%	
	30	1.716	100%	
5Dj10	10	0.768	>50%	< 20
	20	1.231	>75%	
	30	1.845	100%	
5Dk11	10	0.876	>50%	< 20
	20	1.341	>75%	
	30	1.935	100%	
Control	–	0.507	No lysis	–

Based on the structure-activity relationship (SAR), it was concluded that the presence of electronegative atoms, electron-withdrawing effect and hydrophilic properties present in the structure favoured the activity and made a strong binding to the polar recognition region of the active site.

Molecular docking: To know the structural basis of the designed ligands binding to target each ligand and 5-FU docked with the receptors at their active site using the glide program. These docking results disclose that all the designed compounds were fully favourable in terms of the Glide dock score. The results were described in terms of docking score, glide evdw, glide ecoul, glide energy and glide model. The glide docking score for the designed compounds ranges from -7.19 to -6.391.

TABLE-2
DOCKING SCORE OF SYNTHESIZED COMPOUNDS **5Da1- 5Dk11**

S. No	Code	Docking score	Glide evdw	Glide ecoul	Glide E model	Glide energy
3GC7	Co-crystal	-10.669	-57.252	-8.242	-103.95	-65.494
1	**5Da1**	-6.628	-43.417	-4.072	-64.804	-47.489
2	**5Db2**	-6.701	-47.52	-1.681	-67.324	-49.201
3	**5Dc3**	-6.658	-47.039	-2.754	-67.369	-49.793
4	**5Dd4**	-6.873	-47.613	-2.747	-68.223	-50.36
5	**5De5**	-6.706	-47.328	-2.668	-67.684	-49.996
6	**5Df6**	-6.744	-48.302	-2.625	-69.894	-50.927
7	**5Dg7**	-6.547	-46.165	-2.51	-66.013	-48.675
8	**5Dh8**	-6.917	-47.52	-2.026	-69.338	-49.546
9	**5Di9**	-6.81	-45.431	-2.6	-65.5	-48.031
10	**5Dj10**	-6.435	-46.071	-3.687	-66.283	-49.758
11	**5Dk11**	-6.478	-47.435	-3.193	-67.165	-50.628
Standard	**5-FU**	-5.765	-20.13	-3.629	-32.049	-23.759

where designed compounds **5Df6** and **5Dh8** showed the lowest binding energy with -7.19 and -7.027 compared with 5-FU -5.765 (Table-2).

Based on the Lipinski′s rule, all the molecules fulfilled the predictions and shows that LogP, TPSA, HBA and HBD values are within the range (Table-3), thus thereby suggested the good pharmacokinetic permeability and their oral bioavailability, which may allow them to constitute lead compounds for cancer treatment.

Depending on the molecular docking studies, synthesized compounds show greater activity than the standard drug 5-fluorouracil. 5-Fluorouracil shows -5.765 kcal/mol binding energy when docked against the target DNA structure 3GC7. Compounds **5Da1**, **5Db2**, **5Dc3**, **5Dd4**, **5De5**, **5Df6**, **5Dg7**, **5Dh8**, **5Di9**, **5Dj10** and **5Dk11** showed binding energy values -6.621, -6.927, -6.832, -6.82, -6.935, -7.19, -6.723, -7.027, -6.823, -6.391 and -6.413 kcal/mol, respectively. While other compounds (**5Db2**, **5DF6**, **5De5**, **5Dh8** and **5Di9**) having more negative values possess higher potent activity than 5-fluorouracil. Docking figures of 2D and 3D structures are shown in Fig. 1.

Conclusion

In this work, a series of novel anticancer molecules containing 2,3-disubstituted quinazolin-4-one pharmacophore was

TABLE-3
PREDICTED ADME PROPERTIES OF SYNTHESIZED COMPOUNDS BY SWISS ADME

Code	R	[a]Log P	MR	[b]TPSA	HBA	HBD	RB	G.I. absorption & BBB permeant	Log Kp (cm/s)	[c]Lipinski rule	Logs & class	Synthetic accessibility
5-FU	–	0.44	27.64	65.72	3	2	0	High & No	-7.73	Yes	-0.01 & Very soluble	1.52
5Da1	H	3.32	122.1	77.57	3	0	3	High & No	-5.59	Yes	-5.77 & Moderately soluble	3.75
5Db2	3-NH_2	2.9	126.51	103.59	3	1	3	High & No	-6.17	Yes	-5.61 & Moderately soluble	3.85
5Dc3	4-NH_2	2.87	126.51	103.59	3	1	3	High & No	-6.17	Yes	-5.61 & Moderately soluble	3.82
5Dd4	2-OH	3.32	124.13	97.8	4	1	3	High & No	-5.95	Yes	-5.82 & Moderately soluble	3.81
5De5	4-OH	2.81	124.13	97.8	4	1	3	High & No	-5.95	Yes	-5.82 & Moderately soluble	3.76
5Df6	2,4-OH	2.83	126.15	118.03	5	2	3	High & No	-6.29	Yes	-5.88 & Moderately soluble	3.85
5Dg7	4-Cl	3.45	127.11	77.57	3	0	3	High & No	-5.36	Yes	-6.42 & Poorly soluble	3.74
5Dh8	2,4-Cl	3.71	132.12	77.57	3	0	3	High & No	-5.13	Yes	-7.06 & Poorly soluble	3.82
5Di9	4-CH_3	3.5	127.07	77.57	3	0	3	High & No	-5.42	Yes	-6.14 & Poorly soluble	3.87
5Dj10	4-OCH_3	3.63	128.6	86.8	4	0	4	High & No	-5.8	Yes	-5.93 & Moderately soluble	3.85
5Dk11	3-NO_2	2.21	130.93	123.39	5	0	4	High & No	-5.99	Yes	-6.55 & Poorly soluble	3.87

[a]Predicted octanol/H_2O partition coefficient (< 5); [b]van der Waals surface area of polar N & O atoms and carbonyl carbon atoms (Range 7 to 200); [c]Lipinski's violations (≤ 1)

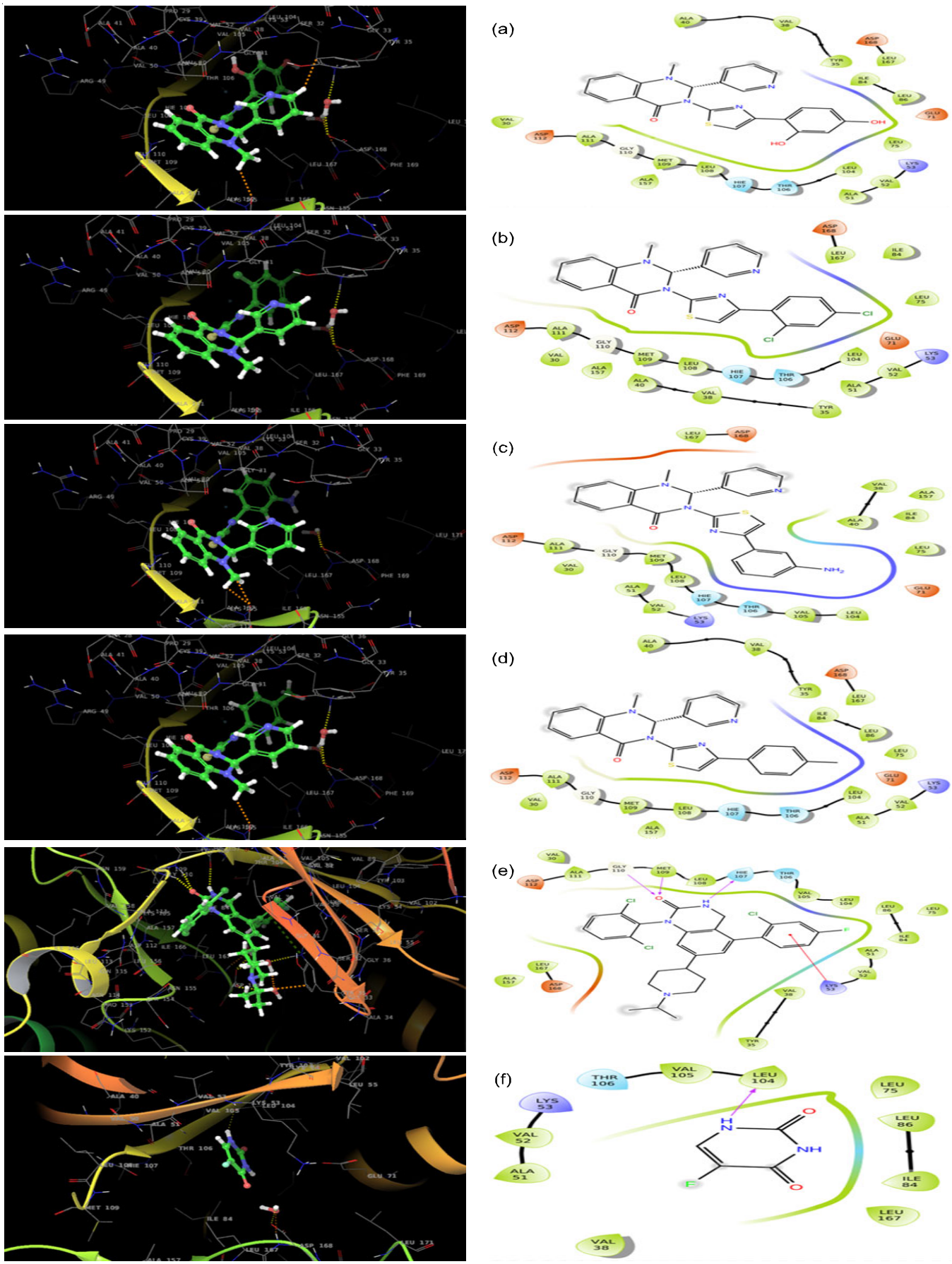


Fig. 1. 3D and 2D molecular docking structures of 5Df6 (a); 5Dh8 (b); 5Db2 (c); 5Di9 (d); 3GC7 (e); and 5-fluorouracil (f)

synthesized and characterized. The cell containing HT-29 HCAC was used to measure the cytotoxicity of the proposed quinazolinone derivatives. Compounds **5Dh8**, **5DF6**, **5Db2** and **5Di9** exhibit a potent antitumor activity against HT-29 HCAC IC_{50} range of 10-30 µg/mL. These derivatives are known to overexpress MAP kinase, which leads to continuous activation of the MAPK pathway in cell proliferation. Docking studies revealed that compounds **5Df6** and **5Dh8** have the highest binding with glide score -7.19 and -7.027 kcal/mol as compared to the target protein -10.67 kcal/mol.

ACKNOWLEDGEMENTS

The authors are thankful to DST-SERB facilities of Molecular Modeling and Drug Discovery Division RERDS -CPR, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, India for providing the docking studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. N.P. McLaughlin, P. Evans and M. Pines, *Bioorg. Med. Chem.*, **22**, 1993 (2014); https://doi.org/10.1016/j.bmc.2014.02.040
2. K.K. Rajasekhar, N.D. Nizamuddin, A.S. Surur and Y.T. Mekonnen, *Res. Rep. Med. Chem.*, **6**, 15 (2016); https://doi.org/10.2147/RRMC.S91474
3. I. Khan, A. Ibrar, N. Abbas and A. Saeed, *Eur. J. Med. Chem.*, **76**, 193 (2014); https://doi.org/10.1016/j.ejmech.2014.02.005
4. U.U. Demirel, A. Yilmaz, H. Türkdagi, B. Öztürk and U. Arslan, *Polycycl. Aromat. Compd.*, **12**, 1 (2019); https://doi.org/10.1080/10406638.2019.1689513
5. V. Alagarsamy, V. Raja Solomon, R.V. Sheorey and R. Jayakumar, *Chem. Biol. Drug Des.*, **73**, 471 (2009); https://doi.org/10.1111/j.1747-0285.2009.00794.x
6. A.M. Alafeefy, A.A. Kadi, A.S. El Azab, S.G. AbdelHamide and M.H. Daba, *Arch. Pharm.*, **341**, 377 (2008); https://doi.org/10.1002/ardp.200700271
7. R. Sompalle, S.M. Roopan, N.A. Al-Dhabi, K. Suthindhiran, G. Sarkar and M.V. Arasu, *J. Photochem. Photobiol. B*, **162**, 232 (2016); https://doi.org/10.1016/j.jphotobiol.2016.06.051
8. A. Ayati, S. Emami and A. Foroumadi, *Eur. J. Med. Chem.*, **109**, 380 (2016); https://doi.org/10.1016/j.ejmech.2016.01.009
9. S. Patil, S.S. Patil, S.D. Jadhav and M.B. Deshmukh, *Indian J. Pharm. Sci.*, **72**, 500 (2010); https://doi.org/10.4103/0250-474X.73934
10. E. Bhargav, P. Ramalingam and M. Gowthami, *Int. J. Res. Pharm. Sci.*, **6**, 3796 (2019).
11. G. Cavaletti, M. Miloso, G. Nicolini, A. Scuteri and G. Tredici, *J. Peripher. Nerv. Syst.*, **12**, 175 (2007); https://doi.org/10.1111/j.1529-8027.2007.00138.x
12. W.T. Zhang, J.L. Ruan, P.F. Wu, F.C. Jiang, L.N. Zhang, W. Fang, X.L. Chen, Y. Wang, B.-S. Cao, G.-Y. Chen, Y.-J. Zhu, J. Gu and J.-G. Chen, *J. Med. Chem.*, **52**, 718 (2009); https://doi.org/10.1021/jm800902t
13. A. Plaper, M. Golob, I. Hafner, M. Oblak, T. Šolmajer and R. Jerala, *Biochem. Biophys. Res. Commun.*, **306**, 530 (2003); https://doi.org/10.1016/S0006-291X(03)01006-4
14. I.J. Bruno, J.C. Cole, P.R. Edgington, M. Kessler, C.F. Macrae, P. McCabe, J. Pearson and R. Taylor, *Acta Crystallogr B Struct. Sci.*, **58**, 389 (2002); https://doi.org/10.1107/S0108768102003324
15. C.E. Fitzgerald, S.B. Patel, J.W. Becker, P.M. Cameron, D. Zaller, V.B. Pikounis, S.J. O'Keefe and G. Scapin, *Nat. Struct. Biol.*, **10**, 764 (2003); https://doi.org/10.1038/nsb949
16. S.B. Patel, P.M. Cameron, S.J. O'Keefe, B. Frantz-Wattley, J. Thompson, E.A. O'Neill, T. Tennis, L. Liu, J.W. Becker and G. Scapin, *Acta Crystallogr. D Biol. Crystallogr.*, **65**, 777 (2009); https://doi.org/10.1107/S090744490901600X
17. C.N. Lungu and M.V. Diudea, *Curr. Comput. Aided Drug Des.*, **14**, 29 (2018); https://doi.org/10.2174/1573409913666170927113813
18. R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, D.E. Shaw, P. Francis and P.S. Shenkin, *J. Med. Chem.*, **47**, 1739 (2004); https://doi.org/10.1021/jm0306430
19. L.L. Schrödinger, Schrödinger Release 2015-4: Schrödinger, New York (2015).
20. X. Zhang, H. Perez-Sanchez and F.C. Lightstone, *Curr. Top. Med. Chem.*, **17**, 1631 (2017); https://doi.org/10.2174/1568026616666161161117112604
21. J. Wang, T. Hou and X. Xu, *Curr. Comput. Aided Drug Des.*, **2**, 287 (2006); https://doi.org/10.2174/157340906778226454
22. S. Bhattacharya, L. Xu and D. Thompson, *ACS Chem. Neurosci.*, **10**, 2830 (2019); https://doi.org/10.1021/acschemneuro.9b00053
23. P.D. Lyne, M.L. Lamb and J.C. Saeh, *J. Med. Chem.*, **49**, 4805 (2006); https://doi.org/10.1021/jm060522a
24. J.L. Banks, H.S. Beard, Y. Cao, A.E. Cho, W. Damm, R. Farid, A.K. Felts, T.A. Halgren, D.T. Mainz, J.R. Maple, R. Murphy, D.M. Philipp, M.P. Repasky, L.Y. Zhang, B.J. Berne, R.A. Friesner, E. Gallicchio and R.M. Levy, *Comput. Chem.*, **26**, 1752 (2005); https://doi.org/10.1002/jcc.20292
25. G.A. Kaminski, R.A. Friesner, J. Tirado-Rives and W.L. Jorgensen, *J. Phys. Chem. B*, **105**, 6474 (2001); https://doi.org/10.1021/jp003919d
26. J. Hodgson, *Nat. Biotechnol.*, **19**, 722 (2001); https://doi.org/10.1038/90761
27. F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee and Y. Tang, *J. Chem. Inf. Model.*, **59**, 4959 (2019); https://doi.org/10.1021/acs.jcim.9b00969
28. A. Daina, O. Michielin and V. Zoete, *Sci. Rep.*, **7**, 42717 (2017); https://doi.org/10.1038/srep42717
29. C.A. Lipinski, F. Lombardo, B.W. Dominy and P.J. Feeney, *Adv. Drug Deliv. Rev.*, **23**, 3 (1997); https://doi.org/10.1016/S0169-409X(96)00423-1
30. D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward and K.D. Kopple, *J. Med. Chem.*, **45**, 2615 (2002); https://doi.org/10.1021/jm020017n