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Synthesis and Molecular Docking Study of Novel Pyrazolo[3,4-*b*]quinoline Derivatives

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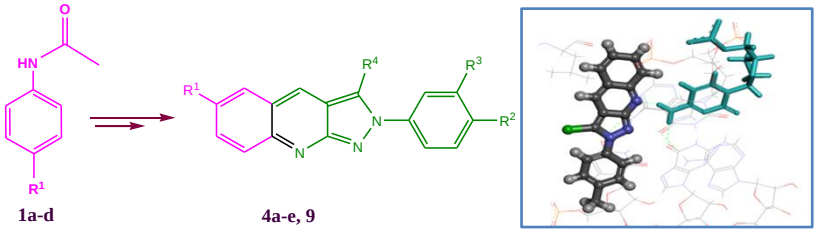
ABSTRACT

*Phenylpyrazolo[3,4-b]quinolin-3-ols were prepared by using 2-chloroquinoline-3-carboxylic acids and phenyl hydrazine hydrochlorides in the presence of $POCl_3$. One of the phenylpyrazolo[3,4-b]quinolin-3-ols underwent chlorination (**9**). To check binding modes and binding affinity of synthesized compounds were docked with the active sites of human telomerase (hTERT). The results indicated that compound **4b** has good affinity to the active site residue of human telomerase, least energy (-23.012 score).*



Keywords: Phenylpyrazolo[3,4-*b*]quinolin-3-ols, 3-Chloro-2-*p*-tolyl-2*H*-pyrazolo[3,4-*b*]quinoline, $POCl_3$, Molecular Docking Studies.

INTRODUCTION

Quinoline derivatives have displayed wide range of biological properties ranging from antimicrobial activity to cytotoxicity [1]. The fused quinoline derivatives like pyrazolo[3,4-*b*]quinolines were used as antiviral [2], antimalarial [3], serum cholesterol lowering agents [4] and vasodilators [5]. Pyrazolo[3,4-*b*]quinoline derivatives were known to possess bactericidal [6], parasiticidal [4], and enzyme inhibitory activity [7]. Moreover, pyrazolo[3,4-*b*]quinolines are used in several optical devices like molecular

sensors [9] and organic light emitting diodes [10] because of their fluorescent behavior [8,11]. Because of the above consequences of pyrazolo[3,4-*b*]quinolines and in continuation of our research [12], we in this reported a study on the synthesis and docking study of new pyrazolo[3,4-*b*]quinoline derivatives (**4a-e**, **9**).

MATERIALS AND METHODS

General information: Required chemicals and reagents were obtained from Sigma-Aldrich and SD-Fine Chemicals, and were used without further purification. Melting points were recorded in an open capillary and are uncorrected. ESI-MS spectra were measured by using a 5 mM ammonium acetate:acetonitrile (95:5 mixture) and 5 mM acetonitrile:ammonium acetate (5:95 mixture) as mobile phases A and B respectively by using an OpenLynx ESI-LCMS spectrometer. The CHN analysis was carried on an ElementarVario MICRO cube. The progress of reactions and the purity of products were monitored via TLC using silica gel 60 F254 thin layer chromatography plates. Column chromatographic separations were performed on silica gel of 60-120 mesh size column, using mixture of ethyl acetate and petroleum ether solvent system. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on Bruker AV-400 spectrometer using TMS as internal reference.

General procedure for the synthesis of the compounds 3a-b.
Preparation of 2-chloro-6-methylquinoline-3-carboxylic acid (3a) as an example: To a stirred mixture of 2-chloro-6-methylquinoline-3-carbaldehyde (**2a**) and 546.0 mg, 9.7 mmol KOH, 5 mL of $KMnO_4$ (770.9 mg, 4.8 mmol in distilled water) was added in portion wise at 0 ^{o}C. After complete addition, the solution was stirred for another 10 h at room temperature. Completion of the reaction was monitored by TLC for the disappearance of reactant **2a**. After completion, the reaction mixture was diluted with 100 mL of ice cold water and neutralized with 1.5N HCl. The precipitate obtained was filtered, washed with water and dried to get pure **3a** in 99% (1050 mg, 4.7 mmol) yield. Similarly, the other carboxylic acid **3a** was prepared [13-14].

General procedure for the synthesis of the compounds 4a-e and 9
Preparation of 2-(3-fluorophenyl)-6-methyl-2*H*-pyrazolo[3,4-*b*]quinolin-3-ol (**4a**) as an example : A mixture of 2771.9 mg, 8.0 mmol, 1.7 mL $POCl_3$ and 2-chloro-6-methylquinoline-3-carboxylic acid (**3a**) (500.0 mg, 2.2 mmol) was refluxed for about 15 min followed by addition of 1-(3-fluorophenyl)hydrazine hydrochloride (366.8 mg, 2.256 mmol). Then continued the reflux for about 8 h. Completion of the reaction was monitored by TLC for the disappearance of reactants. After completion, cooled to room temperature, quenched with ice cold water containing an excess of crushed ice and then neutralized with 10% $NaHCO_3$ solution. The solid obtained was filtered, dried and purified by column chromatography using 0.5:9.5 ratio of ethyl acetate and petroleum ether was yielded 51% (340.0 mg, 1.1 mmol) of pure **4a**. The other products (**4b-e** and **9**) were prepared in a similar manner (Table 1, Scheme 1).

Docking Studies: Structures of synthetic compounds (**4a**, **4b**, **4c**, **4d**, **4e** & **9**) were designed using the Marvink sketch software package [15] and were MM2 optimized using Discovery Studio 3.5 [16]. The bioavailability of synthesized compounds and ascorbic acid control was assessed for ADMET and Lipinski´s rule-of-five [17, 18]. The 3D crystal structure of PDB ID: 3KYL was retrieved from the Protein Data Bank. Telomerase reverse transcriptase activity is an invariable finding where human telomerase (hTERT) has been implicated in tumor oxidative stress and redox-mediated malignancy [19]. The 3KYL structure was first relaxed by 20,000 steps of minimization and a standard relaxation procedure using restrained Molecular Dynamics [20]. The active site prediction was performed based on a receptor cavity method [21]. Molecular docking studies were carried to investigate the binding affinities and interaction modes between the synthesized compounds and the target (3KYL) using Lead IT [22]. The docked ligand-target complexes were analyzed to identify the interactions and binding affinities. The docking score was recorded and docking poses were saved for reference.

Analytical data of the products (**4b-e** and **9**).

*2-(3-Fluorophenyl)-6-methyl-2H-pyrazolo[3,4-b]quinolin-3-ol (**4a**):* ^{1}HNMR **(**400 MHz, DMSO): δ = 2.49 (s, 3H), 6.96-7.01 (m, 1H), 7.51-7.57 (m,1H), 7.69 (dd, J=2, J=8.8Hz, 1H), 7.923-7.99 (m, 2H), 8.24-8.32 (m, 2H), 8.81 (s,1H) ppm; ^{13}C NMR (100 MHz, DMSO): δ = 20.8, 104.6, 104.8, 109.5, 109.7, 110.3, 113.6, 113.6, 123.7, 127.6, 128.1,130.5, 130.7, 130.8, 133.5, 134.0, 141.4, 141.5, 146.6, 149.5, 161.2, 163.6 ppm (shows excess signals in ^{13}C NMR due the presence of F atom); Negative LC-ESI-MS, m/z: 292.30 $[M-H]^+$; Anal. Calcd for $C_{17}H_{12}FN_3O$: C, 69.61; H, 4.12, N, 14.35; Found: C, 69.58; H, 3.92, N, 14.19.

2-(3-Nitrophenyl)-2H-pyrazolo[3,4-b]quinolin-3-ol (**4b*):*** ^{1}HNMR **(**400 MHz, DMSO): δ = 7.55-7.69 (m, 1H), 7.80-7.90 (m, 3H), 7.95-8.01 (m,1H), 8.06-8.13 (m,1H), 8.18-8.24 (m,1H), 8.26-8.31 (m,1H), 8.91-8.97 (m,2H) ppm; ^{13}C NMR (100 MHz, DMSO): δ = 117.23, 118.7, 122.7, 125.0, 128.7, 129.2, 129.7, 133.1, 135.2, 135.9, 136.2, 137.1, 137.9, 145.9, 153.1, 153.6 ppm; Negative LC-ESI-MS, m/z: 305.27 $[M-H]^+$; Anal. Calcd for $C_{16}H_{10}N_4O_3$: C, 62.73; H, 3.29, N, 18.28; Found: C, 62.58; H, 3.12, N, 18.22.

*2-(4-Methoxyphenyl)-2H-pyrazolo[3,4-b]quinolin-3-ol (**4c**):* ^{1}HNMR **(**400 MHz, $CDCl_3$): δ = 3.91 (s, 3H), 7.07-7.09 (m, 2H), 7.33-7.36 (m,1H), 7.64-7.68 (m,1H), 7.78 (dd, J=2, J=7.2Hz, 2H), 7.878 (d, J=8Hz, 1H), 8.09 (d, J=9.2Hz, 1H), 8.67 (s,1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.6, 114.3, 124.1, 125.4, 127.0, 127.3, 128.8, 129.4, 129.5, 130.9, 131.4, 131.6, 152.1, 156.6, 160.5 ppm; Negative LC-ESI-MS, m/z: 290.11 $[M-H]^+$; Anal. Calcd for $C_{17}H_{13}N_3O_2$: C, 70.08; H, 4.51, N, 14.43; Found: C, 69.99; H, 4.44, N, 14.37.

4-(3-Hydroxy-2H-pyrazolo[3,4-b]quinolin-2-yl)benzonitrile (**4d**): ^{1}HNMR **(**400 MHz, $CDCl_3$): δ = 7.35-7.39 (m, 1H), 7.66-7.69 (m, 1H), 7.861-7.92 (m,3H), 8.07-8.10 (m, 3H), 8.69 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 113.6, 114.0, 117.6, 124.8, 126.3, 128.8, 128.9, 129.3, 130.0, 131.8, 133.1, 133.2, 141.5, 152.7, 156.6 ppm; Negative LC-ESI-MS, m/z: 285.11 $[M-H]^+$; Anal. Calcd for $C_{17}H_{10}N_4O$: C, 71.33; H, 3.51, N, 19.57; Found: C, 71.22; H, 3.41, N, 19.10.

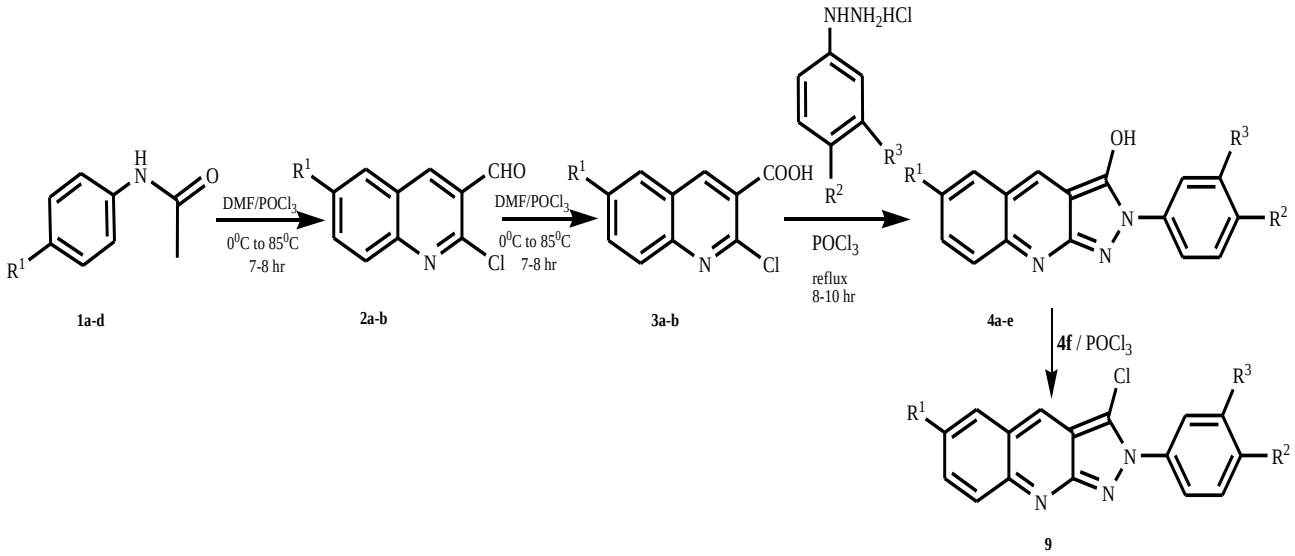
2-(3-Fluorophenyl)-2H-pyrazolo[3,4-b]quinolin-3-ol (**4e**): ^{1}HNMR **(**400 MHz, DMSO): δ = 6.98-7.03 (m, 1H), 7.53-7.59 (m, 2H), 7.84-7.88 (m,1H), 8.08-8.33 (m, 4H), 8.96 (s,1H) ppm; ^{13}C NMR (100 MHz, DMSO): δ = 104.7, 105.0, 109.7, 109.9, 110.5, 113.7, 113.8, 123.8, 124.2, 127.9, 129.8, 130.2, 130.8, 130.9, 131.6, 141.3, 141.5, 147.9, 161.2, 163.6 (shows excess signals in ^{13}C NMR due the presence of F atom) ppm; Negative LC-ESI-MS, m/z: 278.20 $[M-H]^+$; Anal. Calcd for $C_{16}H_{10}FN_3O$: C, 68.81; H, 3.61, N, 15.05; Found: C, 69.04; H, 3.45, N, 15.00.

3-Chloro-2-p-tolyl-2H-pyrazolo[3,4-b]quinoline (**9**): ^{1}HNMR **(**400 MHz, $CDCl_3$): δ = 2.47 (s, 3H), 7.32-7.35 (m, 1H), 7.38 (d, J=8.4 Hz, 2H), 7.63-7.68 (m, 1H), 7.727-7.748 (m,2H), 7.864 (d, J=8.8 Hz, 1H), 8.112 (d, J=8.8 Hz, 1H), 8.67 (s,1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.8, 114.3, 118.9, 124.7, 125.7, 125.9, 129.3, 129.4, 130.2, 130.7, 131.8, 136.3, 140.6, 152.0, 156.5 ppm; Positive LC-ESI-MS, m/z: 294.08 $[M-H]^+$; Anal. Calcd for $C_{17}H_{12}ClN_3$: C, 69.51; H, 4.11, N, 14.31; Found: C, 69.46; H, 4.30, N, 14.21.

RESULTS AND DISCUSSION

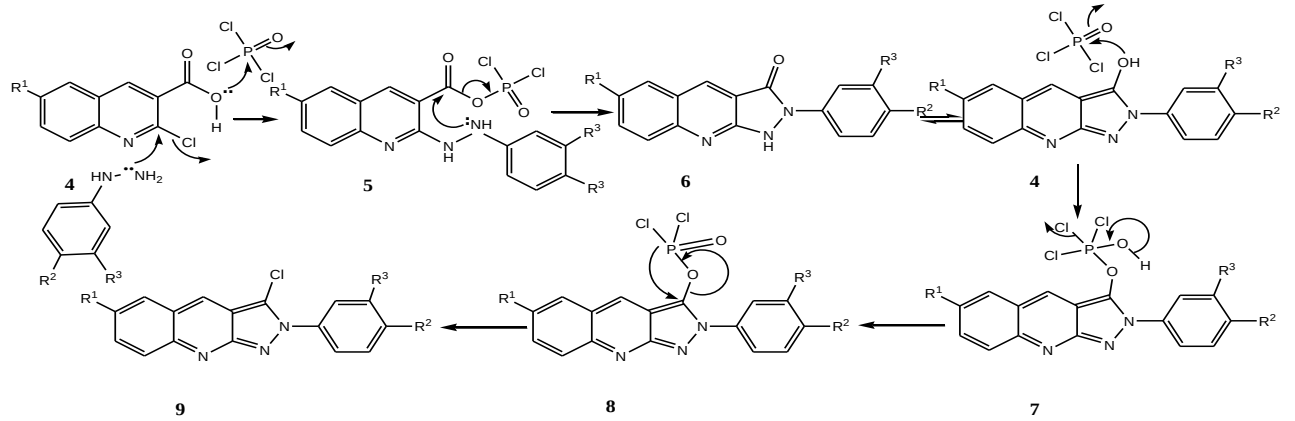
Synthesis: Compounds **4a-e** and **9** were prepared in three step procedure as shown in Scheme-1, Table.1. Required 2-chloroquinoline-3-carbaldehydes (**2a-b**) were acquired through the conventional Vilsmeier-Haack cyclisation procedure [13**]**. The aldehydes **2a-b** obtained were oxidized into corresponding 2-chloroquinoline-3-carboxyllic acids [14] **3** in the presence of $KMnO_4$ and KOH in water medium. These acids (**3**) underwent nucleophillic substitution at the chlorine carbon with $–NH_2$ of phenyl hydrazine hydrochloride and condensation with $POCl_3$ at carboxylic acid carbon into an intermediate compound **6**. Then compound **6** underwent intra-molecular acid amine coupling into the ketone **7** with the elimination of PO_2Cl and HCl. Since, the compound **7** is in keto-enol tautomerism and gets converted into enolic form **4**.

Scheme 1, Table 1: Synthesis of 2-phenyl-2*H*-pyrazolo[3,4-*b*]quinoline derivatives (4a-e and 9).



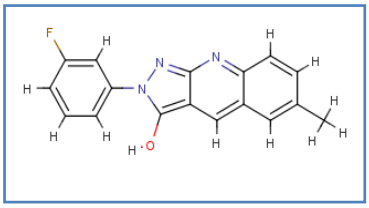
Sl. No	Entry	R^1	R^2	R^3	Yield in %	M.P in ^oC (Reported)
1	**3a**	CH_3	-	-	99	222-225 (224)
2	**3b**	H	-	-	96	233-236 (235)
1	**4a**	CH_3	H	F	51	>220
2	**4b**	H	H	NO_2	40	220
3	**4c**	H	OCH_3	H	38	155-160
5	**4d**	H	CN	H	47	210
6	**4e**	H	H	F	44	223-226
7	**4f**	H	CH_3	H	--	Not isolated
8	**9**	H	CH_3	H	64	160-165

The $POCl_3$ reactions towards **4a-e** were stopped at enolic stage, but the **4f** was continued to react with $POCl_3$ to give chlorinated pyrazoloquinoline **9** via the intermediates **7-8,** Scheme 2. It was observed that, the compound **9** exhibited green fluorescence property in its solution state [8-11]. All these final products were characterized by means of melting point, elemental and spectral analyses. Detailed procedures and analytical data are described in the experimental section.

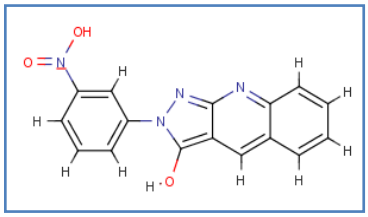


Scheme 2. Plausible mechanism for the formation of products 4a-e and 9

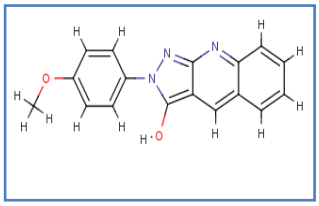
Molecular Docking: High resolution (2.7 Å) crystallographic structures of the human telomerase complex with RNA–DNA ligands has provided the opportunity to expand research targeting small molecules with potential telomerase inhibition activity as a marker of inhibiting the oxidative stress cascade. The designed compounds and control used in this study are presented in (Fig 1).



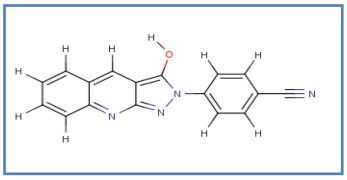
4a



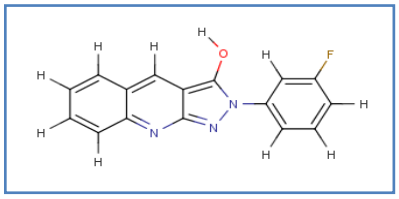
4b



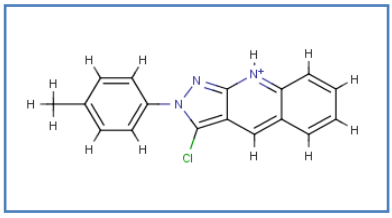
4c



4d



4e



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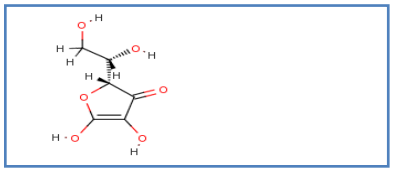


Ascorbic acid

Fig. 1: 2D structure of synthesized products and ascorbic acid

It was found that all compounds satisfied the Lipinski rule, ADME and TOPKAT parameter (Tables 2,3), (Fig 2).

Table 2: Results of ADME properties

S. No	Compound Name	ADMET Solubility Level	ADMET BBB Level	ADMET EXT CYP2D6	ADMET EXT Hepatotoxic	ADMET Absorption Level	ADMET EXT PPB	ADMET AlogP98	ADMET PSA 2D
1	**4a**	1	1	-2.45602	3.39006	0	5.06316	4.61	48.685
2	**4b**	**2**	**2**	**-7.63529**	**5.72677**	**0**	**2.04953**	**3.813**	**91.508**
3	**4c**	2	1	-3.32115	4.10139	0	1.41636	3.902	57.615
4	**4d**	2	1	-2.87025	6.02262	0	4.23095	4.124	48.685
5	**4e**	2	2	-5.39316	4.09629	0	1.00819	3.798	71.62
6	**9**	1	0	-3.62122	2.33283	0	5.82215	4.878	31.664
7	STD	5	4	-5.84825	-6.79965	0	15.0757	-1.36	109.492

Table 3: Results of TOPKAT properties

S. No	Compound Name	NTP Carcinogenicity Call (Male Mouse) (v3.2)	NTP Carcinogenicity Call (Female Mouse) (v3.2)	Developmental Toxicity Potential (DTP) (v3.1)	Skin Irritation (v6.1)	Ames Mutagenicity (v3.1)
1	**4a**	0.000	0.099	0.053	0.000	0.000
2	**4b**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
3	**4c**	0.000	0.099	0.000	1.000	0.000
4	**4d**	0.000	0.099	0.053	0.000	0.000
5	**4e**	0.000	0.099	0.000	1.000	0.000
6	**9**	0.000	0.099	0.053	0.000	0.000
7	STD	1.000	0.000	0.000	1.000	0.000

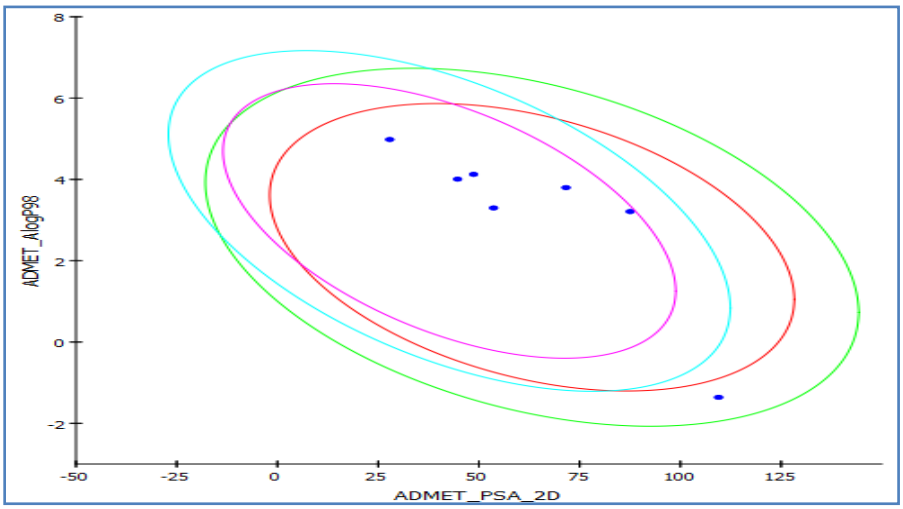
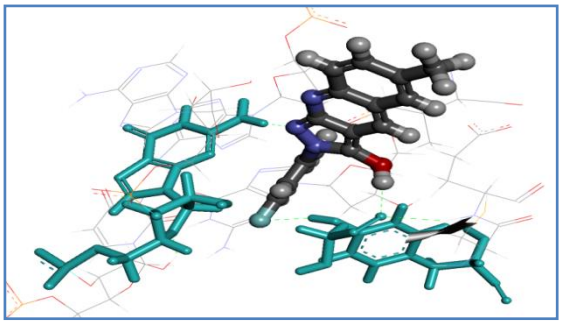
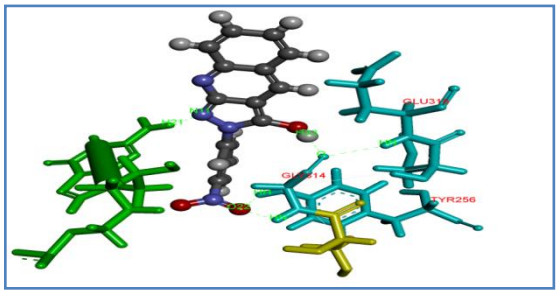


Fig. 2: Results of ADME properties with synthesized and ascorbic acid compounds

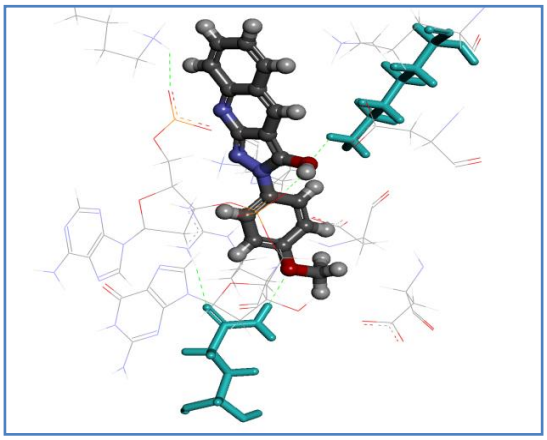
The investigated compounds were subjected to a molecular modeling study to evaluate their recognition profile at the hTERT binding-pocket. The results indicated that synthesized **4b** compound had the least energy (-23.012 score) and exhibited hydrogen bond interaction with TYR256, GLY314, GLU318 and ARG340 residues (Fig 3).



4a



4b



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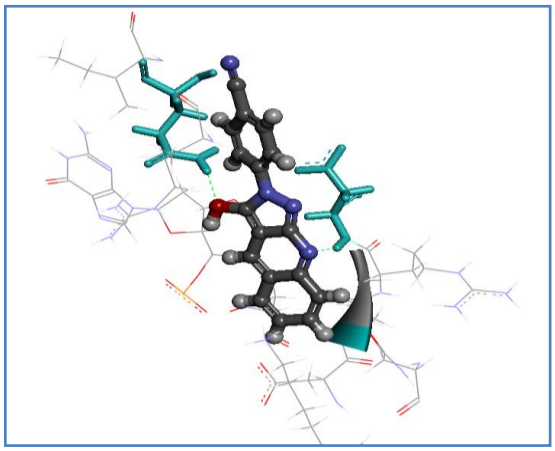

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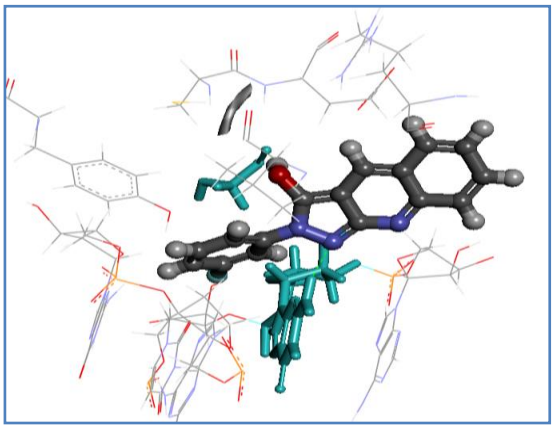

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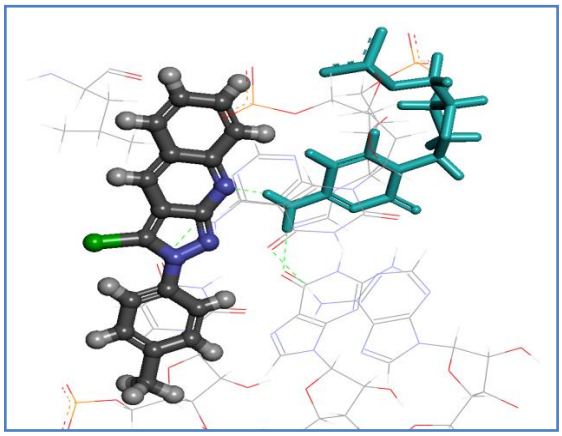

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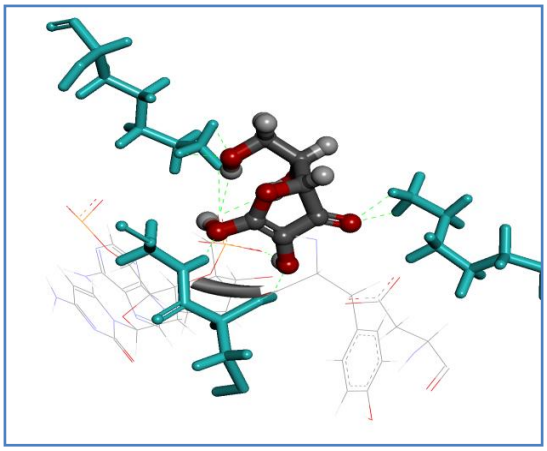

Ascorbic acid

*Balls and Stick model – Ligand molecules

*Stick model (Blue color) – Interacted aminio acids

Fig. 3: Protein – ligand interaction

DNA binding residues are TYR256, GLY314, GLU318 and ARG340. Compound **4b** binds to the above amino acid residues and perhaps inhibits the oxidative stress.

CONCLUSIONS

Different hydroxylated pyrazolo[3,4-*b*]quinolones were prepared. Acquired molecular docking data for the final compounds against human telomerase (hTERT). It was observed that, the compound **4b** has least energy (-23.012 score) perhaps due to good affinity with active site residue of human telomerase (hTERT).

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