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Research Article

SYNTHESIS,CHARACTERIZATION, ANTI- PROSTATE CANCER DOCKING AND IDENTIFICATION OF TARGET SITE OF VARIOUS MANNICH BASES OF QUINAZOLINONES

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ABSTRACT

Aim: Cancer is a serious disease that not fully defined now-a-days. The problem of cancer is the metastatis, and the unnatural proliferation of the cell. Many chemotherapeutic agents are used to treat cancer, but they failed to cure the cancer patient. They may prevent some of the portion but not totally defend against cancer. So our work devoted to design a novel chemotherapeutic agent with minimal sideeffects against prostate cancer.

Methods: Various mannich bases of 2,3-dihydroquinazolin-4(1H)-ones were synthesized in a three step process. The reaction proceeds via reductive cyclization for the formation of quinazolinone nucleus. Further, mannich bases were prepared by the reaction of quinazolinone parent nucleus with various aldehydes and secondary amines. Spectral studies such as IR, NMR and Mass were carried out. Docking was performed by the employment of AutoDock version 4.0 against Androgen receptor obtained from the PDB.

Results: Compounds 3d and 3e had shown good binding energy values and showed that they can act against prostate cancer.

Conclusion: Compounds 3d and 3e were found to be most active when compared to other synthesized derivatives with binding energies of -8.41Kcal/mol and -8.2Kcal/mol respectively. The yield of the two compounds was also satisfactory. Binding energy values obtained were very high when compared with previous analysis as per review of literature.

Keywords: Mannich base, Quinazolinone, Prostate cancer, Docking, PDB.

INTRODUCTION

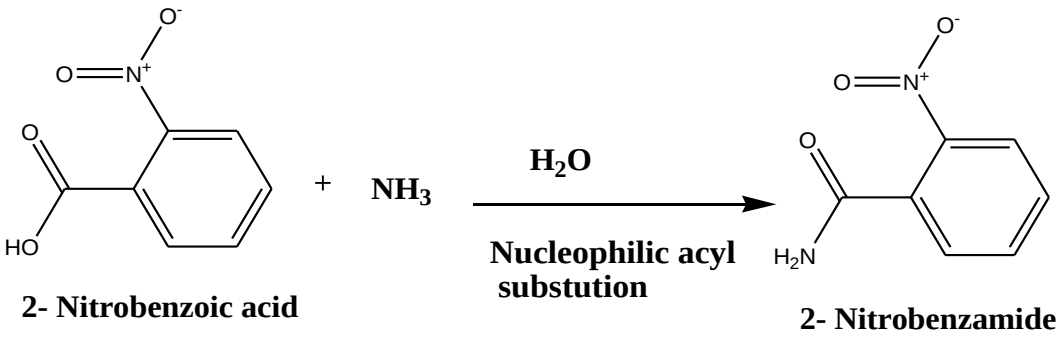
Quinazolines[1] are found to possess a broad spectrum of pharmacological responses, including applications for hypertension, diabetes, cancer, inflammation, ulceration, immunosuppression and also against microorganisms. The quinazoline moiety, in particular, is widely present in natural purines, alkaloids and in many biologically active compounds. This makes the quinazolines to possess a wide role in medicinal chemistry. Quinazoline derivatives have a therapeutic benefit as anti invasive agents with potential activity against all kinds of solid tumors, leukemia's etc. Some of the quinazoline derivatives are found to be highly effective against cancer. Trimetrexate (TMQ) and Piritrexim (PTX) are potent new generation DHFR inhibitors. It is also known that anilinoquinazolines are considered to be highly effective against tyrosine kinase activity. Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system. The cancer cells may metastasize (spread) from the prostate to other parts of the body, particularly the bones and lymph nodes. The androgen receptor[2,3](AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a type of nuclear receptorthat is activated by binding of either of the androgenic hormones testosterone or dihydrotestosteronein the cytoplasm and then translocating into the nucleus. Docking studies[4,5,6] is a modern tool of drug design and drug discovery. Through docking study, the activity of the synthesized compounds is assessed by using AutoDock[7,8] version, 4.0.

MATERIALS AND METHODS

Commercially available reagents and analytical grade solvents were used without purification. All the reactions were under controlled anhydrous condition. Melting points were determined by capillary method. The synthesized compounds were recrystallised using solvent ethanol. All the solvents, chemicals and reagents were obtained from Spectrochem Ltd, Mumbai. IR studies were performed using the instrument ALPHA- BRUKER and KBr as the reagent. NMR studies were carried out with the instrument AVANCE (500MHz)-BRUKER. DMSO was used as the solvent and TMS as the reference standard. MASS spectral studies were obtained by the help of the instrument JEOL GC mate. Docking studies were performed with the aid of Python Molecule Viewer (Pymol) and AutoDock version 4.0 in MGL tools1.5.4. Anticancer[9-11] studies were performed for these synthesized compounds against prostate cancer. Androgen receptor bearing the **pdb id: 1Z95** was taken and docking for the compounds was carried out against this receptor.

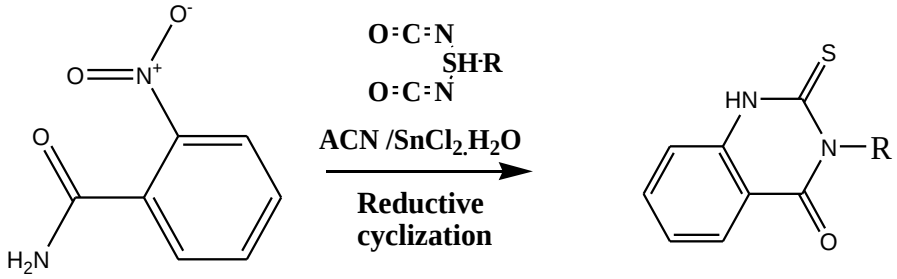
Synthesis scheme

Step-1



2- Nitrobenzoic acid **2- Nitrobenzamide**

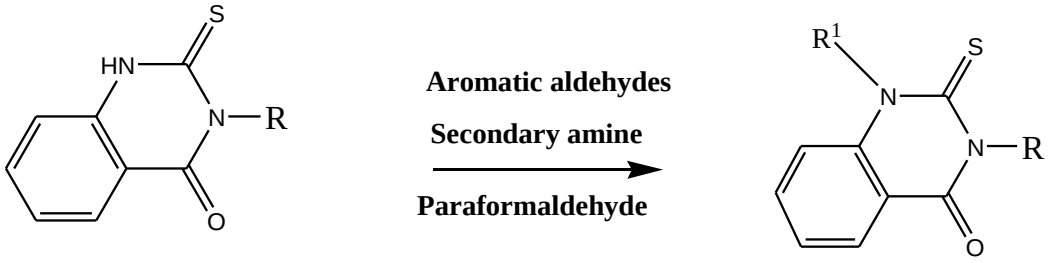
Step-2



2- Nitrobenzamide

3-R-2-thioxo-2,3-dihydroquinazolin-4(1H)-one

Step-3



3-R-2-thioxo-2,3-dihydroquinazolin-4(1H)-one

Mannic base
3a-3f

Synthesis of Mannich bases[12] of 2, 3-dihydroquinazolin-4(1H)-ones[13]

The synthesis commences as a three step reaction:

Step-I: The reaction involves the reaction of 2-nitrobenzoic acid with dil.ammonia which involves nucleophillic acyl substitution that results in the formation of 2-nitrobenzamide. The product was obtained in the form of crystals and recrystallized using ethanol.

Step-II: 2-nitrobenzamide obtained in the above step was added with phenyl thioisocyanate in the presence of acetonitrile and stannous chloride as the reaction medium. Reflux the mixture for 3hrs. This reaction proceeds via the reductive cyclization mechanism. The reaction proceeds by the formation of thioxo-dihydroqinazolin-4-one. The obtained compounds were recrystallised from ethanol. The commencement of the reaction was assessed through TLC analysis between the reactant and the product.

Step-III: This was the final step of the reaction which progressed by the reaction between thioxo-dihydroquinazolin-4-one and various substituted aldehydes and secondary amines in the presence of ethanol and p-formaldehyde. The mixture was refluxed for 6-7hrs. This resulted in the formation of various mannich base derivatives of thioxo-dihydroquinazoli-4-ones. Recrystallisation of the compound was carried out using ethanol. Further, TLC analysis was performed using silica gel plates and by employing ninhydrin as the visualizing agent.

Synthesis of various Mannich bases of dihydroquinazolin-4-ones

Preparation of compound 1: 2g of 2-nitrobenzoic acid was added to 5ml ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide was treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 3g of Tin(II)Chloride medium was used as the reductive system. Then the reaction mixture is allowed to reflux for 3hours. Add 15ml of 30% HCl to this solution, while the reaction mixture was hot. This filtered product was recrystallised with ethanol. This product was added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of 2-nitrobenzaldehyde in ethanol and reflux the resulting solution for 6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

Preparation of compound 2: 2g of 2- nitrobenzoic acid was added with ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide was treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 3g of Tin(II)Chloride medium was used as the reductive system. Then the reaction mixture was allowed to reflux for 3hours. Add 15ml 30% HCl to this solution, while the reaction mixture was hot. This filtered product was recrystallised with ethanol. This product was added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of 3,4,5-trimethoxybenzaldehyde in ethanol and reflux the resulting solution for 6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

Preparation of compound 3: 2g of 2- nitrobenzoic acid was added with ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide was treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 3g of Tin(II)Chloride medium was used as the reductive system. Then the reaction mixture was allowed to reflux for 3hours. Add 30% HCl to this solution, while the reaction mixture was hot. This filtered product was recrystallised with ethanol. This product was added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of p-chlorobenzaldehyde in ethanol and reflux the resulting solution for

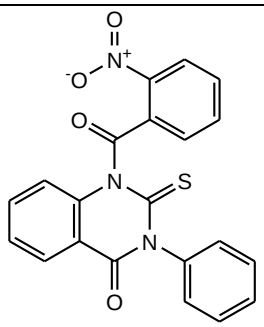
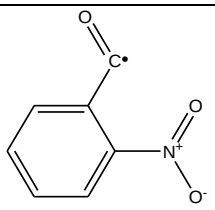
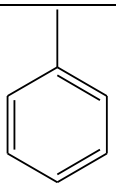
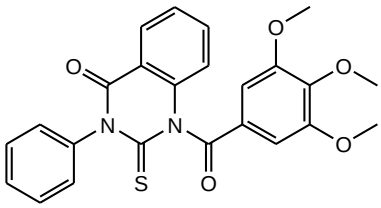
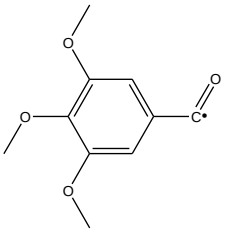
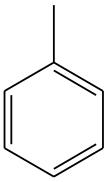
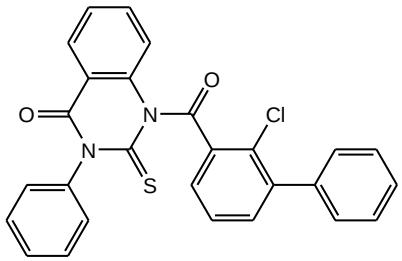
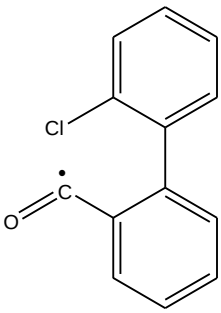
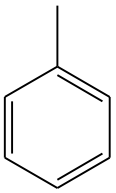
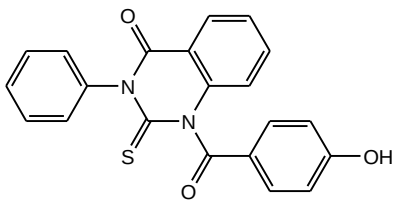
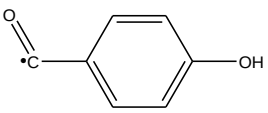
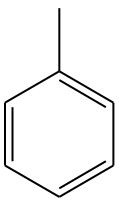
6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

Preparation of compound 4: 2g of 2 - nitrobenzoic acid was added with 5ml of ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide was treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 3g of Tin(II)Chloride medium was used as the reductive system. Then the reaction mixture was allowed to reflux for 3hours. Add 15ml of 30% HCl to this solution, while the reaction mixture was hot. This filtered product is recrystallised with ethanol. This product was added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of p-hydroxybenzaldehyde in ethanol and reflux the resulting solution for 6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

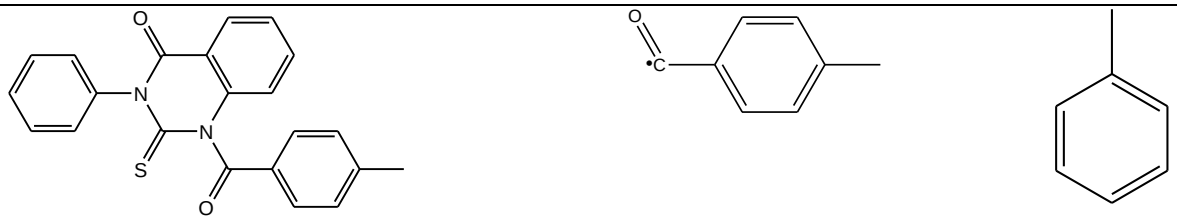
Preparation of compound 5: 2g of 2- nitrobenzoic acid was added with 5ml of ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide was treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 3g of Tin(II)Chloride medium was used as the reductive system. Then the reaction mixture was allowed to reflux for 3hours. Add 15ml of 30% HCl to this solution, while the reaction mixture was hot. This filtered product was recrystallised with ethanol. This product has been added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of 4-methylbenzaldehyde in ethanol and reflux the resulting solution for 6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

Preparation of compound 6: 2 g of 2- nitrobenzoic acid was added with 5ml of ammonia and the reaction yields 2-nitrobenzamide. 2-nitrobenzamide is treated with 5ml of phenyl thioisocyanate. Mixture of 10ml of Acetonitrile and 5ml of Tin (II) Chloride medium was used as the reductive system. Then the reaction mixture was allowed to reflux for 3hours. Add 15ml of 30% HCl to this solution, while the reaction mixture is hot. This filtered product was recrystallised with ethanol. This product was added with 10ml of paraformaldehyde and left to heat for 10min.till paraformaldehyde dissolves. To this solution, add 20ml ethanol and 10ml of dimethyl amine in ethanol and reflux the resulting solution for 6hours. Cool the solution and filter it. The obtained product was recrystallised with ethanol.

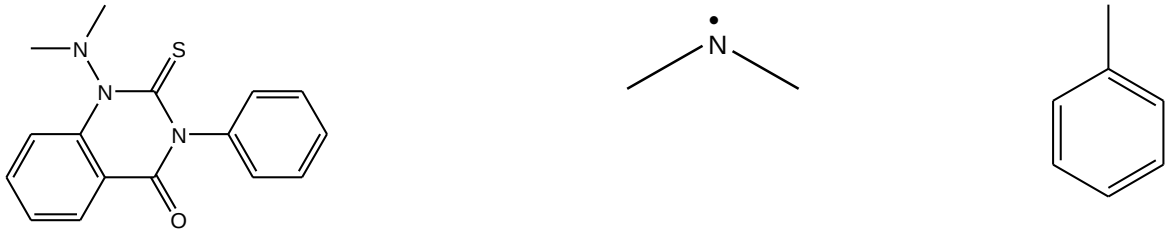
Substitution of compounds

No.	Structure	R1	R
3a			
3b			
3c			
3d			

3e



3f



Spectral Analysis

The structure of the synthesized compounds was characterized by IR, NMR and Mass

3a. 1-(2-nitrobenzoyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin -4(1H)-one: $C_{21}H_{13}N_3O_4S$, IR (KBr, cm^{-1}): 1705 cm^{-1}(N-C=O), 1606 cm^{-1} (N→O), 1445 cm^{-1} (C=S), 1538 cm^{-1} (C=C {aromatic}). NMR(DMSO) δ: 7.24(t, H-benzene), 7.82(m, H-Quinazolinone), 7.94(m, H-nitrobenzene). EI-MS m/z: 403.06.

3b. 1-(3,4,5 trimethoxy benzoyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one: $C_{24}H_{20}N_2O_5S$, IR (KBr, cm^{-1}), 1767cm^{-1} (N-C=O), 1530 cm^{-1} (C=S), 1034 cm^{-1} (C-O), 1621 cm^{-1} (C=O{amide}). ^{1}H-NMR(DMSO) δ: 3.73(t, CH_3), 7.62(m, H-Quinazolinone), 7.24(m, H-benzene). EI-MS m/z: 448.69.

3c. 1-(2-chloro phenyl benzoyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one: $C_{27}H_{17}ClN_2O_2S$, IR (KBr, cm^{-1}), 1795cm^{-1} (C=C-Cl), 1536 cm^{-1} (C=S), 1847 cm^{-1} (N-C=O), 1702 cm^{-1} (C=O{amide}). ^{1}H-NMR(DMSO) δ: 7.74(m, CH_2), 7.82(m, H-Quinazolinone), 7.64(m, H-benzene). EI-MS m/z: 468.61.

3d.1-(4-hydroxybenzoyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one: $C_{21}H_{14}N_2O_3S$, IR (KBr, cm^{-1}), 3223cm^{-1} (Ar-OH), 1315 cm^{-1} (C=S), 1664 cm^{-1} (N-C=O), 1407cm^{-1} (C=C{Ar}). ^{1}H-NMR(DMSO) δ: 5.05(s, -OH), 7.18(m, H-Quinazolinone), 7.49(m, H-benzene). EI-MS m/z: 374.61.

3e. 1-(4-methylbenzoyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin -4(1H)-one: $C_{22}H_{16}N_2O_2S$, IR (KBr, cm^{-1}), 1515 cm^{-1} (C=S), 1764 cm^{-1} (N-C=O), 1357cm^{-1} (C=C{Ar}). ^{1}H-NMR(DMSO) δ: 7.53(t, -CH_3), 7.48(m, H-Quinazolinone), 7.89(m, H-benzene). EI-MS m/z: 297.31.

3f. 1-(dimethylamino)-3-phenyl-2-thioxo-2,3-dihydroquinazolin -4(1H)-one: $C_{16}H_{15}N_3OS$, (KBr, cm^{-1}), 1605cm^{-1}(N-H), 1576 cm^{-1} (C=S), 1354 cm^{-1} (N-C=O), 1557cm^{-1} (C=C{Ar}).

RESULTS AND DISCUSSION

Anti- prostate cancer docking

Docking studies for the synthesized compounds were carried out against androgen receptor for bearing the PDB id- 1Z95 by the use of molecular docking softwares such as PYMOLWIN and AutoDOCK. It proceeded with the preparation of receptor and preparation of ligand. Receptor molecule was obtained from protein data bank (pdb). The receptor was selected based on the activity to be performed. Ligand molecules were drawn in Chemsketch software and further used for docking. Both receptor and ligand molecules were saved in pdb format to perform docking. Binding energy and hydrogen bond interactions were observed through docking studies and found that all the compounds showed good binding interaction with the receptor. The study also showed in **Figure-1** and **Figure-2** that METHIONINE745 is the target site for binding of the compounds 3d and 3e, for anti prostate cancer activity. **Table-1** shows the docking score of the synthesized compounds.

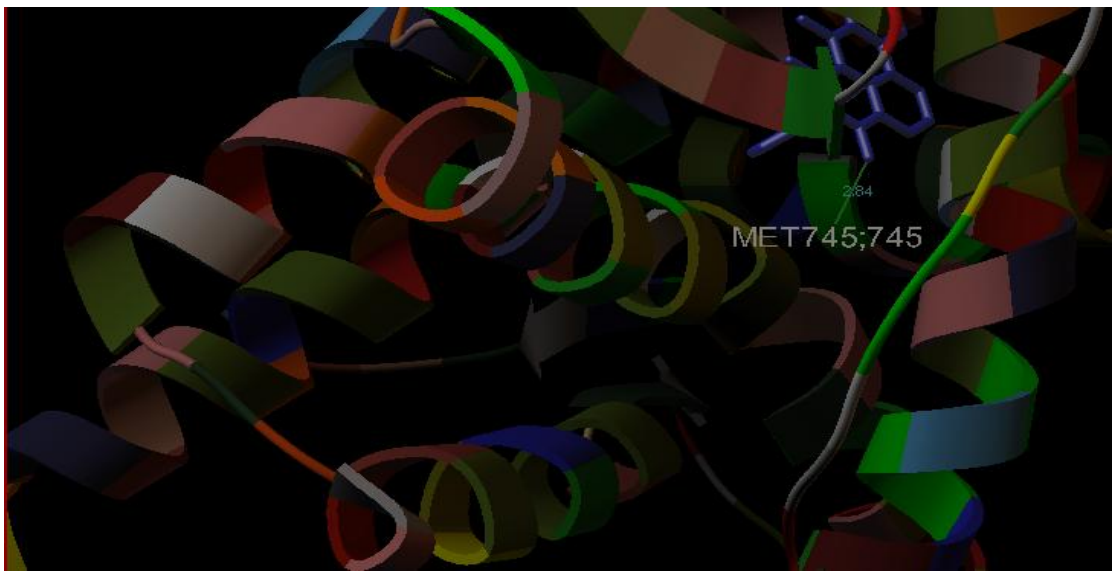


Fig. 1: Docking analysis of compound 3d

Table 1: Docking score of the synthesized compounds

Compound	Binding Energy (Kcal/mol)	Inhibitory constant	The best binding pose (Hydrogen bond interaction and distance)	No. of H-bond interactions (score)	Rank (Based on binding energy)
3a	-7.44	3.51uM	VAL685: 1.96 Å ASN756: 2.055 Å	10	3
3b	-6.79	55.34 uM	VAL685: 2.142 A°	5	5
3c	-7.4	532.78 nM	GLU681: 2.29 A°	6	4
3d	**-8.41**	**689.67 nM**	**MET745: 2.84Å**	**10**	**1**
3e	**-8.2**	**976.91 nM**	**MET745: 2.744A°**	**10**	**2**
3f	-6.31	23.88uM	-----	----	6

Docking studies showed of compound 3d proved to possess the highest binding energy of -8.41 k.cal/mol. Hydrogen bonding was observed and a hydrogen bond length of 2.84A° is noted. Binding interaction was noticed with MET745 as residue.

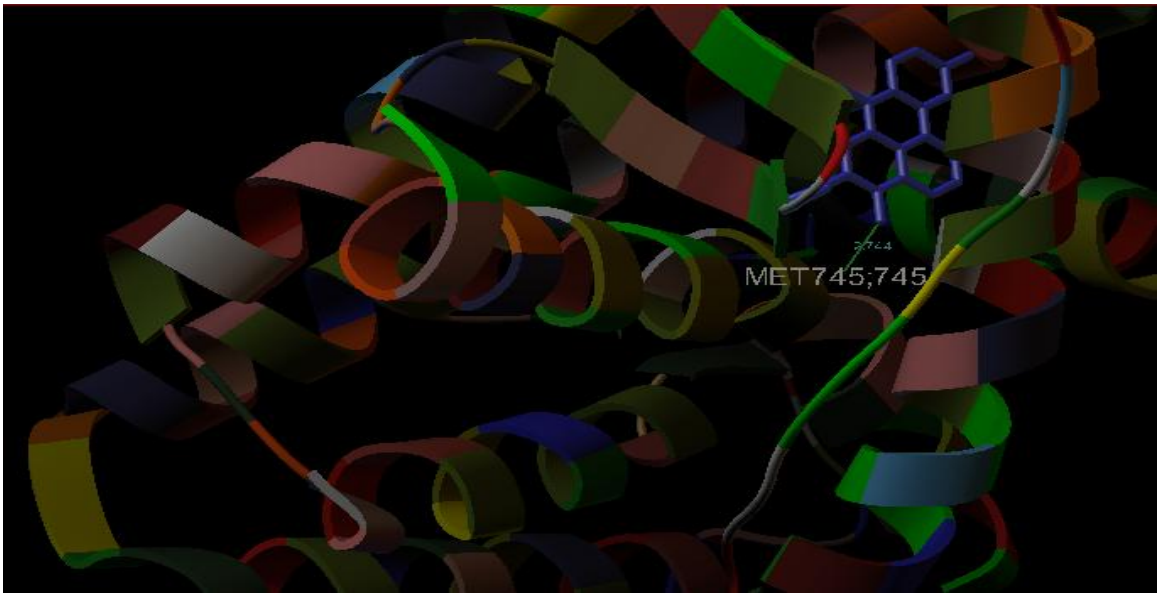


Fig. 2: Docking analysis of compound 3e

Docking study of compound 3e showed binding energy of -8.2 k.cal/mol. A hydrogen bond length of 2.744A° was observed with MET745 as the residue.

The derivatives were synthesized using solution phase synthesis and the physical data are given in **Table-2**.

Table 2: Physiochemical properties of the synthesized compounds

S. No.	Code of the compound	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	R_f Values
1	3a	$C_{21}H_{13}N_3O_4S$	403.41	246-247	90	0.66
2	3b	$C_{24}H_{20}N_2O_5S$	448.49	262-263	85	0.50
3	3c	$C_{27}H_{17}ClN_2O_2S$	468.95	248-249	82	0.63
4	3d	$C_{21}H_{14}N_2O_3S$	374.41	264-265	93	0.60
5	3e	$C_{22}H_{16}N_2O_2S$	372.44	255-256	80	0.49
6	3f	$C_{16}H_{15}N_3OS$	297.37	258-259	63	0.59

CONCLUSION

Drug designing is a modern tool to develop many drug entities of desired pharmacological activity. Employment of softwares in the prediction of the target site is a novel technique which reduces time and is cost efficient. Docking study is a new advancement in the field of molecular modeling and is a novel tool for predicting the activities of the compounds along with the target site.

Quinazolone derivatives were found to possess a broad spectrum of activities. In the present work, mannich bases of thioxo-dihydroquinazolones were synthesized via three step process. Quinazolone nucleus was synthesized by the reaction between 2-nitrobenzamide and phenylthioisocyanate in the presence of acetonitrile and stannous chloride as the reductive system. The mechanism of reaction through which quinazolone nucleus was obtained is Reductive Cyclization. Further, mannich bases of these

quinazolone were synthesized by employing various aldehydes and secondary amines. Yield of mannich bases obtained by the reaction between quinazolone and aldehydes was satisfactory when compared with the yield of mannich bases obtained by the reaction between quinazolone and secondary amines. Various spectral studies such as IR, NMR and MASS were carried out for structural elucidation of the synthesized compounds. Spectral studies proved the presence of various functional groups.

Docking studies for the synthesized compounds were carried out against androgen receptor for bearing the PDB id- 1Z95 by the use of molecular docking softwares such as PYMOLWIN and AutoDOCK and found that all the compounds showed good binding interaction with the receptor. The study also showed that METHIONINE745 is the target site for binding of the drug to show anti cancer activity i.e. against Prostate cancer.

Compounds 3d and 3e were found to be most active when compared to other synthesized derivatives with binding energies of -8.41Kcal/mol and -8.2Kcal/mol respectively. The yield of the two compounds was also satisfactory. Binding energy values obtained were very high when compared with previous analysis as per review of literature.

A further, in vivo study of the synthesized compounds has to be carried out to confirm the pharmacological activity.

ACKNOWLEDGEMENT

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