



ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2018, 10(10): 125-131
(<http://www.derpharmachemica.com/archive.html>)

The In-Silico Screening of Pyrazolo[3,4-E][1,4]Thiazepine Derivatives as Potent Cytotoxic Agents

Srikanth A¹, Hemanth Kumar P¹, Vijayakumar V¹, Sudha², Anand A^{1,2}, Sarveswari S^{1*}

¹Centre for Organic and Medicinal Chemistry, VIT University, Vellore 632 014, India

²Medical and Biological Computing Laboratory, School of Biosciences and Technology, VIT University, Vellore 632 014, India

ABSTRACT

A new series of 1, 4-thiazepinone incorporated pyrazolopyridines has been synthesized using L-proline as a catalyst through multi-component reaction which in turn subjected to the in-silico cytotoxicity studies against various cancer cell lines and almost all the compounds found to show good to moderate activity. Among the tested compounds, compound 4f and 4k are found to have least binding energy value and Z-score value. These compounds found to form more stable ligand-receptor complex amongst other compounds.

Keywords: 1,4-thiazepine derivatives, L-proline catalysis, C-S bond formation, Docking studies.

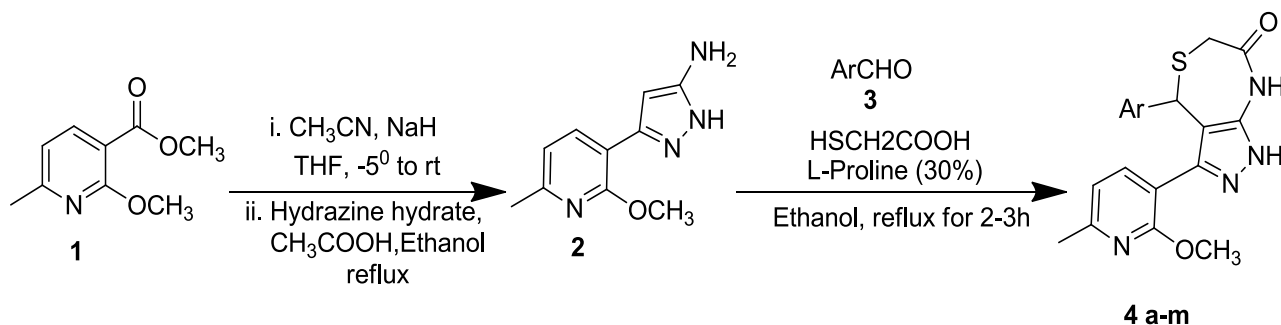
INTRODUCTION

Design and synthesis of new lead structures essentially interacting with novel biological targets are the major challenges for medicinal chemists and biologists. It is necessary to design target oriented synthesis for the development of new molecules even though a large number of drugs are in clinical trials [1-3]. There is a considerable research over design of new drugs which are more selective for cancer cells with lesser side effects. Chemotherapy in cancer treatment is utilizing the intracellular barrier crossing property of drugs [4,5].

3,4-di hydro-5-oxo-1,4-benzothiazepine alkyl derivatives were described as HIV-1 enzyme integrase [6], reverse transcriptase inhibitors [7], and antitumor agents [8]. The core unit hetero ring systems are potent inhibitors of HSV-1 replication [9], compounds possessing H1 antihistamine activity [10], selective antagonists of 5-HT1A and dopamine D2 receptors [11]. Substrate organization, transition state stabilization and product release are the characteristic features of enzymes and catalysts, where convergent construction of molecular frame works for complex skeletons, eco-friendliness and chemo selectiveness are additive features.

MATERIALS AND METHODS

We attempted an L-proline catalyzed synthesis of 1, 4-thiazepinones (Scheme 1) and screened them for their cytotoxic activity [12] based on target binding ability observed in in-silico studies (Table 1). The formation of C-S bond is a common functionality found in various pharmaceutically active and bio molecules [13,14] as the viscous mass.



Scheme 1: L-proline catalyzed synthesis of 1, 4-thiazepine fused pyrazolines (4a-m)

Table 1: Optimization of 1,4-thiazepine fused pyrazolines (4a-m) in ethanol solvent medium

Compound	ArCHO (3a-m)	Time (Hour)	Yield (%) ^b	MP (°C)
4a	2-Methoxybenzaldehyde	2	92	123-125
4b	3, 4, 5-Trimethoxybenzaldehyde	2	89	115-117
4c	3-Nitrobenzaldehyde	2.45	76	130
4d	2,5-Dimethoxybenzaldehyde	2	90	118-120
4e	2,4-Dichlorobenzaldehyde	3	79	238-240
4f	4-Cyanobenzaldehyde	2.3	72	196-198
4g	2-Nitrobenzaldehyde	2.3	75	132-136
4h	3,4-Dimethoxybenzaldehyde	2.15	88	218-220
4i	2, 4, 6-trimethoxybenzaldehyde	2.3	91	110-112
4j	4-Chlorobenzaldehyde	2	86	120
4k	2-Fluorobenzaldehyde	3	82	138-141
4l	4-Methylbenzaldehyde	2	89	190-192
4m	4-Methoxybenzaldehyde	2.15	87	180-182

RESULTS AND DISCUSSIONS

The newly synthesized 1, 4-thiazepines 4a-m was characterized [12] by Proton Nuclear Magnetic Resonance (¹H-NMR), Carbon-13 Nuclear Magnetic Resonance (¹³C-NMR) and HRMS (Figure 1).

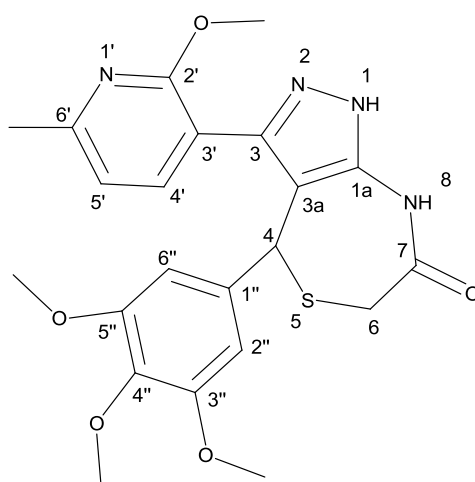


Figure 1: Numbering the position of atoms of 3-(2-methoxy-6-methylpyridin-3-yl)-4-(3,4,5-trimethoxyphenyl)-6,8-dihydro-1H-pyrazolo[3,4-e][1,4]thiazepin-7(4H)-one (4b)

In silico studies of Pyrazolo pyridine fused 1,4-thiazepines (4a-m)

Newly synthesized compounds 4a-m has been subjected to the in-silico studies towards six selected target proteins. The 3-dimension (3D) coordinates of six cancer target proteins were selected and obtained from protein data bank (PDB) [15]. The PDB id 3B8Q (renal cancer) and 4FLH (colon cancer), 1SVC (pancreatic cancer), 1MOX (lung cancer), 2DSQ (normal breast epithelium cancer) and 3TWJ (non-small cell lung cancer) were selected for structural analysis based on its high resolution crystallographic structure. The co-crystallised ligands and water molecules were identified and removed from the atomic coordinate file and the structure was optimized and refined using spdbv viewer [16]. The synthesized compounds structure was sketched with the help of chemsketch [17]. 3-dimensional conversion and geometry optimization of all the compounds were performed using chimera for flexible conformations of the compounds during the docking [18]. Data are given in Tables 2-7. iGEMDOCK2.1(A generic evolutionary method for molecular docking) docking software was used to study the detailed intermolecular interactions between the target protein and the ligand molecule [19]. iGEMDOCK integrated the molecular docking, post screening analysis and visualization steps. We selected Rho-associated protein kinase 1, (PDB Id: 3TWJ), nuclear factor kappa-b (nf-kb) (PDB Id: 1SVC), vascular endothelial growth factor receptor-2 (PDB Id: 3B8Q) and human phosphoinositide 3-kinase (PI3K-gamma) (PDB Id: 4FLH), crystal structure of human epidermal growth factor receptor (PDB id: 1MOX) and crystal structure of the tyrosine kinase domain of the hepatocyte growth factor receptor (PDB Id: 3CE3) as target proteins to carry out the docking analysis of our synthesized compounds. The 3D atomic coordinates of each therapeutic target protein were implemented through the GEMDOCK graphical environment interface. Then the default option was used to import the 3D coordinates of 13 synthesized compounds. Before docking the output path was set. GEMDOCK default parameters included the population size (n=200), generation (g=70) and number of solutions (s=10) to compute the possible binding conformation of synthesized compounds. Then the docking run was started using Gemdock scoring function. After docking, the individual binding conformation of each ligand was observed and their binding affinity with the target proteins was analyzed. The best binding pose and binding energy of each ligand was selected. In the post docking analysis, Vander Waals score, Z-score and the details of interacted residues were saved in output folder. Protein-ligand binding site was analysed and visualized using pymol [20].

The binding affinity of 13 synthesised compounds towards nuclear factor kappa-b has been investigated and compounds 4f and 4k found to exhibit best binding conformation with nuclear factor kappa-b. The binding energy and Z-score values of compound 4f and 4k are given in Table 2. On analysis of the interaction and position of compounds 4f and 4k in the nuclear factor kappa-b binding site, it is observed that compound 4f forms three H-bonds with nuclear factor kappa-b and compound 4k shows three hydrogen bond interaction with the amino acid residues such as Ser 81, Ser 74 and Gly 72. The binding pose and interaction mode of compound 4f and 4k are shown in Figures 2 and 3.

Table 2: Docking results of synthesized compounds in the binding site nuclear factor kappa-b (pancreatic cancer)

Sample Code	Z Score E (pharma)	Total Energy	VDW
4a	-132.3	-106.1	-94.9414
4b	-104.8	-104.8	-80.6809
4c	-107.9	-107.9	-81.1659
4d	-139.6	-105.4	-110.049
4e	-100.4	-100.4	-69.5653
4f	-159.5	-120.5	-115.7825
4g	-110.2	-115.3	-75.694
4h	-111	-111	-81.1596
4i	-107.9	-109.9	-108.515
4j	-136.4	-108.5	-101.622
4k	-120	-116.5	-88.9061
4l	-103.5	-104	-82.4252
4m	-100.4	-102.2	-76.556

The binding energy is calculated for 13 synthesized ligand molecules with vascular endothelial growth factor receptor 2 which has play important role renal cancer. From the docking results, compound 4f and compound 4k has shown best conformation with vascular endothelial growth factor receptor 2. The binding energy and Z-score value of compound 4f and compound 4k is given in Table 3. Compound 4f binds to binding site of vascular endothelial growth factor receptor 2. It can be seen in Figure 4 and the nitrogen atoms of compound 4f forms only one hydrogen bond with Glu 885. On analysis of the interaction and position of compound 4k in the vascular endothelial growth factor receptor 2 binding site, it is observed that compound 4k forms six H-bonds with Lys1070, Tyr1106, Pro1105, Ser1104 and Ala1103. The best possible binding mode of compound 4f and 4k with vascular endothelial growth factor receptor 2 corresponding 2D interaction models are depicted in Figures 4 and 5.

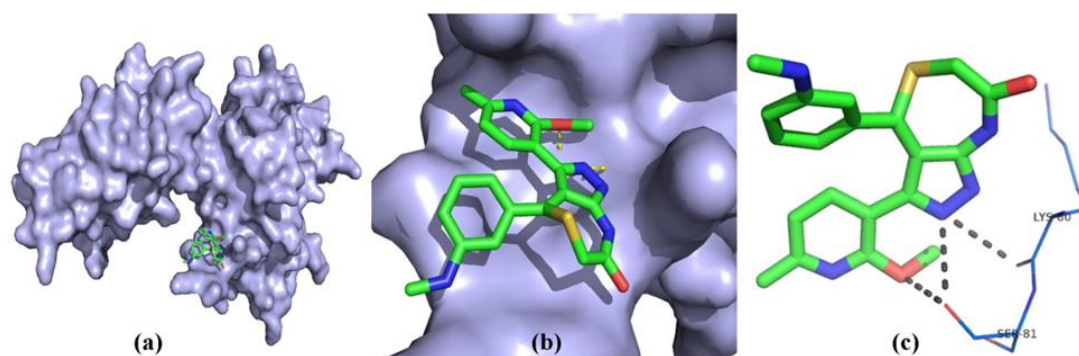


Figure 2: Docking results of compound 4f (a) Binding pose of compound 4f in the Nuclear factor kappa-b. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

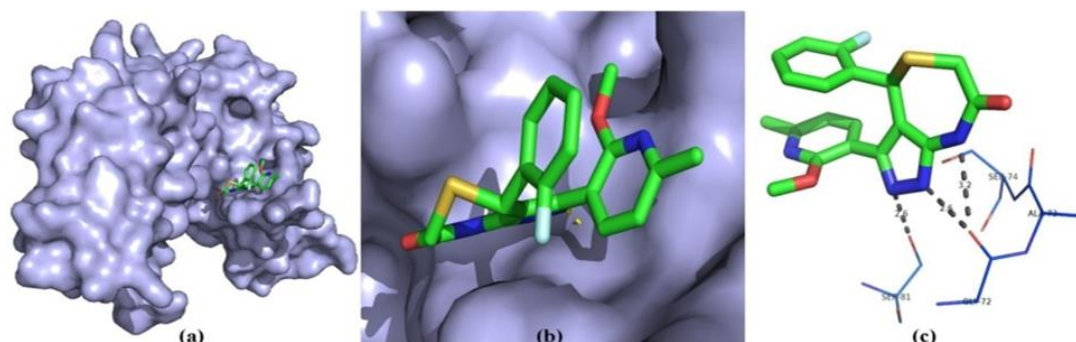


Figure 3: Docking results of compound 4k (a) Binding pose of compound 4k in the Nuclear factor kappa-b. (b) A close-up view of the binding pose of compound 4k; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown.

Table 3: Docking results of synthesized compounds in the binding site vascular endothelial growth factor receptor 2 (Renal cancer)

Sample Code	Z Score E(pharma)	Total Energy	VDW
4a	-117.7	-96.8184	-82.7337
4b	-135.1	-107.591	-96.591
4c	-104.4	-101.006	-77.4242
4d	-105.3	-103.284	-77.612
4e	-138.8	-109.491	-109.491
4f	-150.6	-124.516	-118.3808
4g	-138.3	-107.206	-100.556
4h	-140	-110.913	-107.413
4i	-141.7	-107.642	-97.5849
4j	-101.1	-97.7	-106.654
4k	-148.2	-118.243	-102.321
4l	-107.4	-105.821	-98.458
4m	-130.2	-108.287	-101.347

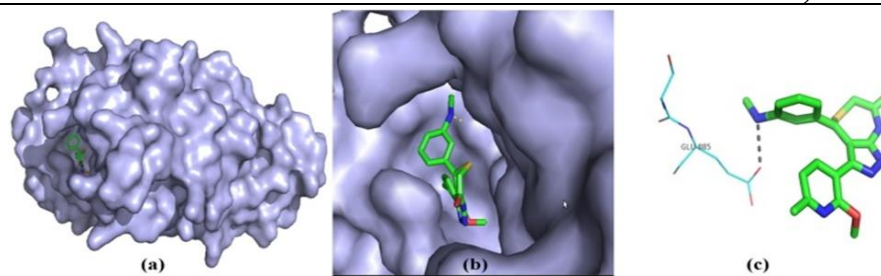


Figure 4: Molecular docking result of compound 4f (a) Binding pose of compound 4f in the vascular endothelial growth factor receptor 2. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

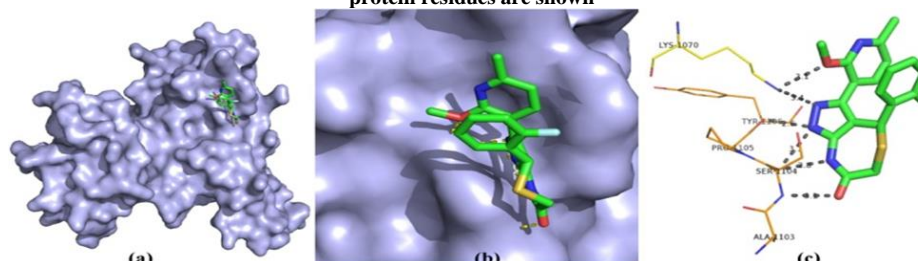


Figure 5: Molecular docking results of compound 4k. (a) Binding pose of compound 4k in the vascular endothelial growth factor receptor 2. (b) A close-up view of the binding pose of compound 4k; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

The binding energy is calculated by iGEMDOCK for synthesised compounds. Compound 4f and 4k has shown best conformation with human phosphoinositide-3-kinase involved in colon cancer. The binding energy and Z-score value of the compound 4f and 4k is given in Table 4. Compound 4f binds to the binding site and forms five hydrogen bonds with human phosphoinositide-3-kinase. Glu176, Ser342, Arg403, Ile341 and Glu293 are involved in the interaction. From the analysis of interaction and position of compound 4k in the human phosphoinositide-3-kinase, it is observed that four hydrogen bonds are found and the amino acid residues Tyr1050, Asp946, Gly945 and Ile944 and Tyr1067 are participated in the interaction. The hydrogen bond network and binding pose of compound 4f and compound 4k are shown in Figures 6 and 7.

Table 4: Docking results of synthesized compounds in the binding site human phosphoinositide-3-kinase (pi3k)-gamma (colon cancer)

Sample code	Z Score E(pharma)	Total Energy	VDW
4a	-171.5	-113.767	-85.8677
4b	-128.7	-117.116	-101.019
4c	-122.2	-114.124	-82.8858
4d	-146.2	-108.713	-88.4499
4e	-146.2	-122.03	-101.328
4f	-176.3	-130.02	-101.1308
4g	-176.8	-109.369	-70.0512
4h	-156.8	-117.387	-70.187
4i	-134.1	-123.515	-90.7239
4j	-156.6	-113.767	-85.8677
4k	-176.6	-124.116	-113.019
4l	-109.4	-120.211	-100.337
4m	-147.5	-108.713	-88.4499

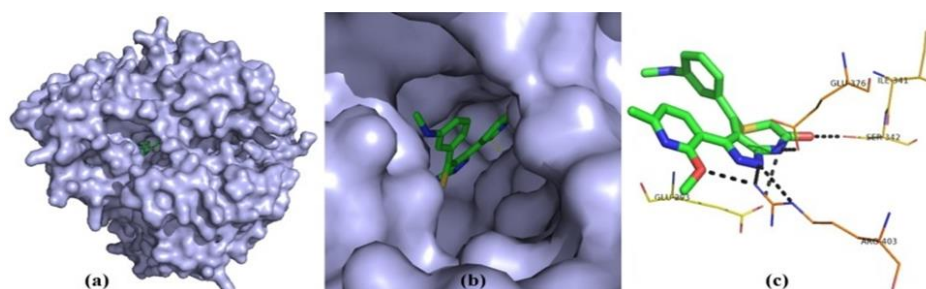


Figure 6: Molecular docking results of compound 4f. (a) Binding pose of compound 4f in the Human phosphoinositide-3-kinase (pi3k)-gamma. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown.

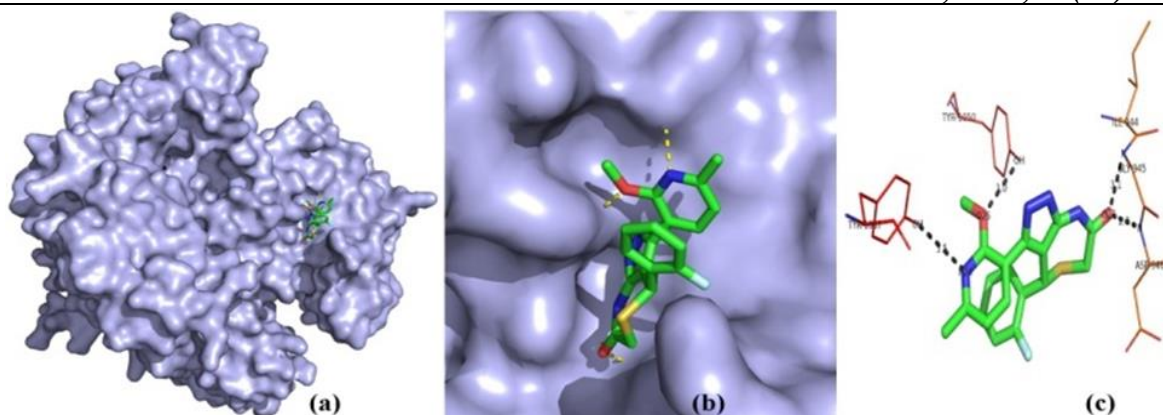


Figure 7: Molecular docking results of compound 4k. (a) Binding pose of compound 4k in the Human phosphoinositide-3-kinase (pi3k)-gamma. (b) A close-up view of the binding pose of compound 4k; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

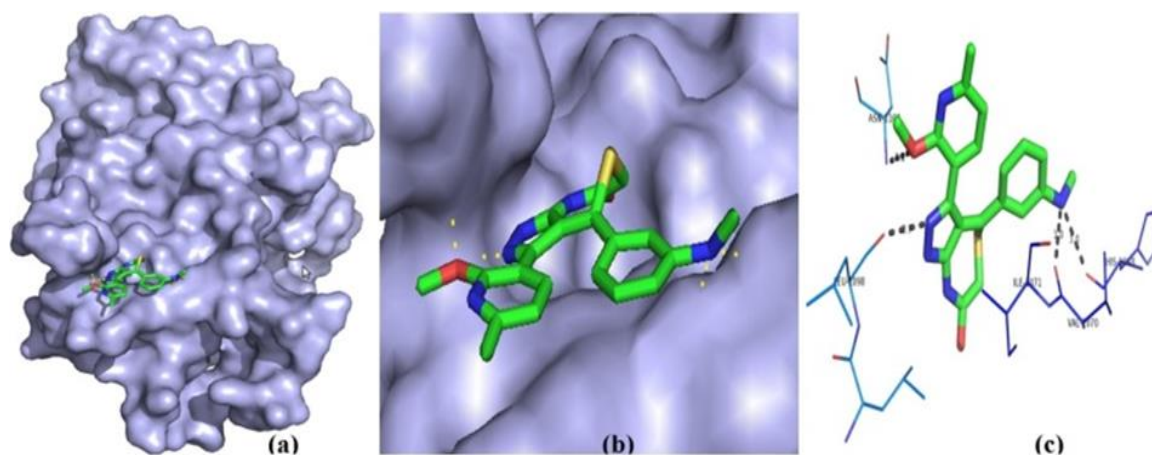


Figure 8: Molecular docking results of compound 4f. (a) Binding pose of compound 4f in the Hepatocyte growth factor receptor. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

Table 5: Docking results of synthesized compounds in the binding site Hepatocyte growth factor receptor (Non-small cell lung cancer)

Sample code	Z Score E (pharma)	Total Energy	VDW
4a	-132.4	-101.064	-83.905
4b	-104.2	-110.164	-89.7346
4c	-111.8	-109.114	-75.5307
4d	-127.4	-99.2605	-83.5389
4e	-106.6	-104.302	-84.7897
4f	-112.2	-119.094	-89.0001
4g	-110.4	-110.386	-67.4132
4h	-109.6	-108.446	-79.125
4i	-147.1	-102.852	-90.1644
4j	-125.6	-109.055	-89.5523
4k	-130.7	-85.516	-97.7281
4l	-115	-105.664	-83.4292
4m	-132.4	-108.867	-91.9362

Docking analysis of compound 4f shows best binding conformation with hepatocyte growth factor receptor which has crucial role in non-small cell lung cancer (energy=-119.094 K. cal/Mol, Z-score=-112.2) (Table 5). From the Figure 8, it has shown that the compound 4f forms four hydrogen bonds with hepatocyte growth factor receptor. The binding pose and hydrogen bond network of compound 4f in hepatocyte growth factor receptor are shown in Figure 8.

The post docking analysis of compound 4f shows best binding pose in epidermal growth factor receptor which has key role in lung cancer development (Total energy=-127.3 Kcal/Mol, Z-score=-136.3) (Table 6). Compound 4f binds to epidermal growth factor receptor and form three H-bond interactions with His658, Gln846 and Gln291 residues. The best binding pose of compound 4f in the epidermal growth factor receptor and corresponding 2D interaction models, hydrogen bonds and bond distance are depicted in Figure 9.

Table 6: Docking results of synthesized compounds in the binding site of epidermal growth factor receptor (human epidermal growth factor receptor; lung cancer)

Sample code	Z Score E (pharma)	Total Energy	VDW
4a	-175.9	-124.5	-93.7778
4b	-121.7	-121.7	-103.5
4c	-122.6	-120.6	-78.7755
4d	-113.9	-113.9	-94.5327
4e	-152.4	-105.5	-84.8099
4f	-136.3	-127.3	-81.0741
4g	-166.8	-115.5	-88.6983
4h	-157.9	-108.4	-83.0514
4i	-112.3	-112.3	-74.7006
4j	-105.8	-105.8	-88.7077
4k	-160.1	-95.1	-98.4659
4l	-164.7	-117.9	-87.9991
4m	-136.5	-102.1	-81.0066

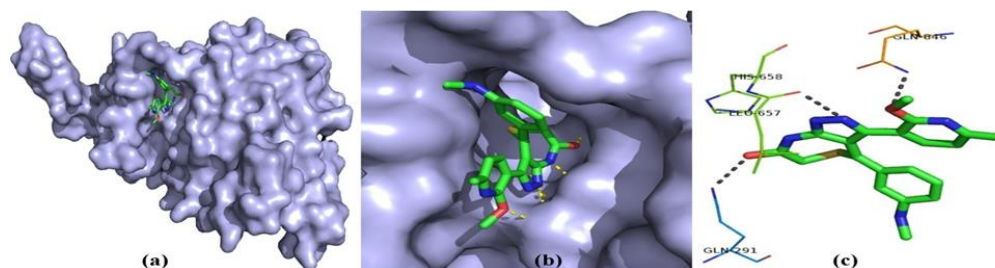


Figure 9: Molecular docking results of compound 4f. (a) Binding pose of compound 4f in the epidermal growth factor receptor. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

From the docking analysis of synthesized compounds and Rho-associated protein kinase, compound 4f has high binding affinity (energy=-105.169 Kcal/Mol, Z-score=-183.5) and shows three hydrogen bond interactions with the residues Thr219, Arg197 and Ala234. The docking results of all 13 ligand molecules with Rho-associated protein kinase are given in Table 7. The possible binding mode of compound 4f in the Rho-associated protein kinase binding site and corresponding 2D interaction models, hydrogen bonds are shown in Figure 10.

Table 7: Docking results of synthesized compounds in the binding site of Rho-associated protein kinase (Normal breast epithelium cancer)

Sample code	Z Score E (pharma)	Total Energy	VDW
4a	-173.6	-101.931	-98.0808
4b	-181	-90.642	-103.906
4c	-183.3	-97.482	-92.023
4d	-180.8	-100.698	-103.063
4e	-169.7	-94.953	-88.1788
4f	-183.5	-105.169	-102.391
4g	-119.7	-101.374	-92.0233
4h	-171.7	-92.479	-86.6902
4i	-159.2	-103.653	-89.329
4j	-112	-96.03	-91.1223
4k	-120.8	-80.364	-80.9456
4l	-156	-102.064	-95.9908
4m	-103	-101.509	-96.8364

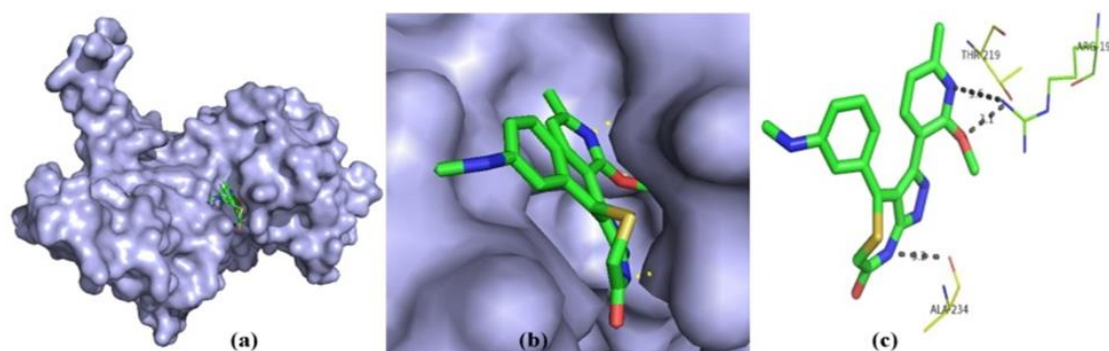


Figure 10: Molecular docking results of compound 4f. (a) Binding pose of compound 4f in the Rho-associated protein kinase. (b) A close-up view of the binding pose of compound 4f; Protein structure is shown in surface model and the ligand is shown in stick model. (c) H-bond network with protein residues are shown

Among the 13 compounds, compound 4f and 4k are found to have least binding energy value and Z-score value. These compounds found to form more stable ligand-receptor complex amongst other compounds. Compound 4f shows best binding pose with all six selected cancer target proteins and compound 4k shows best binding pose with nuclear factor kappa-b (pancreatic cancer), vascular endothelial growth factor receptor 2 (renal cancer) and human phosphoinositide-3-kinase (colon cancer). In examining the interaction of compound 4f with all six cancer target proteins, it is observed that compound 4f shows more than three hydrogen bond interactions with the target proteins except vascular endothelial growth factor receptor 2.

CONCLUSION

In summary a series of pyrazolopyridine fused 1, 4-thiazepinones has been synthesized using MCR with L-proline as a catalyst, which in turn subjected to the in-silico studies against various cancer cell lines and almost all the compounds found to possess good to moderate activity. Among these, the compounds 4f and 4k found to possess activity equal to the control in all tested cell lines docking analysis.

ACKNOWLEDGEMENT

The authors are thankful to VIT University Vellore, India for providing facilities to carry out research work, and also thankful to SAIF, Vellore Institute of Technology, SAIF, IIT-Madras and VIT-TBI for providing NMR, Mass and IR spectral facilities respectively.

REFERENCES

- [1] M.D. Burke, S.L. Schreiber, *Angew. Chem. Int. Ed.*, **2004**, 43, 46.
- [2] S. Oh, H.J. Jang, S.K. Ko, Y. Ko, S.B. Park, *J. Comb. Chem.* 2010, 12, 548.
- [3] S.L. Schreiber, *Science.*, **2000**, 287, 1964.
- [4] S.P. Gupta, *Chem. Rev.*, **1994**, 94, 1507.
- [5] P. Martins, J. Jesus, S. Santos, L.R. Raposo, C.R. Rodrigues, P.V. Baptista, A.R. Fernandes, *Molecules.*, **2015**, 20, 16852.
- [6] N. Neamati, J.A. Turpin, H.E. Winslow, J.L. Christensen, K. Williamson, A. Orr, W.G. Rice, Y. Pommier, A. Garofalo, A. Brizzi, G. Campiani, I. Fiorini, V. Nacci, *J. Med. Chem.*, **1999**, 42, 3334.
- [7] H. Maruenda, F. Johnson, *J. Med. Chem.*, **1995**, 38, 2145.
- [8] A. Garofalo, G. Campiani, I. Fiorini, V. Nacci, *Farmaco.*, **1993**, 48, 275.
- [9] S.L. Boulware, J.C. Bronstein, E.C. Nordby, P.C. Weber, *Antivir. Res.*, **2001**, 51, 111.
- [10] A.D. Cale, T.W. Gero, K.R. Walker, Y.S. Lo, W.J. Welstead, L.W. Jaques, A.F. Johnson, C.A. Leonard, J.C. Nolan, D.N. Johnson, *J. Med. Chem.*, **1989**, 32, 2178.
- [11] T. Nakao, H. Tanaka, H. Yamato, T. Akagi, S. Takehara, Patent 5, 141, 930, **1992**, 1.
- [12] A. Srikanth, S. Sarveswari, V. Vijayakumar, P. Gridharan, S. Karthikeyan, *Med. Chem. Res.*, 2014, 24, 553.
- [13] L. Liu, J.E. Stelmach, S.R. Natarajan, M. H Chen, S.B. Singh, C.D. Schwartz, C.E. Fitzgerald S.J. O'Keefe, D.M. Zaller, D.M. Schmatz, J.B. Doherty, *Bioorg. Med. Chem. Lett.*, **2003**, 13, 3979.
- [14] S.W. Kaldor, V.J. Kalish, J.F. Davies, B.V. Shetty, J.E. Fritz, K. Appelt, J.A. Burgess, K.M. Campanale, N.Y. Chirgadze, D.K. Clawson, B.A. Dressman, S.D. Hatch, D.A. Khalil, M.B. Kosa, P.P. Lubbehusen, M.A. Muesing, A.K. Patick, S.H. Reich, K.S. Su, J.H. Tatlock, *J. Med. Chem.*, **1997**, 40, 3979.
- [15] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, *Nucleic Acids Res.*, **2000**, 28, 235.
- [16] N. Guex, M.C. Peitsch, *Electrophoresis.*, **1997**, 18, 2714.
- [17] Z. Li, H. Wan, Y. Shi, P. Ouyang, *J. Chem. Inf. Comput. Sci.*, **2004**, 44, 1886.
- [18] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, *J. Comput. Chem.*, **2004**, 25, 1605.
- [19] J.M. Yang, C.C. Chen, *Proteins.*, **2004**, 55, 288.
- [20] D. Seeliger, B.L. de Groot, *J. Comput. Aided Mol. Des.*, **2010**, 24, 417.