EJPMR, 2026, 13(4), 135-151 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

IN-SILICO DRUG DESIGN AND SYNTHESIS OF NOVEL 2- AMINOTHIOPHENE AS ANTI-CANCER AGENTS

***Prof. Surabhi K. V.**

Associate Professor, Westfort College of Pharmacy.

***Corresponding Author: Prof. Surabhi K. V.**

Associate Professor, Westfort College of Pharmacy.

DOI: https://doi.org/10.5281/zenodo.19308906

How to cite this Article: *Prof. Surabhi K. V. (2026). In-Silico Drug Design And Synthesis Of Novel 2- Aminothiophene As Anti-Cancer Agents. European Journal of Biomedical and Pharmaceutical Sciences, 13(4), 135–151.

Article Received on 28/02/2026 **Article Revised on 18/03/2026** **Article Published on 01/04/2026**

ABSTRACT

Cancer is the major worldwide problem. It arises due to uncontrolled growth of cells. While there have been many remarkable breakthroughs in cancer research, there is still a need for the development of new therapies that target the different mechanisms of cancer cells. Therefore, the focus of our research is identifying novel hit molecules targeting Poly (ADP-ribose) polymerase 1(PARP-1). PARP-1 is a critical enzyme involved in DNA damage repair and recombination, and shows great potential for drug development in the treatment of cancers with defective DNA repair. Thiophene and its derivative can lead in the future to synthesize varieties of chemotherapeutic entities in the field of cancer treatment. Chemistry of 2-aminothiophenes is arguably one of the most extensive and dynamic field of present-day thiophene research. A series of novel PARP-1 inhibitor derivatives were designed and computationally optimized to investigate the maximum interaction between designed ligands and proteins from protein data bank (PDB) with an advantage of reduced cost and time. Docking studies were performed using PyRx software. All the designed ligands showed mild to excellent binding with protein(4RV6) and it was found that out of 4 designed ligand, PR2 ligand showed the best binding with 4RV6. Gewald reaction is the universal method for synthesis of substituted 2 aminothiophenes. Consequently, details about the proposed mechanism of Gewald-like reactions and the wide scope of substituted 2-aminothiophenes for real life applications. The substituted 2-aminothiophene-3-carboxamide was synthesized in our laboratory.

KEYWORDS: Anticancer activity, PARP 1, PDB, 2-aminothiophenes, PyRx, Gewald reaction.

TABLE OF CONTENTS

LIST OF TABLES

ADME	Absorption, Distribution, Metabolism, Excretion
ADP	Adenosine Diphosphate
ART	ADP Ribosyl Transferase
ATPC	2-Aminothiophenone-3- carboxylate
BER	Base excision repair
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
DMF	Dimethyl formamide
DNA	Deoxyribonucleic acid
DSB	Double stranded breaks
FDA	Food and Drug Administration
FEP+	Free energy perturbation
GLY	Glycine
HD	Helical domain

HRR	Homologous recombination repair
LID	Ligand interaction diagram
NAD+	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NSCLC	Non - small cell lung carcinoma
PAR	Protease activator receptor
PARP	Poly ADP ribose polymerase
PARPi	PARP inhibitor
PDB	Protein Data Bank
PRD	PARP Regulatory domain
RNA	Ribonucleic Acid
RNase L	Ribonuclease L
SAR	Structural activity relationship
SSB	Single strand breaks
QSAR	Quantitative structural activity relationship

INTRODUCTION
TRIVENI INSTITUTE OF PHARMACY
IN-SILICO DRUD DESIGN AND SYNTHESIS OF NOVEL 2- AMINOTHIOPHENE AS ANTI-CANCER AGENTS

1. INTRODUCTION

Cancer is a disease characterized by uncontrolled growth of abnormal cells disregarding the normal cell division rules. Normal cells are continually subject to signals that regulate the cell division, differentiation or death. Cancer cells deregulate these signals, leading to uncontrolled division and proliferation.[1]

Poly (ADP-ribose) polymerase (PARP) inhibitors represent an interesting novel class of targeted cancer therapies. Poly (ADP-ribose) polymerase-1 (PARP-1) is a chromatin-bound nuclear enzyme capable of DNA repair as it recognizes and binds rapidly to DNA single or double strand breaks. The inhibition of PARP enhances the effects of cytotoxic drugs such as topoisomerase I inhibitors and DNA alkylating agents as well as ionizing radiation. Furthermore, some PARP inhibitors have been shown to display single agent activity against tumors exhibiting BRCA1- or BRCA2- mutations. Accordingly, development of PARP inhibitors is an attractive research area providing advanced cancer treatment possibilities.[1]

Few PARP-1 inhibitors have been discovered and FDA approved e.g., Olaparib **II,** Niraparib **III**, Rucaparib **IV** and Talazoparib **V**.[1] Nevertheless, effort of pursuing new inhibitors with high potency, significant aqueous solubility and good oral bioavailability against PARP-1 is still needed. To verify the rationale of the design and to approach the development of potent inhibitors, the molecular docking study of all of the compounds to the catalytic domain of PARP-1 was first conducted. Affinity of the compounds to the target enzyme was judged by comparing the binding free energy (docking score) and the binding mode of the target compounds with that of the co-crystallized ligand. Analysis of the docking results showed that all the designed compounds fit into the active site of PARP-1, most of them displayed comparable docking scores and binding modes and were able to reproduce all the main interactions accomplished by the co-crystallized ligand.[1]

Poly-(ADP-ribose) (PAR) is a large, negatively-charged and branched polymer that can exceed the mass of the unmodified protein. PARylation creates binding sites for PAR-specific binding proteins and changes the electrostatic properties of the modified protein, with the notable capacity to change DNA binding properties of enzymes, histones, and structural proteins. PARP-1 itself is the target of most of the poly-(ADP-ribosylation) (PARylation) occurring in response to DNA damage. Auto modification of PARP-1 increases its association with a variety of repair and signalling proteins that are recruited to sites of DNA damage by PARP-1 activity. In turn, some of these proteins are PARylated by PARP-1. PARP enzymes responding to damage can consume substantial amounts of cellular NAD+ within minutes, changing a cell's metabolic status while modifying vast numbers of proteins, many of which have been only recently identified by proteomic surveys. For most of these proteins, the effects of PARylation remain to be functionally characterized.[2]

PARP presence and activity at the damage site then can contribute to the efficiency of the repair process and the repair pathway choice. A key role of the DNA- damage dependent PARPs and the PAR modification they produce is to recruit repair factors to the site of damage. Several motifs and domains have been identified in repair proteins that mediate the interaction with PAR and the recruitment to sites of PAR synthesis.[3]

The DNA-damage dependent PARPs have similar catalytic domain structures, but they differ somewhat in the domains that contact DNA damage. In the catalytic domain, they share a conserved structural feature known as the helical domain (HD) (also referred to as the PARP regulatory domain – PRD). The HD is only found in the DNA-damage dependent PARPs, and it plays an important role in regulating PARP catalytic activity, as described later. The HD is adjacent to the ADP-ribosyl transferase (ART) fold, which is common to all PARP family members. The ART contains the binding site for NAD+, which donates ADP-ribose, and a second binding site for an ADP-ribose unit, which accepts the next ADP-ribose during the PAR extension reaction that can result in both linear and branched polymers.

Detailed structural views of PAR biosynthesis (NAD+ binding, initiation on target protein, polymer have not been obtained, thus our complete understanding of PAR synthesis is limited. The NAD+ binding sites for the DNA-damage dependent PARPs are similar and have the conserved His-Tyr-Glu (HYE) amino acids that define catalytically active PARP members capable of forming PAR (as opposed to mono- ADP-ribose).[2]

The docking process is carried out using PyRx software. PyRx is a virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enable medicinal chemists to run virtual screening from any platform and helps users in every step of this process – from data preparation to job submission and analysis of results. While it is true that there is no magic bottom in the drug discovery process, PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design.[24]

In organic chemistry, due to their many applications, derivatives of thiophene stand out among biomolecules used in trials to determine biological activity. Substituted 2-aminothiophene-3-carboxamides are versatile building

blocks for the synthesis of agrochemicals, dyes, and pharmacologically active compounds. Several methods were reported in the literature for the preparation of these materials. The most convergent and well-established classical approach for the preparation of 2-aminothiophenes is Gewald's method. Our review deals with the effective use of 2- aminothiophene-3-carboxamide in the synthesis of different poly functional heterocyclic compounds.[1] The substituted 2-aminothiophene-3-carboxamide was synthesized in our laboratory.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

1. Christopher J Lord and Alan Ashworth et-al (2008)

Poly (ADP-ribose) Polymerase (PARP) has a well-established role in DNA repair processes, and small molecule inhibitors of PARP have been developed as chemotherapy sensitizers for the treatment of cancer. The subsequent demonstration that PARP inhibition is selective for BRCA1 or BRCA2 deficiency suggests that PARP inhibitors may be particularly useful for the treatment of cancer with BRCA mutations.[4]

2. Nicola Curtin et-al (2014)

PARP-1 [poly (ADP-ribose) polymerase-1], which plays a key role in DNA repair, was discovered 50 years ago. PARPi (PARP inhibitors), originally made to probe the function of the enzyme, inhibit DNA repair and increase the potency of anticancer cytotoxic agents. PARPi of increasing potency were developed as chemo- and radio-sensitizers and first entered clinical trial in cancer patients in 2003.[5]

3. Alan D. D'Andrea(2005)

BRCA1 and BRCA2 deficient tumor cells are sensitive to inhibitors of Poly ADP Ribose Polymerase (PARP1) through the mechanism of synthetic lethality. Several PARP inhibitors, which are oral drugs and generally well tolerated, have now received FDA approval for various ovarian cancer and breast cancer indications.[6]

4. Andreza Conception Véras of Aguiara, Ricardo Olímpio of Moura b, Jaime Francisco Bezerra Mendonça Juniorc et-al (2017)

In spite of great progress in understanding cancer biology, current therapeutic procedures remain unsatisfactory. Chemotherapy is often followed by secondary effects with cellular toxicity negatively affecting the results. The discovery and development of new safe and efficient antitumor agents is necessary.[7]

5. Aziz 1 2, Gamal El-Din A Abuo-Rahma

Over the past decades, cancer has been a challenging domain for medicinal chemists as it is an international health concern. In association, small molecules such as 2-aminothiophenes and their derivatives showed significant antitumor activity through variable modes of action.[8]

6. JiminHwang, XiaqiuQiu, Lydia Borgelt et-al (2022): Aminothiophene is a scaffold that is widely present in drugs and biologically active small molecules as chemical probes. In this study, 43 compounds sharing a 2-aminothiophenone-3-carboxylate (ATPC) scaffold, known to activate the ribonuclease L (RNase L), were synthesized and selected ATPCs showed enhancement of thermal stability of RNase L upon binding.[9]

7. Bryan A. Gibson and W. Lee Kraus (2012)

Poly (ADP-ribose) polymerases (PARPs) are enzymes that transfer ADP-ribose groups to target proteins and thereby affect various nuclear and cytoplasmic processes. The activity of PARP family members, such as PARP1 and PARP2, is tied to cellular signaling pathways, and through poly (ADP-ribosyl) ation (PARylation) they ultimately promote changes in gene expression, RNA and protein abundance, and the location and activity of proteins that mediate signaling responses.[10]

8. LucioTentori, GraziaGraziani (2005)

Poly (ADP-ribose) polymerases (PARP) constitute a family of enzymes involved in the regulation of many cellular processes such as DNA repair, gene transcription, cell cycle progression, cell death, chromatin functions and genomic stability. Among the 18 members identified so far, PARP- 1 and PARP-2 are the only proteins stimulated by DNA strand breaks and implicated in the repair of DNA injury.[11]

9. Ram W. Sabnis (2016)

The chemistry of 2-aminothiophenes is arguably one of the most extensive fields of present-day thiopheneresearch. The bulk of the literature dealing with the chemistry of 2-aminothiophene has forced us to restrict this review to only one superior and efficient preparative method called the Gewald reaction, which leads to the synthesis of 2- aminothiophenes.[1]

10. Anthony J. Chalmers (2009)

Since many anti-cancer agents act by inflicting DNA damage on tumors cells, there is increasing interest in the use of inhibitors of DNA repair to increase the cytotoxicity of these agents.[13]

11. Hui Ling Ko and Ee Chee Ren (2012)

Poly (ADP-ribose) polymerase 1 (PARP1) is an ADP-ribosylating enzyme essential for initiating various forms of DNA repair. Inhibiting its enzyme activity with small molecules thus achieves synthetic lethality by preventing unwanted DNA repair in the treatment of cancers.[14]

12. Moustafa A. GOUDA et al (2011)

Substituted 2-aminothiophene-3-carboxamides are versatile building blocks for the synthesis of agrochemicals, dyes, and pharmacologically active compounds. 1−3 Several methods were reported in the literature for the preparation of these materials.[15]

13. Marie-France Langelier and John M Pascal (2013)

Poly (ADP-ribose) polymerase 1 (PARP-1) regulates gene transcription, cell death signaling, and DNA repair through production of the post- translational modification poly (ADP-ribose). During the cellular response to genotoxic stress PARP-1 rapidly associates with DNA damage, which robustly stimulates poly (ADP-ribose) production over a 16 low basal level of PARP-1 activity.

14. Dana V. Ferraris (2010)

Poly (ADP-ribose) polymerase-1 (PARP-1a) has been an actively pursued drug discovery target for almost 3 decades. Often referred to as the "guardian angel of DNA", this abundant nuclear enzyme has been the focus of over 20 medicinal chemistry programs in a wide range of therapeutic areas encompassing stroke, cardiac ischemia, cancer, 17inflammation, and diabetes.

15. Virág L, Szabó C (2002)

Poly (ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family consisting of PARP-1 and several recently identified novel poly (ADP-ribosylating) enzymes. PARP-1 is an abundant nuclear protein functioning as a DNA nick-sensor enzyme.[18]

16. Junko Murai , Shar-yin N Huang et –al (2012)

Small-molecule inhibitors of PARP are thought to mediate their antitumor effects as catalytic inhibitors that block repair of DNA single- strand breaks (SSB). However, the mechanism of action of PARP inhibitors with regard to their effects in cancer cells is not fully understood.[19]

17. Emma Bolderson et-al (2009)

Damage to genetic material represents a persistent and ubiquitous threat to genomic stability. Once DNA damage is detected, a multifaceted signaling network is activated that halts the cell cycle, initiates repair, 20 and in some instances induces apoptotic cell death.

18. ZitaPuterová et-al (2010)

Chemistry of 2-aminothiophenes is arguably one of the most extensive and dynamic field of present-day thiophene research. Since 1961 when first report on the Gewald reaction was reported it became a universal method for synthesis of substituted 2-aminothiophenes and has gained 21 prominence in recent times.

19. Heng Zhu1, MiaoyanWei (2020)

Pancreatic cancer is a highly lethal disease with a poor prognosis, and existing therapies offer only limited effectiveness. Mutation gene sequencing has shown several gene associations that may account for its carcinogenesis, revealing a promising research direction. Poly (ADP- ribose) polymerase (PARP) inhibitors target tumor cells with a homologous recombination repair (HRR) deficiency based on the 22 concept of synthetic lethality.

20. Dea Slade (2020)

Oxidative and replication stress underlie genomic instability of cancer cells. Amplifying genomic instability through radiotherapy and chemotherapy has been a powerful but nonselective means of killing cancer cells. Precision medicine has revolutionized cancer therapy by putting forth the concept of selective targeting of cancer cells.[23]

21. Chin J Cancer. (2011)

PARP is an important protein in DNA repair pathways especially the base excision repair (BER). BER is involved in DNA repair of single strand breaks (SSBs). If BER is impaired, inhibiting poly (ADP-ribose) polymerase (PARP), SSBs accumulate and become double stand breaks 24 (DSBs).

22. J. Mateo1, C.J. Lord, V. Serral

Genomic instability is a hallmark of cancer, and often is the result of altered DNA repair capacities in tumors cells. DNA damage repair defects are common indifferent cancer types; these alterations can also induce tumour-specificvulnerabilities that can be exploited 25 therapeutically.

23. Amy Drean Christopher J. Lord Alan Ashworth (2016)

In 2014, Olaparib (Lynparza) became the first PARP (Poly (ADP-ribose) polymerase) inhibitor to be approved for the treatment of cancer. When used as single agents, PARP inhibitors can selectively target tumors cells with BRCA1 or BRCA2 tumors suppressor gene mutations through 26 synthetic Lethality.

24. He Li, Zhao-Yi

Liu Due to the DNA repair defect, BRCA1/2 deficient tumor cells are more sensitive to PARP inhibitors (PARPi) through the mechanism of synthetic lethality. At present, several PAPRi targeting poly (ADP- ribose) polymerase (PARP) have been approved for ovarian cancer and breast cancer indications. However, PARPiresistance is ubiquitous in clinic. More than 40% BRCA1/2- deficient patients fail to respond to PARPi.[27]

25. Aneta Kolaczek *et al* (2014)

The synthesis of sulfonamide derivatives has been reported in many ways. These classes of compounds are considered as "scalffolds" in medicinal chemistry to drug development with different biological activities. In organic chemistry, these compounds have a functional application in the industry in some products of health, food colorants and others; therefore, it is necessary to continue with research projects that 28 help to synthesize new compounds with Sulfonamide group.

26. Aastha Pareek *et al* (2013)

Sulphonamides are one of the organ Sulphur compounds containing the-SO_2NH_2 and/or-SO_2NH- group(s). The sulphonamides or sulpha drugs competitively inhibit folic acid synthesis in micro- organisms and

subsequently inhibit multiplication of bacteria but do not actively 29 kill them.

27. Raghav Mishra *et al* (2011)
Thiophene nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. The knowledge of various synthetic pathways and the diverse physicochemical parameters of such compounds draw the especial attention of medicinal chemists to produce combinatorial library and carry out exhaustive efforts in the search of 30 lead molecules.

28. Bu-Bing Zeng *et al* (2010)
An efficient one-pot procedure allows the synthesis of various functionalized 2-aminothiophene scaffolds catalyzed by L-proline in high yields under mild conditions. Low catalyst loading, simple procedure, and high yields are the important attributes of this methodology.[31]

29. Senra *et al* (2011)
PARP-1 is a critical enzyme in the repair of DNA strand breaks. Inhibition of PARP-1 increases the effectiveness of radiation in killing tumor cells. Olaparib, a potent PARP-1 inhibitor, enhances radiotherapy, not only by inhibiting DNA repair but also by changing tumor vascular 32 hemodynamics in non-small cell lung carcinoma (NSCLC).

30. A.P. Leal *et al* (2009)
Poly (ADP-ribose) polymerases (PARPs) are defined as cell signaling enzymes that catalyze the transfer of ADP-ribose units from NAD (+) to a number of acceptor proteins. PARP-1, the best characterized member of the PARP family, which currently comprises 18 members, is an abundant nuclear enzyme implicated in cellular responses to DNA injury provoked by genotoxic stress.[33]

AIM AND OBJECTIVES
AIM
3. AIM AND OBJECTIVES
In silico design and synthesis of novel 2-aminothiophenes.

OBJECTIVES
- Study the structural features of PARP 1 inhibitor.
- Develop SAR based on literature survey.
- In silico design of novel PARP 1 inhibitor.
- Synthetic methodology.

TRIVENI INSTITUTE OF PHARMACY
PLAN OF WORK
4. PLAN OF WORK
- ❖ DESIGN
- ❖ PROPOSED SCHEME FOR 2-AMINOTHIOPHENE-3- CARBOXAMIDE DERIVATIVES
- ❖ IN SILICO DESIGN: SOFTWARES
- DRAWING TOOL: CHEMSKETCH
- PREDICTION OF BIOLOGICAL ACTIVITY:
- ➢ PASS ONLINE
- ➢ MOLINSPIRATION
- DOCKING: PYRX
- ❖ SYNTHETIC METHODOLOGY

5. DESIGN
From the review of PARP-1 enzyme we retrived a collection of PARP-1 protein from protein data bank (PDB). PARP-1 inhibitors have an aromatic ring with carboxamide and amino group, that are located at 1, 3 positions (Meta to each other). Therefor we have taken 3-aminobenzamide as the lead molecule. But analysis of the ligand interaction diagrams (LIDs) of PARP-1 inhibitors from important PDB entries lead us to the observation that the meta-amino group has no significant interactions like H bond or Ionic interactions with any of the amino acids in the active site.

The design of 2-(Phenylsulphonylamino) thiophene-3-carboxamides, in which the amino group (secondary) is at ortho position, rather than in the meta position.

Since, a large number of inhibitors possess an electronegative atom attached directly to the aromatic ring, Meta to carboxamido group; we decided to keep a highly electronegative atom, sulphonyl oxygen at the same position. This is achieved by the design of 2-(Phenylsulphonylamino) thiophene-3- carboxamides.

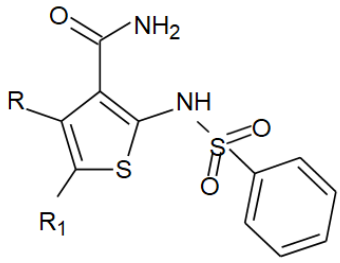


Fig. No. 1.

It has been also noted that a bulkier substituent in the same side to the carboxamido group [(methylamino)methyl] phenyl substituent in the second position of azepinoindole ring. Therefore, we have taken Rucaparib as the lead molecule. The key SAR aspect taken from literature survey for further design is as follows.

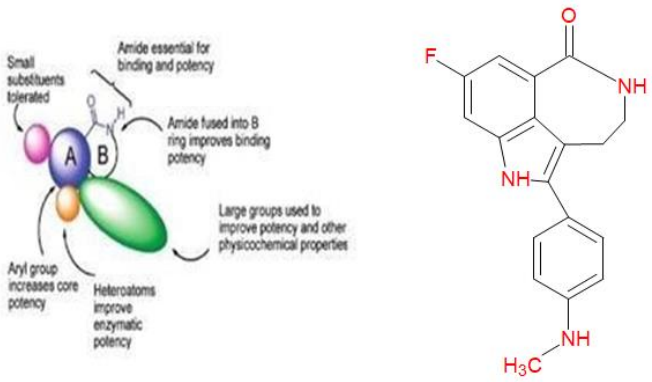


Fig. No. 2.

Docking studies also strongly supports this as the bulkier group is located in a large hydrophobic pocket. To study the stereochemical aspects of Rucaparib and the designed molecule, we superimposed the designed molecule with Rucaparib.

The challenge in our design was to orient the phenyl ring of phenyl sulphonyl amino group to the same hydrophobic pocket in which the 4[(methylamino)methyl] phenyl substituent of Rucaparib lies. This is because the the C-2 carbon atom of azepinoindole ring of Rucaparib, which hold the bulkier substituent is sp^2 hybridized whereas the Sulphur atom of sulphonyl amino group which hold the bulkier substituent in the designed molecule was sp^3 hybridized. At the earlier stage of our design, we have already used a disconnection approach by removing bond that connect indole nitrogen to the benzene ring of indole nucleus of Rucaparib which has given more flexibility to the designed molecule to tackle the challenge discussed above.

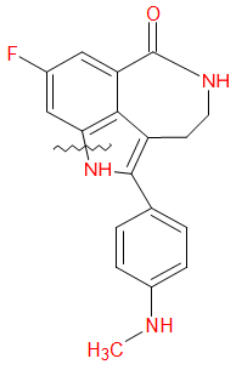


Fig. No. 3.

Another aspect taken into consideration in design is the introduction of additional amino group to the nitrogen of carboxamido group which convert the molecule to acid hydrazides. The literature survey also shows an additional H-bond interaction of acid hydrazides with Gly 863 at activesite.

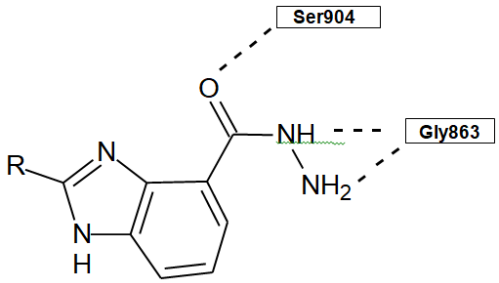


Fig. No. 4.

Through SAR analysis, docking studies and molecular modelling we designed some novel PARP-1 inhibitors. The general structure of the designed novel PARP-1 inhibitors is as follows.

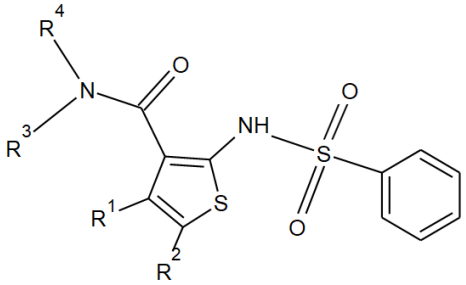


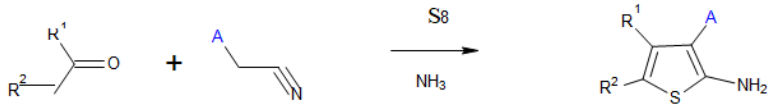
Fig. No. 5.

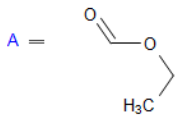
As mentioned above we opted 4RV6 as our protein which have all the required properties.

6. EXPERIMENTAL SECTION

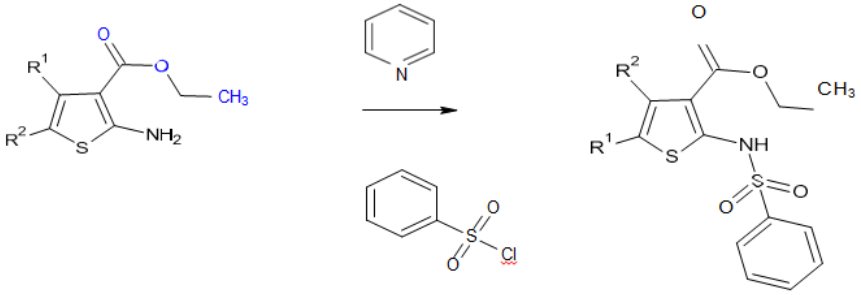
PROPOSED SCHEME FOR 2-AMINOTHIOPHENE-3-CARBOXAMIDE

STEP 1





Step 2



Step 3

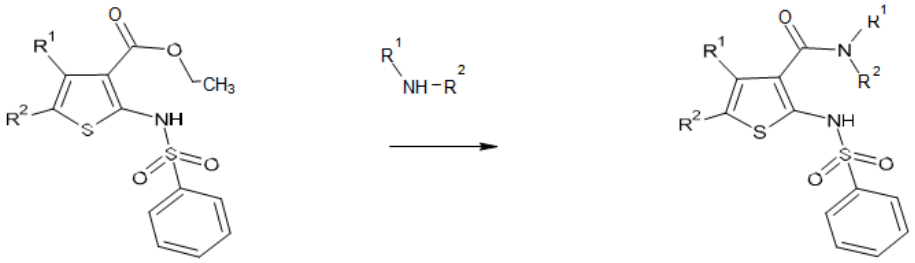


Fig. No. 6.

DOCKING STUDY[24]

Docking studies were carried out using PyRx Auto Dock vina which is a free software. PyRx is a multiple ligand docking software. Main steps for docking in PyRx:

- Downloading PyRx software.
- Loading the molecules in to PyRx: Load your protein and ligand.
- Right click on the ligand> AutoDock> Make ligand.
- Right click on the protein> AutoDock>Macromolecule.
- Now the protein and ligand files ready for docking.
- Click start botton under vina wizard.
- Select protein and ligand by clicking on them.
- Click forward to run vina.
- You will see a grid box in 3D seen.
- Click the molecule and select amino acid residue.
- Make sure to select the grid box size big enough to allow the ligand to move freely in search space.

Click forward botton to start vina calculation.
- Calculation is tabulated with binding affinity values.
- By using output files let's analyses the results with Pymol.

MOLINSPIRATION: LIPINSKI RULE OF FIVE

The simplest and most used approach is developed by Chris Lipinski and his colleagues at Pfizer, which is generally referred either as the Lipinski Rules or the Rule of Five (ROF). Rule of five ROF is a rule of thumb to evaluate drug likeness or determine it a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans (Lipinski et al., 1997).

The biologically active molecule must implement five conditions to be potentially used as a drug for oral administration.

Poor absorption or permeation are most likely if:

- Molar mass >500,
- Number of H-bond acceptors > 10.
- Number of H-bond donors > 5.
- Log> 5 (or MlogP>4.15)

Based on the ROF, the rating of an orally active drug is between „0* and „4" which means that a potential drug has no more than one violation of the exposed criteria. However. Lipinski points out that such molecules should not be completely removed from further consideration; it is known that many drugs do not undergo ROF.

Although the rule of five has a wide application, there are certain deficiencies. The two major weaknesses are the equal weight given to each of the rules and the sharp boundary that marks the violation of a given rule. Another disadvantage of this rule is that it does not include natural and biological compounds. ROF does not incorporate criteria relevant to metabolism.[18]

DRUG LIKENESS

Drug likeness is a key consideration when selecting compounds during the early stages of drug discovery. However, evaluation of drug likeness in absolute terms does not adequately reflect the whole spectrum of compound quality. The concept of drug likeness provides useful guidelines for early-stage drug discovery. Analysis of the observed distribution of some key physicochemical properties of approved drugs, including molecular weight, hydrophobicity and polarity, reveals they preferentially occupy a relatively narrow range of possible values. Compounds that fall within this range are described as "druglike." Indeed, drug likeness is often used as a proxy for oral bioavailability.[18]

MOLECULAR DESCRIPTOR ANALYSIS

Molecular descriptors can be defined as mathematical representations of molecules' properties that are generated by algorithms. The numerical values of molecular descriptors are used to quantitatively describe the physical and chemical information of the molecules. The molecular descriptors that are used in ADMET models can be classified as being one-dimensional (1D), two- dimensional (2D), or three-dimensional (3D) descriptors based on the level of molecular representation required for calculating the descriptor.[20]

PASS DATA

The acronym PASS stands for Prediction of Activity Spectra for Substances. Using structural formula of a drug-like substance as an input, one obtains its estimated biological activity profile as an output. The predicted biological activity list includes the names of the probable activities with two probabilities: Pa – likelihood of belonging to the class of "Actives" and Pi – likelihood of belonging to the class of "Inactive".

SYNTHESIS OF SUBSTITUTED 2-AMINOTHIOPHENES

The chemistry of 2-aminothiophenes has received much attention upon their convenient availability through the most versatile synthetic method developed by Gewald and his co-workers.[17] Many methods of synthesis of substituted 2- aminothiophenes published before the Gewald are generally unsuitable because they involve difficult preparation of the starting materials, multi-step synthesis and do not produce high yields.[12]

STEP 1

PRINCIPLE

The 2–Amino thiophene is synthesized by using Gewald Reaction. In this reaction, α-substituted ketone is treated with α-activated acetonitrile in the presence of a basic catalyst (usually triethylamine or piperidine). Reaction performed in the solvents like methanol, ethanol or DMF at 50 °C takes place in two subsequent steps – Knoevenagel-Cope condensation.[11,18] and intramolecular ring closure of formed sulfanyl substituted α, β-unsaturated nitrile.

α, β- unsaturated nitrile first prepared with knovengel cope condensation and then treated with sulphur and amine to form 2- aminothiophenes.

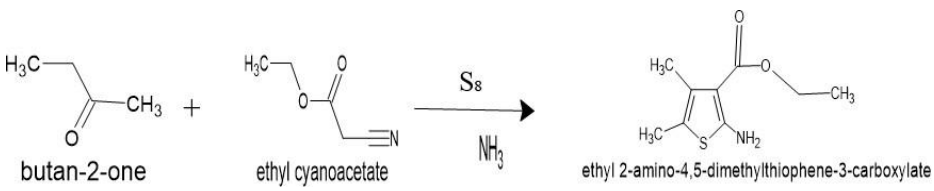


Fig. No. 7.

PROCEDURE

A mixture of butanone(3.6ml), ethyl cyano acetate (5.6ml) and elemental sulphur (1.6g) in 5ml ethanol were stirred and while on stirring ammonia solution(28.4ml) was added drop wise in about 30min. during this period Sulphur completely dissolved. Stirred the reaction mixture for 3hrs or heat at 50˚C for 1hr. The reaction mixture while hot, was poured on to crushed ice with stirring. The precipitate was filtered, washed and dried. The product was recrystallized from ethanol.

STEP 2

PRINCIPLE

In the second step, through schotten-baumann reaction 2-aminothiophenes react with benzene sulphonyl chloride in the presence of strong base like pyridine to form 2-(phenyl sulphonyl amino) thiophene-3- carboxylate derivatives.

Here strong base act a catalyst, solvent and scavenging agent. Scavenging action of base remove hydrochloric acid formed during reaction from reaction site as salt of base.

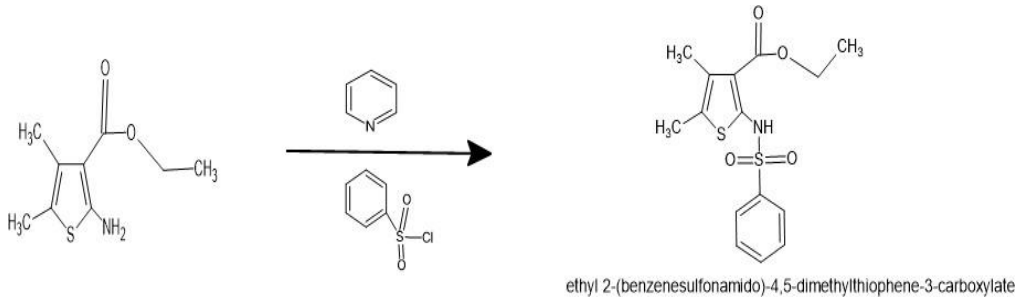


Fig. No. 8.

PROCEDURE

A solution of ethyl 2-amino-4,5-dimethyl thiophene-3-carboxylate(2g) and benzene sulphonyl chloride(4ml) in 12ml pyridine was refluxed at 50˚C for 30min with stirring. The reaction mixture, while hot, was poured on to crushed ice with stirring. The precipitate was filtered, washed and dried. The product was recrystallized from ethanol.

STEP 3

PRINCIPLE

Synthesis of 2-(phenyl sulphonyl amino) thiophene 3-carboxamide derivatives is a type of nucleophilic substitution reaction at carbonyl carbon. Secondary amine (diethyl amine) attack carbonyl carbon of carbethoxy group from amide.

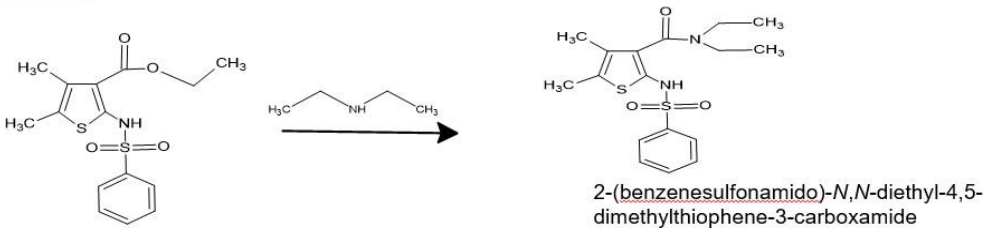


Fig. No. 9.

PROCEDURE

A solution of ethyl2-(benzenesulphonamido) 4,5-dimethylthiophene-3- carboxylate(1g) and diethylamine(2ml) in 5ml ethanol and stirred well. Then the solution is subjected to microwave irradiation 420W for 5min.

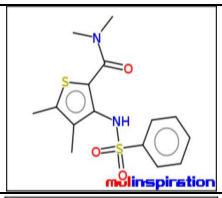
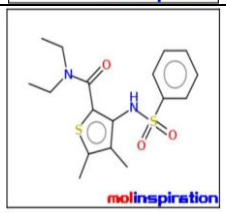
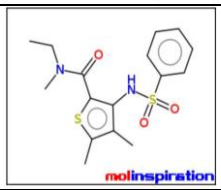
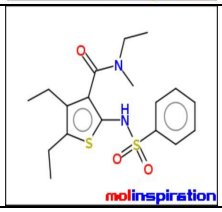
After irradiation the reaction mixture was cooled to get solid precipitate. The precipitate was filtered, washed and dried. The product was recrystallized from ethanol.

7. RESULTS AND DISCUSSION

INSILICO DESIGN

a) SMILES AND LOG P VALUE

Table No. 1.

COMPOUND CODE	STRUCTURE	SMILES NOTATIONS	LOG P
PR1		Cc2sc(NS(=O)(=O)c1ccccc1)c(C(=O)N(C)C)c2C	2.57
PR2		Cc1sc(NS(=O)(=O)c2ccccc2)c(C(=O)N(CC)CC)c1C	3.32
PR3		CN(CC)C(=O)c1c(sc(C)c1C)NS(=O)(=O)c1ccccc1	2.94
PR4		CN(CC)C(=O)c1c(sc(CC)c1CC)NS(=O)(=O)c1ccccc1	3.39

b) MOLECULAR DESCRIPTORS ANALYSIS

Table No. 2.

COMPOUND CODE	MOLAR REFRACTIVIY	MOLAR VOLUME	REFRACTIVE INDEX	PARACHOR	POLARISIBI-LITY	SURFACETENSION
PR1	81.20 ± 0.4 cm^3	255.8 ± 3.0 cm^3	1.614 ± 0.02	696.6 ± 6.0 cm^3	35.36 ± 0.5 10 -24 cm^3	54.9 ± 3.0 dyne/cm
PR2	98.46 ± 0.4 cm^3	288.8 ± 3.0 cm^3	1.597 ± 0.02	776.8 ± 6.0 cm^3	39.03 ± 0.5 10 -24 cm^3	52.2 ± 3.0 dyne/cm
PR3	93.83 ± 0.4 cm^3	272.3 ± 3.0 cm^3	1.605 ± 0.02	736.7 ± 6.0 cm^3	37.19 ± 0.5 10 -24 cm^3	53.5 ± 3.0 dyne/cm
PR4	103.09 ± 0.4 cm^3	305.4 ± 3.0 cm^3	1.590 ± 0.02	816.9 ± 6.0 cm^3	40.86 ± 0.5 10 -24 cm^3	51.1± 3.0 dyne/cm

c) LIPINSKI RULE OF FIVE

Table No. 3.

COMPOUND CODE	LOG P	MOLECULAR WEIGHT	No. of HBA	No. of HBD	No. of ROT.B	TBSA
PR1	2.77	333.45	3	1	5	103.10 A^2
PR2	3.32	366.50	3	1	7	103.10 A^2
PR3	3.05	352.47	3	1	6	103.10 A^2
PR4	3.66	380.52	3	1	8	103.10 A^2

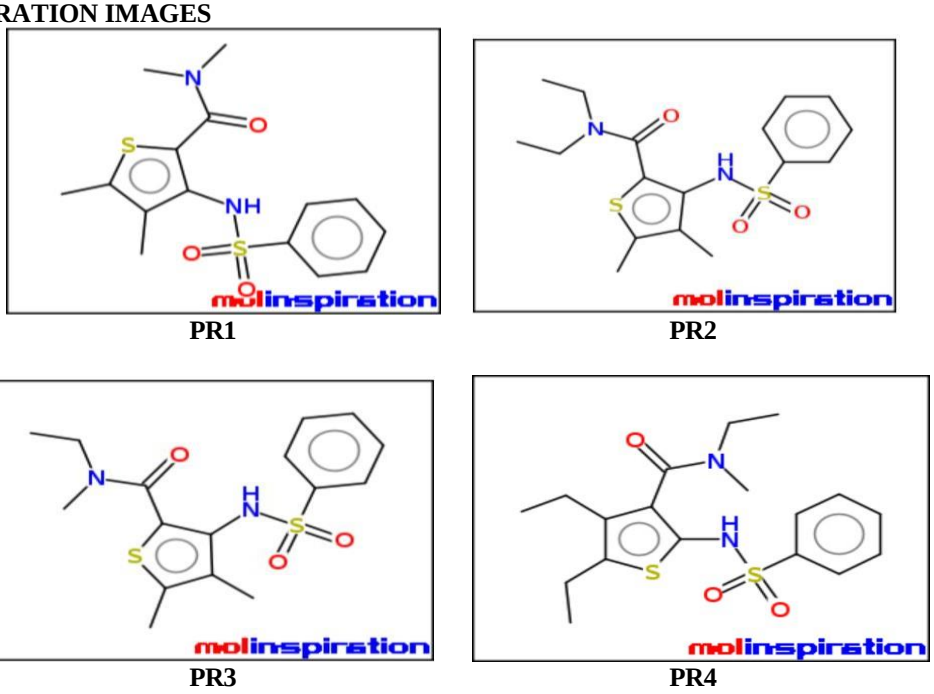
d) ADRUG LIKENESS

Table No. 4.

COMPOUND CODE	GPCR	ION CHANNEL MODULATOR	KINASE INHIBITOR	NUCLEAR RECEPTOR LIGAND	PROTEASE INHIBITOR	ENZYME INHIBITOR
PR1	0.10	0.35	0.43	0.42	0.23	0.07

PR2	0.44	0.89	0.55	0.84	0.54	0.60
PR3	0.09	0.34	0.51	0.36	0.34	0.01
PR4	0.36	0.77	0.49	0.72	0.43	0.48

e) MOLINSPIRATION IMAGES



PR1 **PR2**

PR3 **PR4**

Fig. No. 10.

f) PASS DATA

Table No. 5.

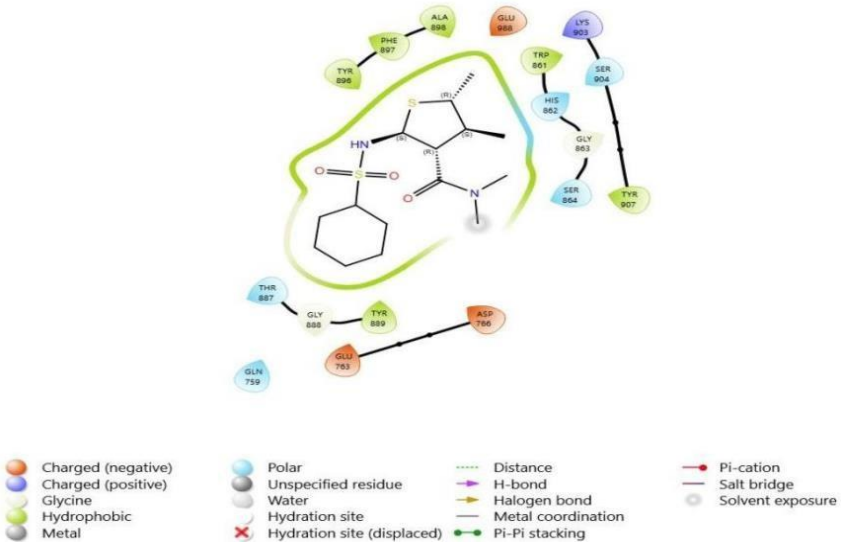
COMPOUND CODE	EFFECT	Pa	Pi
PR1	Analgesic, non-opioid	0,753	0,005
	Analgesic	0,708	0,009
	Anti-inflammatory	0,628	0,026
	NADPH peroxidase inhibitor	0,398	0,103
PR2	Analgesic, non-opioid	0,704	0,006
	Analgesic	0,698	0,010
	Anti inflammatory	0,592	0,033
	Anti neoplastic (small cell cancer)	0,202	0,187
	NADPH Peroxidase inhibitor	0,233	0,225
PR3	Analgesic	0,454	0,051
	Anti neoplastic (pancreatic cancer)	0,317	0,047
	NADPH Peroxidase inhibitor	0,290	0,168
PR4	Analgesic	0,481	0,042
	Analgesic, non-opioid	0,454	0,037
	Antineoplastic (pancreatic cancer)	0,302	0,057
	Anti inflammatory	0,347	0,125
	Anti neoplastic (small cell lung cancer)	0,208	0,172

g) DOCKING

Table No. 6.

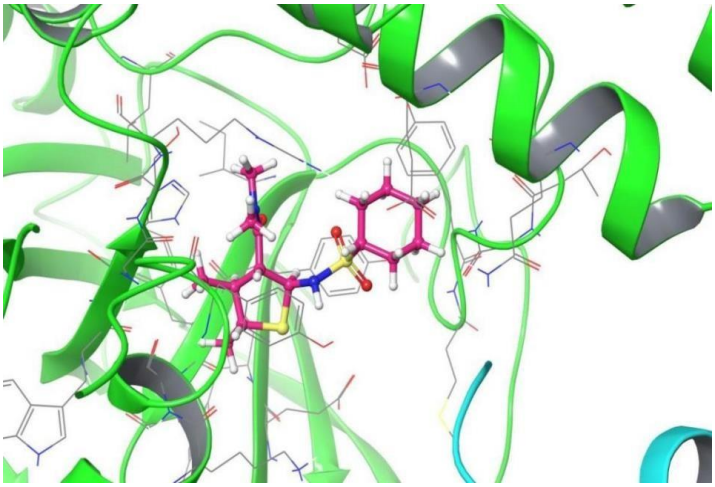
COMPOUND CODE	GLIDE SCORE
RUCAPARIB	-5.9
PR 1	-6.0
PR 2	-6.2
PR 3	-5.8
PR 4	-5.7

PR1



2D

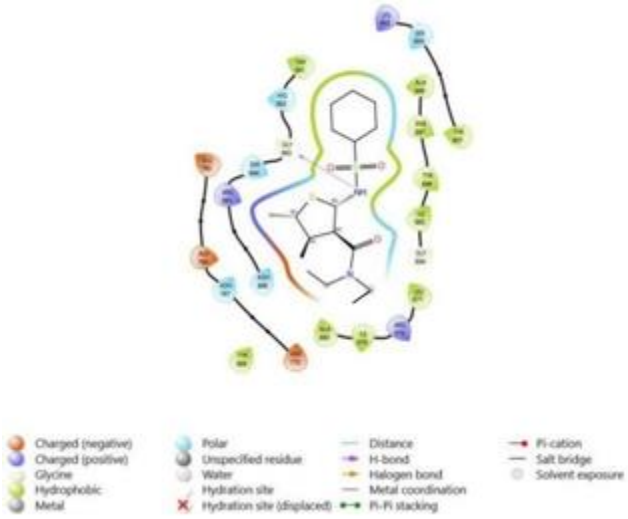
Fig. No. 11.



3D

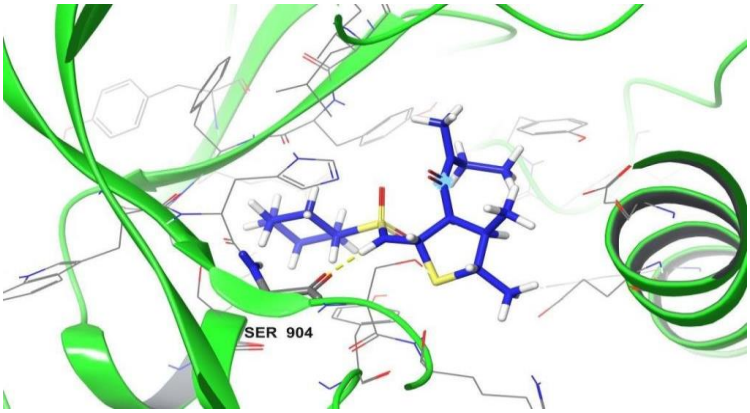
Fig No. 12.

PR2



2D

Fig No. 13.



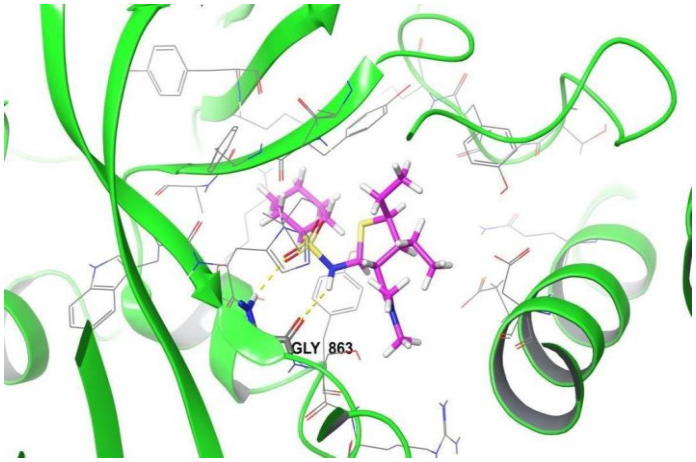
3D

Fig no. 14.

PR3

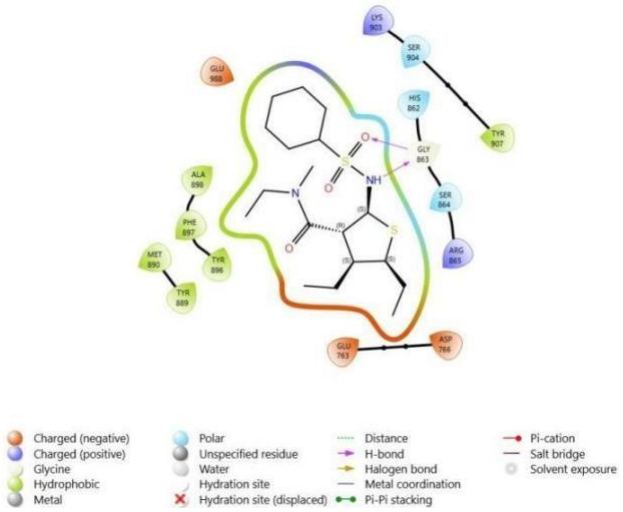
2D

Fig No. 15.



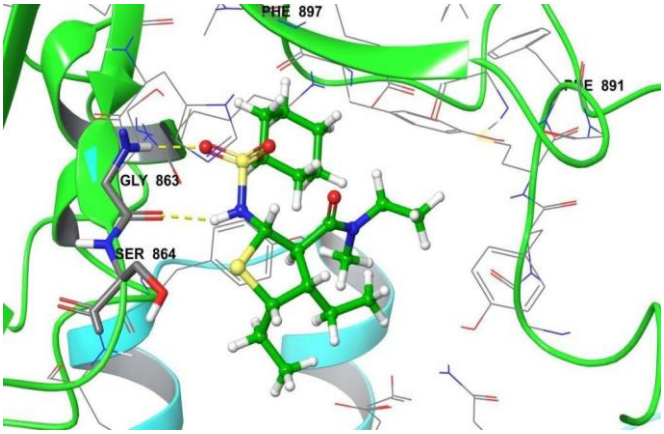
3D

PR4



2D

Fig No. 17.



3D

Fig. No. 18.

DISCUSSION

In this study, a combined approach involving Molinspiration, Pass Data and Docking simulation was applied to predict the properties governing effective inhibition of PARP 1. Molinspoiration software gives us the Smiles and log P value of our different four derivatives of PARP 1 inhibitors (table no.1). Comparatively from the four derivatives we have assessed PR2 contains the most suitable log P value. Smile notations obtained help us to translate our chemical's three-dimensional structure into a string of symbols that is easily understood by various computer software. Through this inhibitory mechanism were compared between them. Also, molinspiration software is used to determine whether our derivatives obey the Lipinski rule of 5 (table no.3). Drug likeness is analyzed which is a qualitative concept used for how "drug like" a substance is with respect to factors like bioavailability (table no.4). This method also used to check compliance of Lipinski's rule of five.

Further we analyzed Molecular discriptors analysis of the four derivatives of PARP 1 inhibitors. It denotes the mathematical representations of molecules and properties are generated by algorithms. From this numerical values we can quantitatively describe the physical and chemical information of the molecules such as parachor, molar refractivity etc. (table no 2). Prediction of activity spectrum of substance [PASS DATA] enables to know the anticancer activity of four derivatives of PARP 1 inhibitor (table no.5). The prediction is based on an analysis of structural activity relationships of our four derivatives. We have analysed this prediction using PASS ONLINE software.

Docking is carried out on PyRx Software and docking scores obtained were Rucaparib (-5.9), PR1 (- 6.0), PR2 (-6.2), PR3 (-5.8), PR4 (- 5.7). From the above obtained results the docking score with least score shows best activity.

From all the above studies conducted we conclude that the compound PR2 shows the best PARP inhibitor activity with optimal receptor interaction within the binding site. The synthesis of compound PR2 was carried out in our laboratory.

8. SUMMARY AND CONCLUSION

In this study using Insilco drug designing we targeted PARP 1 enzyme to obtain some insight into the conformations, structure and interactions for binding with the PARP 1 inhibitors.

PARP 1 enzyme having major role in cancer. Using the market available PARP 1 inhibitors we designed our four derivatives of PARP 1 inhibitors (PR1, PR2, PR3, PR4) using various computer software's. An approved protein was retrived from Protein data bank. protein preparation was done using protein preparation wizard and ligprep for preparing the ligands of PyRx. Molecular docking studies were performed with the glide docking program. The docking score of compounds was found to be -5.9, -6.0, -6.2, -5.8, and -5.7 for Rucaparib, PR1, PR2, PR3 and PR4 respectively. And it was found that the best derivative of inhibitors is PR2 having least docking score and optimal binding interaction with the targeted protein .The synthesis of compound PR2 was carried out in our laboratory.

ACKNOWLEDGMENT

We consider this as an opportunity to express our gratitude to all the dignitaries and kind people who have extended their hand directly or indirectly with the successful completion of this dissertation.

First and foremost, we thank **GOD ALMIGHTY** who gave this opportunity, showed his blessings, strength to pursue and abundance of requisite determination to complete this course and dissertation successfully.

In addition, we would like to express our appreciation to beloved **PARENTS** for all the efforts, care, support, encouragement and prayers throughout our life. We extend our gratefulness to our sibilings who have always been an encouragement for us.

We consider this as a great privilege to express our heartfelt gratitude, appreciation and sincere thanks to our esteemed guide **Mrs. SURABHI K.V.** Triveni Institute of Pharmacy, Kechery. We consider our self-most lucky to work under her, who supervised us with her valuable suggestions, guidance, interest, moral support and endless co-operation throughout our dissertation work.

We would like to express our appreciation to **LIBRARIAN** and **LAB ASSISTANTS** for their sincere help and support.

We would like to express our sincere and utmost gratitude to **Prof. Dr. ANOOP K.R,** Principal, Triveni Institute of Pharmacy, Kechery, who always guide us with his profound knowledge and experience for providing all the opportunities and facilities to accomplish our thesis work on time.

We convey our wholehearted thanks to our friends for their support, enquiries, encouragement and prayers, Last but not least, we express our heartiest thanks to several people knowingly and unknowingly for helping us in the successful completion of this thesis. We thank everyone.

9. REFERENCE

1. Ghada F. Elmasry, Enayat E. Aly, Fadi M. Awadallah, Samir M. El- Moghazy, Design and synthesis of novel PARP-1 inhibitors based on pyridopyridazinonescaffold, Bioorganic Chemistry, 2019; 87: Pages.
2. Pascal JM, Ellenberger T. The rise and fall of poly (ADP-ribose): An enzymatic perspective. DNA Repair (Amst). 2015; 32: 10-16.
3. Barkauskaite E, Jankevicius G, Ladurner AG, Ahel I, Timinszky G. Therecognition and removal of cellular poly (ADP-ribose) signals, Feb 2013; 280: 3491–3507.
4. Nicola J. Curtin. PARP inhibitors for cancer therapy. Cambridge University Pres., 2005; 1-14
5. Murai J, Huang SY, Das BB, et al. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer Res., 2012; 72(21): 5588-5599.
6. Raghav Mishra et al. Synthesis, properties and biological activity of thiophene: A review. Der Pharma Chemica, 2011; 38-54
7. Thomas Helledaya. The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. Molecular oncology, 2011; 387-393.
8. Ko HL, Ren EC. Functional Aspects of PARP1 in DNA Repair and Transcription. Biomolecules, 2012; 2(4): 524-548. Published 2012 Nov 12.
9. Steffen et al. Targeting PARP-1 Allosteric Regulation offers Therapeutic Potential against Cancer. AACR., 2013; 31-36
10. J. Chalmers et al. The potential role and application of PARP inhibitors in cancer treatment. British Medical Bulletin., 2009; 24-37.
11. Sabnis, R. W.; Rangnekar, D. W.; Sonawane, N. D. J. Heterocycl. Chem., 1999; 36: 333.
12. Gewald, K. Angew. Chem., 1961; 73: 114.
13. Puterova, Zita & Krutosíková, Alzbeta & Véghc, D. (2010). Gewald reaction: Synthesis, properties and applications of substituted 2- aminothiophenes. Arkivoc. 2010; 209-246.
14. Bu-Bing Zeng et al. An Efficient One-Pot Synthesis of Substituted 2- Aminothiophenes via Three Component Gewald Reaction Catalyzed by L-Proline. Accounts and Rapid Communications in Synthetic Organic Chemistry, 2009; 1-44.
15. Gouda, Moustafa & Berghot, Moged & Emad Alden, Ghada & Elattar, Khaled & Khalil, Abd. (2012). ChemInform Abstract: Chemistry of 2-rAminothiophene-3-carboxamide and Related Compounds. ChemInform. 43. no-no. 10.1002/chin.201214238
16. Sabnis, Ram. (2016). The Gewald reaction in dye chemistry. Coloration Technology. 132: 49-82. 10.1111/cote.12182.
17. Peralta-Leal A, Rodríguez-Vargas JM, Aguilar-

Quesada R, Rodríguez MI, Linares JL, de Almodóvar MR, Oliver FJ. PARP inhibitors: new partners in the therapy of cancer and inflammatory diseases. Free Radic Biol Med., 2009 Jul 1; 47(1): 13-26.
18. Cope, C. A.; Hofmann, C. M.; Wyckoff, C.; Hardenbergh, E. J. Am. Chem. Soc., 1941; 63: 3452.
19. Ivanović, V., Rančić, M., Arsić, B.B., & Pavlović, A. (2020). Lipinski's rule of five, famous extensions and famous exceptions. *Chemia Naissensis*.
20. Chandrasekaran, B., Abed, S. N., Al-Attraqchi, O., Kuche, K., & Tekade, R. K. (2018). Computer-Aided Prediction of Pharmacokinetic (ADMET) Properties. Dosage Form Design Parameters, 731– 755.
21. Kołaczek, A. & Fusiarz, I. & Lawecka, J. & Branowska, Danuta. (2014). Biological activity and synthesis of sulfonamide derivatives: A brief review. Chemik., 68: 620-628.
22. Aastha Pareek, Priyanka Rani and D. Kishore. A short review on: sulphonamides. Int J Pharm Bio Sci., 2013; 812 – 820.
23. Chen A. PARP inhibitors: its role in treatment of cancer. *Chin J Cancer*, 2011; 30(7): 463-471.
24. Dallakyan, S., & Olson, A. J. Small- molecule library screening by docking with PyRx. Methods in molecular biology (Clifton, N.J), 2015; 1263: 243-250.
25. Sree Karani Kondapuram, Sailu Sarvagalla, Mohane Selvaraj Coumar. Docking-based virtual screening using PyRx tool: Autophagy Target Vps34 as a case study, 2021; 463-477.
26. Darwish DG, El-Sherief HAM, Abdel-Aziz SA, Abuo-Rahma GEA. A decade's overview of 2-aminothiophenes and their fused analogs as promising anticancer agents. *Arch Pharm (Weinheim)*, 2024; 357(6): e2300758.
27. Hwang J, Qiu X, Borgelt L, et al. Synthesis and evaluation of RNase L- binding 2-aminothiophenes as anticancer agents. *Bioorg Med Chem*., 2022; 58: 116653.
28. Hans-peter Buchsteller, Carsten D. siebert, Ralf H. Lyssy, Ina Frank, Adil Duran, Rudolf Gottschlich & Christian R.Noe. Synthesis of novel 2-Aminothiophene-3-carboxylates by variation of the Gewald reaction, Feb 2001; 132: 279-293
29. Altaf S Almatani, Ali Saeed, Ghada E. Abdel-Ghani, Mahmood M.S. Abdullah, Hamad A. Al- Lohedan, Ehab Abdul-Latif, and Amr El- Demerdash. Synthesis of some novel thiophene analogues as potential anticancer agents, March 2024; 21(7): 26-32
30. Heba M. Metwally, Norhan A. Khalaf, Ehab Abdel-Latif and Mohammed A. Ismail. Synthesis, DFT investigations, antioxidant, antibacterial activity and SAR-study of novel thiophene-2-carboxamide derivatives, Feb 2023; 17: 30-39.