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Synthesis and INSILICO Evaluation of Methyl-4(-4-substituted phenyl)-2-oxo-1,2,3,4-Tetrahydropyrimidine-5-Carboxylate Derivatives

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Abstract

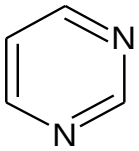
Pyrimidine derivatives are an important class of heterocyclic compounds known for their diverse pharmacological activities. In the present study, a series of **methyl-4-(4-substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives (1–5)** were synthesized through a condensation reaction involving ethyl acetoacetate, thiourea, substituted aromatic aldehydes, glacial acetic acid, and ethanol. The synthesized compounds were obtained in good yields ranging from **65–90%.** Structural characterization of the compounds was carried out using physicochemical parameters, **thin layer chromatography (TLC),** and **Infrared (IR) spectroscopy**. The ir spectra showed characteristic absorption bands corresponding to **C–H (3108–3110 cm^{-1}), C=C (1440–1630 cm^{-1}), C=N (1660–1699 cm^{-1}), and aromatic C–H (672–698 cm^{-1}),** confirming the successful formation of the pyrimidine scaffold. To further understand the biological potential of the synthesized derivatives, **Insilico studies** were performed using computational tools such as **molinspiration, swissadme, and pyrx** for molecular docking analysis. The calculated molecular descriptors revealed that all synthesized compounds obeyed **lipinski's rule of five,** indicating favorable drug-likeness and good oral bioavailability. The derivatives exhibited **milogp values ranging from 1.12 to 2.67, topological polar surface area (TPSA) values between 67.43–96.89 $å^2$**, and **molecular weights from 245.56–292.29**, along with acceptable numbers of hydrogen bond donors and acceptors and **no lipinski rule violations**. These properties suggest good absorption potential and suitable pharmacokinetic profiles.furthermore, molecular docking studies were carried out against the **sars-cov-2 3cl protease,** using **tegafur** as a reference drug. Among the synthesized compounds, **compound 5** exhibited the highest binding affinity and favorable interactions with the target protein, indicating promising antiviral potential. Overall, the findings highlight the medicinal significance of pyrimidine derivatives and suggest their potential as lead molecules for future antiviral drug development.

Key words: Lipinski rule five,Bioavailability, Insilico evaluation,Binding affinity, Drug likeness, Thin Layer Chromatography, Infrared Spectroscopy, Pharmacokinetics, Molecular docking, Molecular discriptors

1. Introduction

Pyrimidine is one of the most important heterocyclic compounds in organic and medicinal chemistry. Heterocyclic compounds are cyclic structures that contain at least one atom other than carbon in the ring, commonly nitrogen, oxygen, or sulfur. Among nitrogen-containing heterocycles, pyrimidine occupies a prominent position due to its wide occurrence in nature and its extensive application in pharmaceuticals, biochemistry, and material science.

Pyrimidine forms the basic structural framework of several biologically significant molecules, including nucleic acids such as DNA and RNA. Many naturally occurring compounds, vitamins, coenzymes, and synthetic drugs contain the pyrimidine nucleus. Because of this, pyrimidine and its derivatives have attracted considerable attention in pharmaceutical research and drug development.



Structure and Properties

Molecular formula: $C_4H_4N_2$

Molecular weight: 80.09g/mol

Physical properties: It is colourless crystaline solid, slightly unplesant odour, it is soluble in water and polar solvents.Pyrimidine is a six-membered aromatic heterocyclic compound. It consists of a benzene-like ring in which two nitrogen atoms replace carbon atoms at the 1st and 3rd positions. The structure is planar and aromatic, obeying Huckel's rule of aromaticity with six π-electrons.[(2)(4)]

The presence of nitrogen atoms in the ring significantly influences the chemical behavior of pyrimidine. These nitrogen atoms are electron-deficient, making pyrimidine less basic than pyridine.[(6)] The aromatic nature of pyrimidine provides stability, while the nitrogen atoms allow it to participate in hydrogen bonding and various substitution reactions. The compound shows weak basicity due to the lone pair of electrons on the nitrogen atoms being involved in aromaticity.

History and Background

Pyrimidine research dates back to the late 19th century, initially focusing on the structure and behavior of heterocyclic compounds. Advancements in organic chemistry and biochemistry identified pyrimidine's role in nucleic acids, marking a pivotal moment in molecular biology. Further studies uncovered various pharmacological activities of pyrimidine derivatives, leading to the development of many therapeutic agents. Pyrimidine is a key component in essential biological molecules, with three major bases—cytosine, thymine, and uracil—integral to nucleic acids. Cytosine and thymine are found in DNA, while cytosine and uracil are present in RNA. These bases are essential for genetic information storage, replication, and protein synthesis, with alterations in pyrimidine metabolism potentially causing

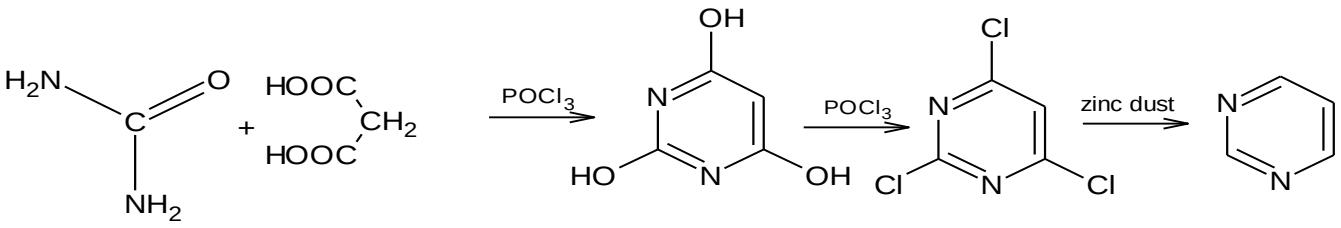
significant genetic and metabolic disorders. Additionally, pyrimidine derivatives appear in some vitamins and coenzymes involved in metabolic processes.

Pyrimidine is known for its diverse biological activities.[8]

1. Antibacterial activity
2. Antiviral activity
3. Antifungal activity
4. Anti neoplastic activity
5. Antitubercular activity

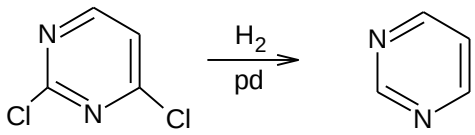
Chemitsry

1.Gabriel synthesis:it involves reaction between urea and malonic acid to give barbituric acid which an reaction with $PoCL_3$ and zinc dust produces pyrimidine.



Urea Malonic acid Barbituric acid Pyrimidine

2. Whittaker synthesis: It involves reduction of 2-6 dichloropyrimidine by using pd- catalyst



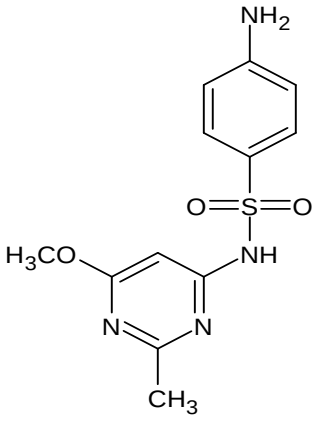
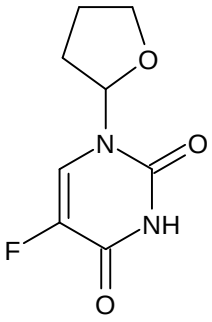
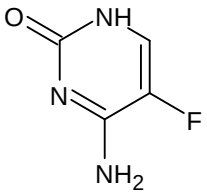
2,6,dichloropyrimidine pyrimidine

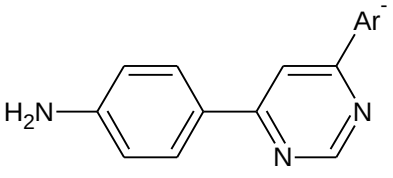
TABLE 1: Marketed drugs conataining Pyrimidine

Review of literature

1.ANTI BACTERIAL ACTIVITY:

i. Neama Abolllah Abd El-tawab et al. synthesized 2,4,6-trisubstituted pyrimidine derivatives were shown to have significant antibacterial activity when compared with standard antibiotics amikacin and penicillin G, against bacteria such as Bacillus pumilis and Escherichia coli.[12]

SI.NO	Marketed product	Structure	Therapeutic uses
1.	Sulfadimethoxine		Used as veterinary medicine for treating wide range of bacterial and some protozoal infections.
2.	Tegafur		A primary use, often as adjuvant therapy after surgery or for metastatic disease.
3.	Flucytosine		Used as antifungal agnet Used to treat severe, systemic fungal and yeast Infections



Ar = 4-Chlorophenyl

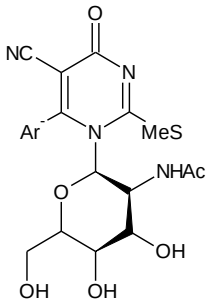
3-Bromophenyl

2-Pyridinyl

4-Pyridinyl

2. ANTIVIRAL ACTIVITY:

Ramiz et al, tested for their antiviral activity against HBV using the HepG 2.2.2.15-Cell a human hepatoplastoma Cell line producing HBV viral particles, and showed moderate viral replication inhibition & mild Cytotoxicity line.[15]

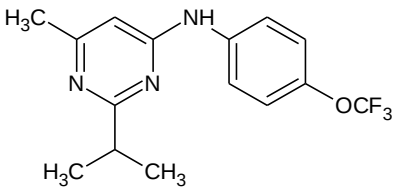


Ar= P-MeC_6H_4

P-MeOC_6H_4

3. ANTI FUNGAL ACTIVITY:

Antifungal activity - Neama Abdallah Abd El-tawah et al, synthesizeds new pyrimidine derivatives were synthesized & their antifungal activities were evaluated in vitro against fourteen phytopathogenic fungi by poisoned food technique, and showed better activity than the lead compound, pyrimethanil to Gibberellafujikuroi (GF).[11][14]



Experimental

Materials and Methods

Materials:

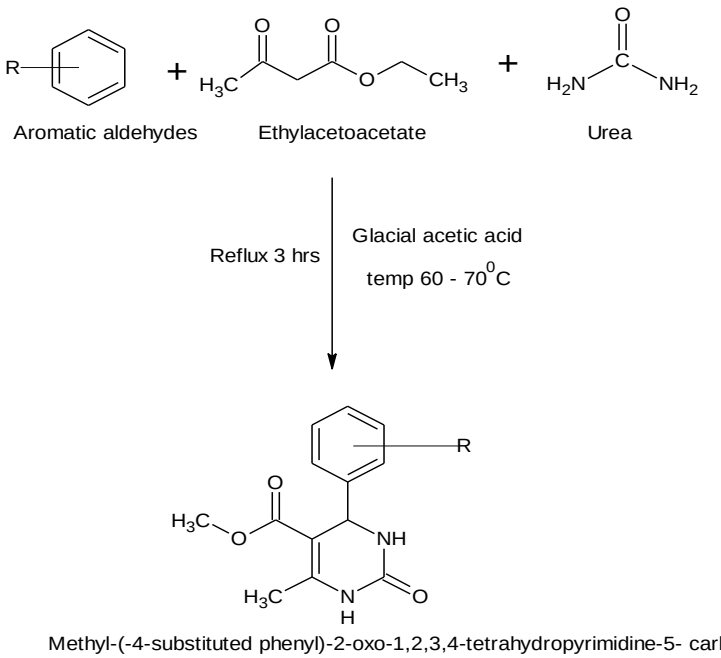
The chemicals required were obtained from HiMedia Chem. Ltd, SD Fine Ltd., and Sigma Aldrich Pvt. Ltd. and were used as such. Melting points were determined using open capillary tube melting point apparatus and are uncorrected. Reaction progress was monitored by performing thin-layer chromatography on silica gel plates. After physical characterization, the compounds were subjected to spectral analysis. IR spectra were recorded on a Nicolet Summit X_using KBr pellets.

Ethyl aceto acetate, benzaldehyde,anisaldehyde,vanillin, p- Chlorabenzaldehyde, thiourea,glacial acetic acid, ethanol.

Method: General Procedure for the synthesis of Methyl-4(-4-substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives:

A mixture of , ethyl acetoacetate (1 mmol), the appropriate aldehyde (1 mmol), and thiourea (1.2 mmol) are taken in a round-bottom flask and dissolved in ethanol (10–15 ml). To this mixture, a few drops of concentrated hydrochloric acid catalyst is added. The reaction mixture is then heated under reflux at about 60-70^{0}C for 3–4 hours, and the progress of the reaction is monitored by TLC using a suitable solvent system such as hexane and ethyl acetate. After completion, the reaction mixture is allowed to cool to room temperature, during which the product usually precipitates. The mixture is then poured into ice-cold water, and the solid obtained is filtered, washed with cold water, and purified by recrystallization from ethanol to afford the desired derivative in good yields, typically ranging from 65 to 90%.

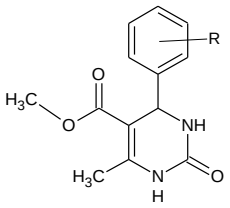
SCHEME



Methyl-(-4-substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5- carboxylate

SI.NO	R
1	-H
2	4-CH_3O
3	4-OH 3-CH_3O
4	4-Cl
5	3-NO_2

Physical data and yields of Methyl-4(-4-Substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives



Compound	R	Molecular formula	Molecular weight	% yield	Melting point	R_F value
1.	-H	$C_{14}H_{16}N_2O_3$	246.27	72.7%	202°C	0.82
2.	4-CH_3O	$C_{15}H_{18}N_2O_4$	276.29	75.5%	160°C	0.89
3.	4-OH 3-CH_3O	$C_{15}H_{18}N_2O_5$	292.29	70.1%	158°C	0.96
4.	4-Cl	$C_{14}H_{15}ClN_2O_3$	280.71	71.3%	200°C	0.94
5.	3-NO_2	$C_{14}H_{15}N_3O_5$	245.56	73.2%	210°C	0.94

TLC:

General procedure for Thin layer chromatography

1.Preparation of TLC plate

Take a pre-coated silica gel TLC plate. Draw a lightpencil line about 1 cm from the bottom to mark the baseline. Do not use ink.

2. Preparation of sample solution

Dissolve a small amount of the sample (and standard, if required) in a suitable volatile solvent such as methanol, ethanol, or ethyl acetate.

3. Spotting the sample

Using a capillary tube, apply a small, concentrated spot of the sample on the baseline. Allow the spot to dry completely.

4. Preparation of mobile phase

Prepare the mobile phase in a TLC chamber (e.g.,hexane :ethyl acetoacetate,1:4). Close the chamber and allow it to saturate with solvent vapors for a few minutes.

5. **Development of the plate**

Place the TLC plate vertically in the chamber, ensuring the spot remains above the solvent level. Close the chamber and allow the solvent to rise.

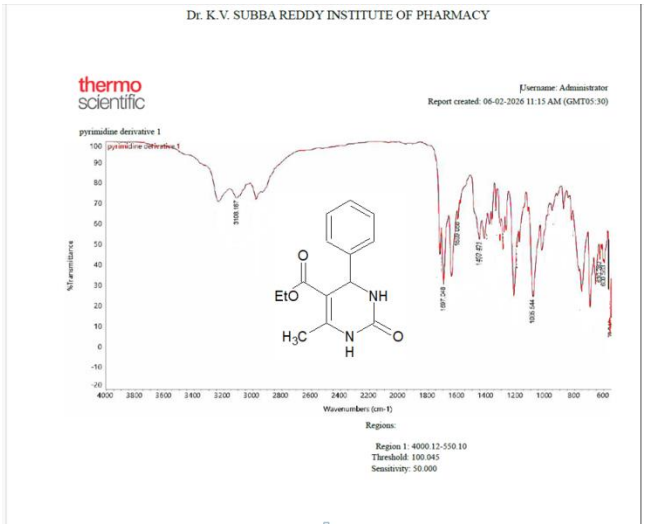
6. Removal of plate

When the solvent front reaches about ¾ of the plate, remove the plate and immediately mark the solvent front with a pencil.

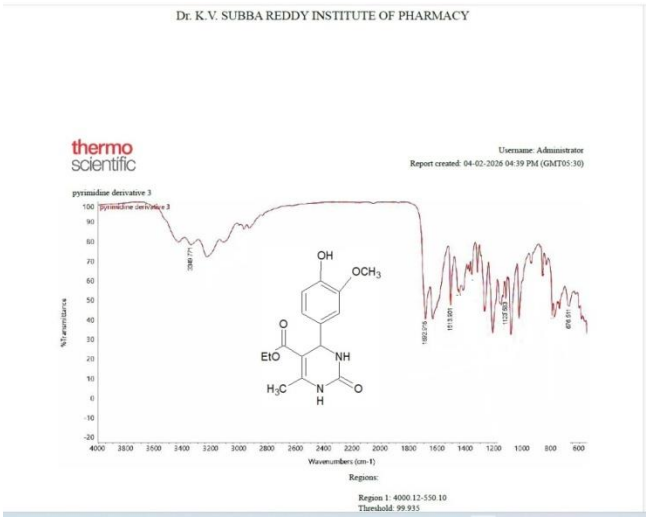
7. Drying and visualization

Dry the plate and visualize the spots under UV light or by using a spraying reagent or iodine chamber.

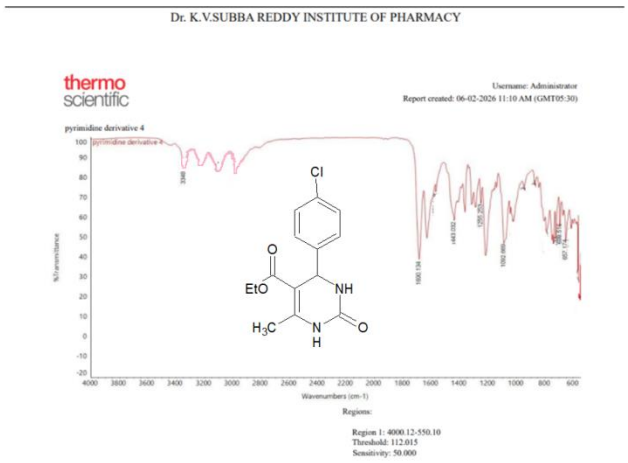
IR Spectra for Compound 1:



IR Spectra for Compound 3:



IR Spectra for Compound 4:



FT-IR Spectral Data of different derivatives

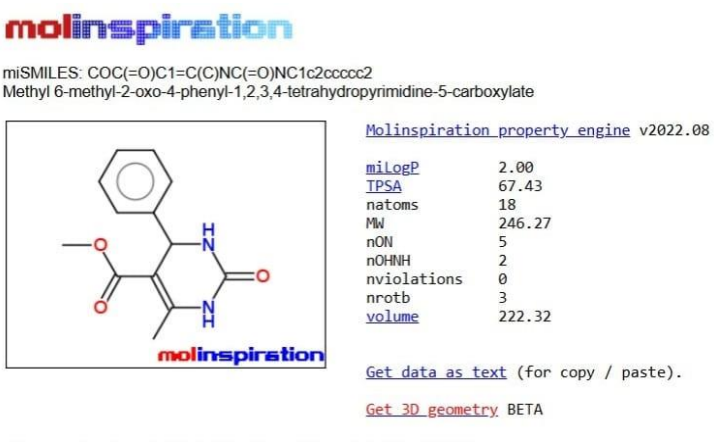
COMPOUND	FT-IR
1	Stretchind bonds 3108(C-H) 1697 (C=N)1452(C=C)1179(C-N) aromatic bonds 696(C-H)
3	Stretchind bonds 3349(C-OH)1639(C=N)1452(C=C)1155(C-N) aromatic bonds 676(C-H)
4	Stretchind bonds 618(C-Cl)1690(C=N)1443(C=C)1092(C-N) aromatic bonds 698(C-H)

Insilico Evalutions:

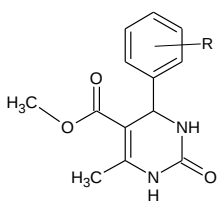
1. Molinspiration:

Molinspiration is an independent research organization focused on development and application of modern chem informatics techniques, especially in connection with the internet. Molinspiration offers broad range of chem, informatics software tools supporting molecule manipulation and processing, including SMILES and SD file conversion, normalization of molecules, generation of tautomer's, molecule fragmentation, calculation of various molecular properties needed in OSAR, molecular modelling and drug design, high quality molecule depiction, molecular data base tools supporting substructure search or similarity and pharmacophore similarity search. Molinspiration tools are therefore platform independent and may be run on any PC, Mac, UNIX or LINUX machine. The software is distributed in a form of toolkits, which may be used as stand-alone computational engines, used top over web-based tools, or easily incorporated into larger in-house Java applications. Molinspiration mi screen engine allows fast prediction of biological activity- virtual screening of large collections of molecules and selection of molecules with the highest probability to show biological activity. The screening is based on identification of fragments or substructure features typical for the active molecules. The Molinspiration virtual screening is fast and therefore allows processing of very large molecular libraries. Validation tests performed by our company, as well as results of our customers on various target classes show 10 to 20-fold increase in hit rate in comparison with random selection of molecules for screening.

Compound :1



Molecular properties of Methyl-4(-4- substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives by using Molinspiration



Compound	R	Mi LogP	TPSA	N Atom	N ON	N OHNH	N Violations	N rotb	Volume	MW
1.	-H	2.00	67.43	18	5	2	0	3	222.32	246.27
2.	4-CH_3O	2.05	76.66	20	6	2	0	4	247.87	276.29
3.	4-OH 3-CH_3O	1.33	96.89	21	7	3	0	4	255.89	292.29
4.	4-Cl	2.67	67.43	19	5	2	0	3	235.86	280.71
5.	3-NO_2	1.12	67.43	19	5	2	0	3	232.01	245.56

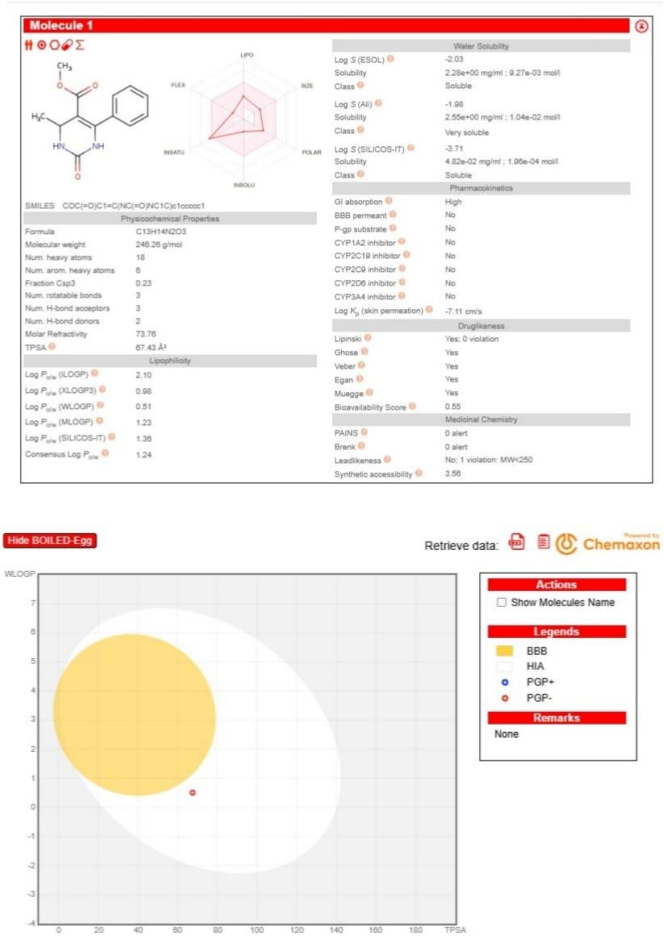
Swiss ADME

A large variety of Insilco methods share the objective of predicting ADME parameters from molecularstructure. Noteworthy, the pioneer work of Lipinski et al. Examined orally active compounds to definephysicochemical ranges for high probability to be an oral drug. This so called Rule-of-five delineated the relationship between pharmacokinetic and physicochemical parameters. Whereas physicochemical parameters give a global description of the structure, molecules can be directly described by substructure searches.

Compound	R	Physico-chemical properties							Pharmacokinetics		
		No. Of Heavy Atoms	No. Of arom. Heavy Atoms	N-Rb	NH-a	NH-d	Molar Refractivity	TPSA	GI Absorption	BBB	Drug likelyness
1	-H	18	6	3	3	2	73.76	67.43 A^2	High	No	Yes
2	4-CH_3O	20	6	4	4	2	79.46	76.66 A^2	High	No	Yes
3	4-OH 3-CH_3O	21	6	4	5	3	81.48	96.89 A^2	High	No	Yes
4	4-Cl	19	6	3	3	2	77.98	67.43 A^2	High	No	Yes
5	3-NO_2	21	6	4	5	2	81.79	113.25 A^2	High	No	Yes

In turn, these ADME parameters can be evaluated separately by dedicated methods. It has been demonstrated that early estimation of ADME in the discovery phase reduces drastically the fraction of pharmacokinetics-related failure in the clinical phases. Computer models have been fostered as a valid alternative to experimental procedures for prediction of ADME, especially at initial steps, when investigated chemical structures are numerous but the availability of compounds is scarce.

Compound :1

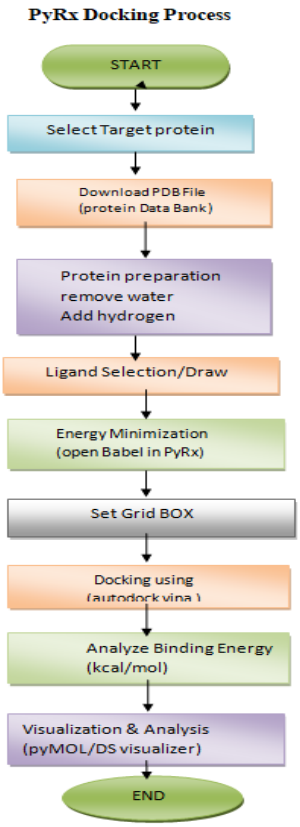


Molecular properties of Methyl-4(-4- substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives by using Swiss ADME

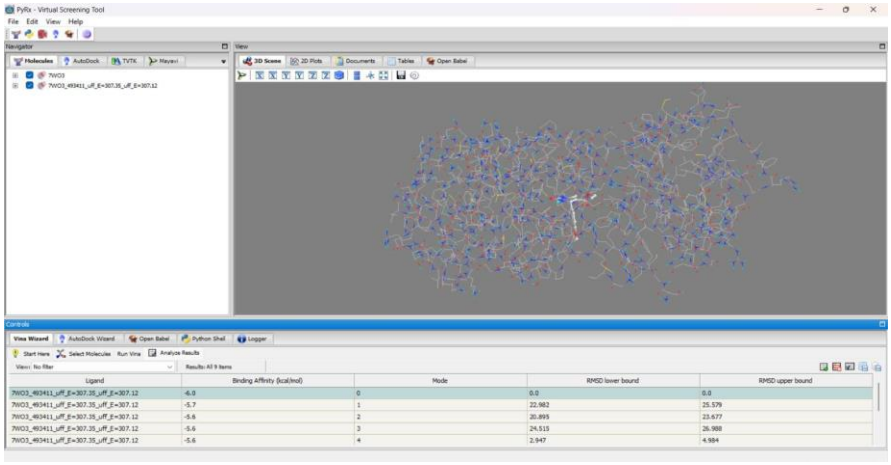
Docking by using PyRx software tool

PyRx is a type of borosilicate glass that is highly resistant to heat, thermal shock, and chemical reactions. It is commonly used in laboratories, pharmaceutical industries, and kitchenware because it does not crack easily when exposed to sudden temperature changes. It is mainly used for virtual screening and molecular docking, allowing scientists to predict the binding affinity and orientation of drug-like compounds within the active site of a target protein. PyRx provides a user-friendly graphical interface and integrates powerful tools like Auto Dock and Auto Dock Vina, making complex docking studies easier even for beginners. Being free and open-source, it is commonly used in academic research for lead identification and optimization, helping to reduce time, cost, and experimental effort in drug development.

One of the major advantages of PyRx is its easy-to-use graphical interface, which reduces the need for advanced programming knowledge. It supports ligand and protein preparation, energy minimization, and file format conversion using Open Babel.



Compounds :1



2. Results and Discussion:

Chemistry:

Synthesis of Methyl-4(-4-substituted phenyl)-2-oxo-1,2,3,4- tetrahydropyrimidine-5-carboxylate derivatives:

The Methyl-4(-4-Substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives are synthesized by Gabriel reaction by using various homogenous mixctures such as ethylacetoacetate,thiourea,glacial acetic acid,ethnol and substited aromatic aldehydes is which the mechanism followed is condensation process.

A series of 1 to 5 compounds for synthezied the compounds obtained in good yelid range from 65-90%.All the synthezied compounds were characterazied byTLC and IR. The physical data such as percentage yield and the melting point and the spectral data of the compounds.

The IR spectra of compounds showed bands such as 3108 to 3110(C-H), 1440 to 1630 (C=C) strecthing bands observed at 1660 to 1699 (C=N), aromatic bands at 672 to 698(C-H).

Molecular Description

The derivatives were also evaluated for its Inslico properities by using software programmes like molinspiration, swiss ADME, molecular docking by using PyRx. Which are helpful to predict molecules general properities, drug likeness to binding affinities. All the results were tabulated and it was observed the compounds obey the LIPINSKI rule of five.

All the molecular properties of the synthesized derivatives were calculated by using molinspiration and swiss ADME. All the compounds obey LIPINSKI rule of five.

Molecular properties of the title compounds include mi log p (1.12-2.67) TPSA (67.43 -96.89) MW (245.56-292.29) HBA(5-7) HBD (2to3) M violate (0) ROB (3to4). All the synthesized compounds showed good TPSA which represents good overall absorption and crosses blood brain barrier also n atoms represents all the derivatives are orally active. The molecular weight was less than 500 for all the synthesized compounds. Number of H bond acceptors are less than 10 and number of H bond donars are

less than 5, number of violations are zero. So all these compounds represents that all synthesized derivatives follow LIPINSKI rule of five.

3. Conclusion

A series of Methyl-4(-4-substituted phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives were synthesized good yields.The Insilico evaluate revealed that all 5 compouds adhered to LIPINSKI rule of five. Indicates favourable pharmacokinetics properties and good oral absorption potential. Molecular docking studies showed good binding interactions, particularly compound 5, which exhibited the highest binding effinity with target protein. These finding collectively highlighted the multi functional biological significance of pyrimidine derivatives reinforcing their values in medicinal chemistry. They serve as promising leads for further development in treating viral infections, inflammation,cardiac and other disorders.

Bibilography

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