

“Design, Docking, Synthesis, Characterization and Biological Evaluation of Isoindole 1,3-Dione Fused With 1-H Pyrazole Derivative”

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ABSTRACT

Isoindole and pyrazole scaffolds represent two privileged heterocyclic frameworks widely recognized for their broad pharmacological potential. In this study, a new series of isoindole-1,3-dione fused 1H-pyrazole derivatives were rationally designed and computationally evaluated to identify candidates with improved drug-likeness and biological activity. Molecular modelling tools, including Mol-Inspiration, SwissADME and ProTox-III, were employed to assess physicochemical properties, Lipinski compliance, pharmacokinetic behaviour, and predicted toxicity. The designed molecules were subsequently synthesized through a multi-step synthetic route starting from phthalic anhydride, followed by condensation with substituted aromatic aldehydes. Structural confirmation of the synthesized derivatives was achieved using UV-Visible and FT-IR spectroscopy.

The compounds were subjected to a comprehensive biological evaluation to assess anti-inflammatory, antimicrobial and antioxidant properties. Inhibition of protein denaturation assay demonstrated moderate to significant anti-inflammatory activity among selected derivatives, whereas antimicrobial screening revealed measurable inhibition against Mycobacterium tuberculosis H37RV strain, with activity comparable to standard anti-TB agents such as isoniazid and ciprofloxacin. Antioxidant potential assessed using hydrogen peroxide radical scavenging indicated favourable free-radical quenching ability for several compounds.

Overall, the integration of computational design, synthetic chemistry and biological assays provides strong evidence that isoindole-pyrazole hybrid

scaffolds constitute promising lead structures for further optimization. These findings support their potential development as multifunctional therapeutic agents with anti-inflammatory, antimicrobial and antioxidant properties.

Keywords

Isoindole-1,3-dione; Pyrazole derivatives; Molecular docking; In-silico ADME; ProTox-III; Heterocyclic synthesis; Anti-inflammatory activity; Antioxidant activity; Antidiabetic activity; α -amylase inhibition; Protein denaturation assay; Hydrogen peroxide scavenging; Spectroscopic characterization; Medicinal chemistry; Multi-target drug design.

I. INTRODUCTION MEDICINAL CHEMISTRY

Medicinal chemistry is a chemistry-based discipline, also involving aspects of biological medical and pharmaceutical science. “It is concerned with the invention discovery, design, identification and preparation of biologically active compound, the study of their metabolism, the interpretation of their mode of action at the molecular level and construction of structural activity relationship”.

Medicinal chemistry involves the lead molecule potency selectivity, reduce toxicity, pharmacokinetic and pharmaceutical properties. It is a science the earlier sources of drugs were from plants, animals and minerals. But, due to the lack of potential action, definite cure, dose level, sometime cause more toxicity, the discovery of new drugs that are more potential and less toxic is essential. The synthesis of new derivatives is an important part of drug development and is aimed at modifying the

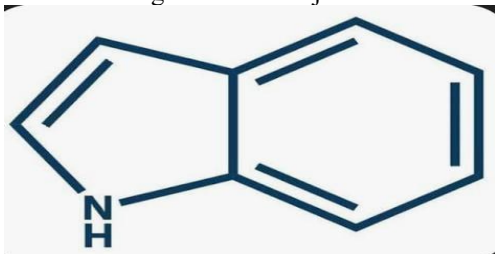
action of drugs, particularly to reduce side effects and potentiate drug action. Today more than 60% drugs used in practices are synthesized derivatives and day by day the scope of synthetic medicinal chemistry is broadening.

Once the new pharmaceutical lead has been discovered, extensive and costly efforts usually are made to prepare a series of analogue in the hope that even better activity will be found. Such programs included the branching lengthening shortening of chain structure, the variation of the kinds and positions of substituents the replacement of rings by similar cyclic structures and other empirical molecular modifications within the frame work of reasonably close analogue.

BASIC NUCLEUS PROFILE

ISOINDOLE:

The basic isoindole nucleus is a bicyclic aromatic structure with a benzene ring fused to a five-membered pyrrole ring. The free molecule itself is highly reactive and not easily isolated, but its structure can be represented for educational purposes. Isoindole Nucleus Structure. A five-membered heterocyclic ring containing one nitrogen atom. The two rings share two adjacent carbon atoms.

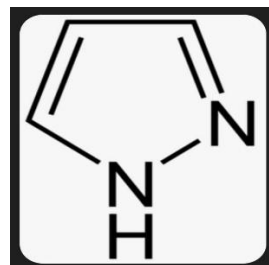


Isoindole is a bicyclic nitrogen-containing heteroaromatic compound consisting of a benzene ring fused to a five-membered pyrrole ring, sharing two adjacent carbon atoms, with the molecular formula C_8H_7N . The isoindole nucleus serves as a key scaffold in medicinal chemistry due to its ability to interact with enzyme active sites through π - π interactions, hydrogen bonding, and van der Waals forces.

PYRAZOLE:

Pyrazole is a five-membered heterocycle bearing two adjacent nitrogen atoms. Both pharmaceutical agents and natural products with pyrazole as a nucleus have exhibited a broad spectrum of biological activities. In the last few decades, more than 40 pyrazole-containing drugs have been approved by the FDA for the treatment of a broad range of clinical conditions including celecoxib (anti-inflammatory), CDPPB

(antipsychotic), difenamizole (analgesic), etc.



Pyrazole is a five-membered aromatic heterocycle with two adjacent N heteroatoms. Its N-1 atom has similar properties to the NH of pyrrole which can serve as a hydrogen bond donor, and its N-2 atom behaves similarly to the nitrogen atom of pyridine which can serve as a hydrogen bond acceptor. Due to different chemical environments, all five bonds have different bond lengths. The aromaticity of pyrazoles lies at an intermediate level among the other aromatic heterocycle

MICROBIAL ACTIVITY

Microorganisms, also known as microbes, are organisms that are too small to be seen by the naked eye and can only be viewed with a microscope. They can be unicellular, multicellular, or acellular, and include a wide range of organisms, such as:

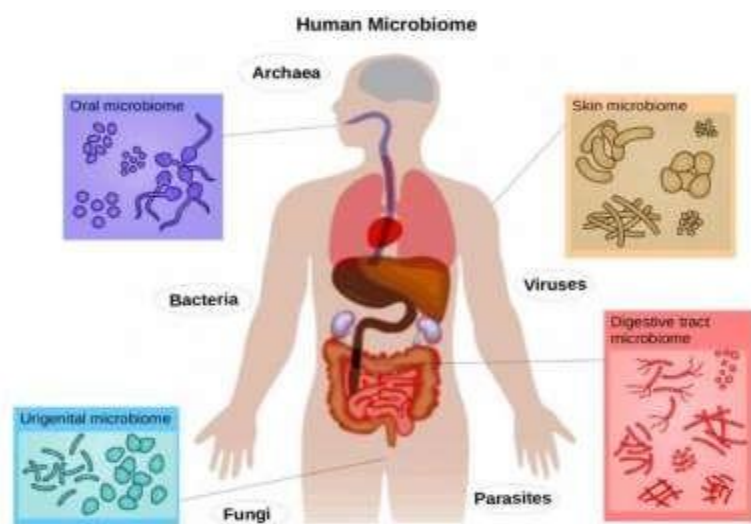
- **Bacteria:** Single-celled microbes that lack a nucleus
- **Archaea:** Similar to bacteria, but with different structures and properties
- **Protists:** Unicellular microorganisms with nuclei
- **Fungi:** Includes mushrooms, molds, and yeasts, and many are multicellular
- **Viruses:** Noncellular entities that consist of a core of DNA or RNA surrounded by protein.

There is a mighty but invisible kingdom of microbes present within your body. Small yet incredibly powerful, these thousands of species and trillions of inhabitants live in all parts of your body and make up the diverse human microbiome. These microbiomes support and maintain your health but also, when the microbiome is disturbed in some fashion, have been linked to hundreds of ailments such as cancers, and autoimmune and cardiovascular diseases.

MECHANISM:

The mechanism of microbial activity encompasses

the attraction of NPs to the cells followed by the release of poisonous ions that bind to the sulfur-containing proteins as a result of the interaction with DNA during the replication process



DIABETIC ACTIVITY

Definition:

Diabetic activity refers to the impaired regulation of blood glucose levels due to defects in insulin secretion, insulin action or both, resulting in chronic hyperglycemia (high blood sugar levels).

Causes:

The causes of diabetic activity include destruction of insulin-producing beta cells of the pancreas (Type 1 diabetes), insulin resistance combined with inadequate insulin production (Type 2 diabetes), genetic factors, unhealthy lifestyle habits, obesity, and lack of physical activity.

Mechanism:

The mechanism involves insufficient insulin or reduced cellular response to insulin, which prevents glucose from entering body cells. As a result, glucose accumulates in the bloodstream while cells are deprived of energy, leading to metabolic disturbances and long-term damage to organs and tissues.

INFLAMMATORY ACTIVITY

Inflammation is a protective biological response of body tissues to injury, infection, toxins, or irritants. Its purpose is to eliminate the cause of injury, remove damaged cells, and initiate healing.

TYPES OF INFLAMMATION:

A. Acute Inflammation

- Rapid onset (minutes to hours)
- Short duration

- Characterized by redness, heat, swelling, pain

B. Chronic Inflammation

- Long-lasting (weeks to years)
- Causes tissue damage
- Associated with diseases like:
 - Arthritis
 - Diabetes
 - Cardiovascular disease
 - Cancer

MECHANISM OF INFLAMMATORY ACTIVITY:

Inflammatory activity begins when tissues are injured or infected, leading to the release of danger signals that activate immune cells such as mast cells and macrophages. These cells release inflammatory mediators including histamine, cytokines (TNF- α , IL-1, IL-6), and chemokines, which cause vasodilation and increased vascular permeability, resulting in redness, heat, and swelling. Simultaneously, activation of phospholipase A₂ releases arachidonic acid from cell membranes, which is metabolized through the cyclooxygenase (COX) pathway to form prostaglandins and through the lipoxygenase (LOX) pathway to form leukotrienes; these mediators are responsible for pain, fever, and recruitment of leukocytes.

OXIDANT ACTIVITY

The process of oxidation in the human body damages cell membranes and other structures, including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates unstable molecules called 'free radicals', which steal electrons from other molecules, causing damage to DNA and other cells.

The body can cope with some free radicals and needs them to function effectively. However, the damage caused by an overload of free radicals over time may become irreversible and lead to certain diseases (including heart and liver disease) and some cancers (such as oral, oesophageal, stomach and bowel cancers).

Oxidation can be accelerated by:

- stress
- cigarette smoking
- alcohol
- sunlight
- pollution > other factors.

The effect of free radicals:

Some conditions caused by free radicals include:

- deterioration of the eye lens, which contributes to vision loss
- inflammation of the joints (arthritis)
- damage to nerve cells in the brain, which contributes to conditions (such as Parkinson's or Alzheimer's disease)
- acceleration of the ageing process
- increased risk of coronary heart disease, since free radicals encourage low-density lipoprotein (LDL) cholesterol to stick to artery walls > certain cancers triggered by damaged cell DNA.

PYRETIC ACTIVITY

Pyretic activity is the activity of causing a fever. The word pyretic comes from the Greek word pyretikos, which comes from the word pyretos meaning fever.

A fever is a temporary increase in body temperature, usually caused by an infection or illness, and is part of the body's immune response. A fever is when your body temperature is higher than normal, usually 100.0°F (37.8°C) or 100.4°F (38°C).

Causes:

Fevers are caused by pyrogens, which can be endogenous or exogenous. Infections, inflammatory disorders, allergic reactions, medications, and illicit drugs can all cause fevers.

Mechanism:

The hypothalamus, the body's thermostat, resets to a higher temperature in response to an infection or illness. This increases heat production and decreases heat loss.

Effects:

Fevers can cause discomfort, chills, sweats, and flushed skin. They usually go away within a few days and aren't a cause for concern, unless you have a fever of 104°F or higher, or you experience other symptoms like confusion, loss of consciousness, or difficulty br

AIM AND OBJECTIVE

AIM:

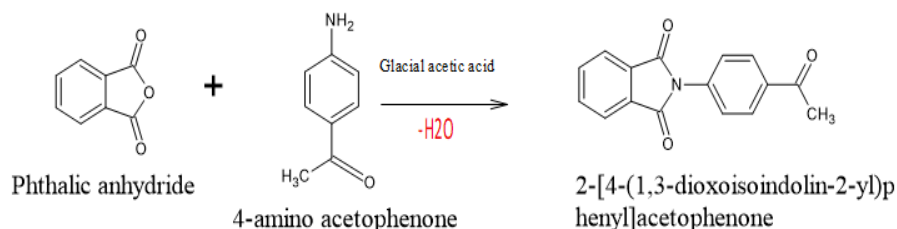
To design, computationally evaluate, synthesize, characterize, and biologically assess a novel series of isoindole-1,3-dione fused 1H-pyrazole derivatives as potential therapeutic agents with enhanced potency and selectivity.

OBJECTIVE:

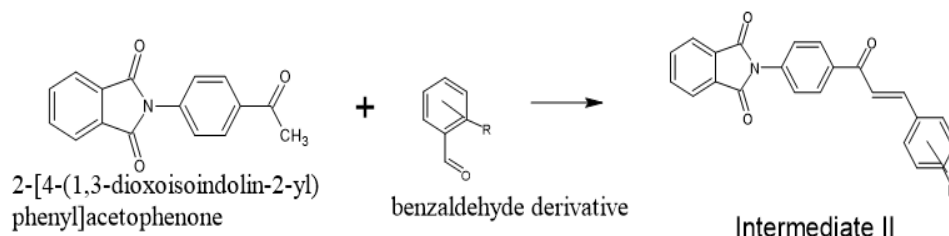
- To design isoindole-1,3-dione fused 1H-pyrazole derivatives using molecular modelling and docking.
- To synthesize the designed derivatives through an optimized synthetic route.
- To characterization of the synthesized compounds by using spectroscopy methods as following IR and UV.
- The biological evaluation of the synthesized compounds to be tested against the Anti-inflammatory, anti-microbial and anti-oxidant
- The Minimum inhibitory concentration of the 5 synthesized compounds against mycobacterium-H37RV strain, which is compared to that of the certain known Anti-TB agents Isoniazid Pyrazinamide, Ciprofloxacin and Streptomycin.

SYNTHETIC SCHEME

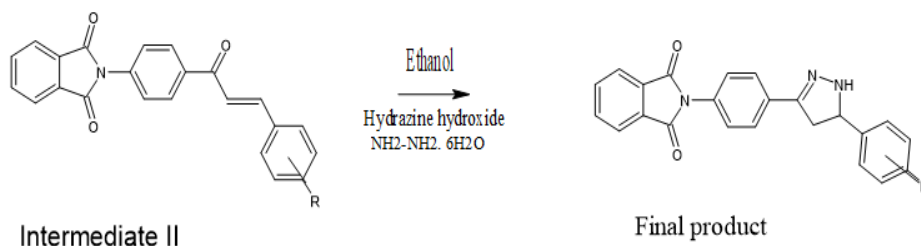
STEP 1



STEP 2



STEP 3



AROMATIC ALDEHYDES

COMPOUND C1- 2 Nitro Benzaldehyde

COMPOUND C2- 4 Nitro Benzaldehyde

COMPOUND C3- 4 Hydroxy Benzaldehyde

COMPOUND C4- Benzaldehyde

COMPOUND C5- 4 N-dimethyl amino Benzaldehyde

MOLECULAR DESIGN:

Molecular design is the process of finding new medicines based on the knowledge of a biological target, it enabled the chemist to predict the structure and the it also allows the medicinal chemist to evaluate the interaction between a compound and its

target site before synthesizing a compound so as to increase the ability by reducing the side effects.

Various software used:

Chem Sketch
Mol inspiration
Swiss ADME
Pro Tox 3.0

MOL INSPIRATION

This software is used to calculate the following properties

- Log P
- Molecular weight
- Number of H-bond donor
- Number of H-bond acceptor
- Number of rotatable bonds

In addition to "LIPINSKI'S RULE" another rule

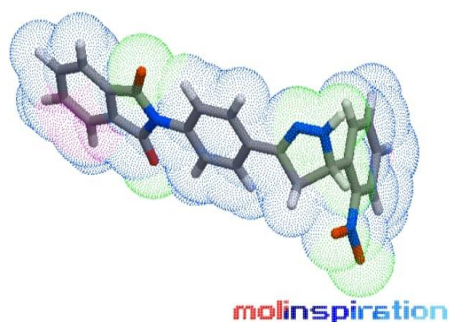
was proposed by VEBER HE states that the number of rotatable bonds should be less than 10. This rule is more appropriate for oral drug only.

ACCORDING to the Vebers rule

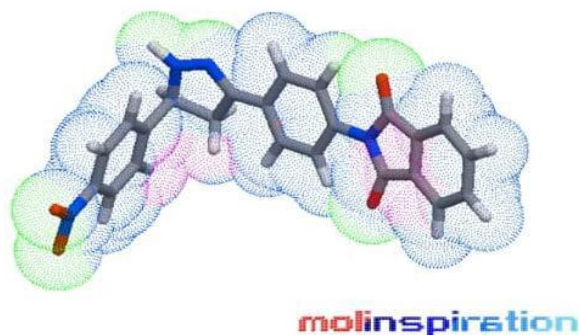
1. The Log P value should not be more than 5
2. The molecular weight of the compound should not more than 500
3. No. of H-bond donor not more than 5
4. No. of rotatable bonds should not be more than 10

3D VIEW STRUCTIRE OF COMPOUNDS

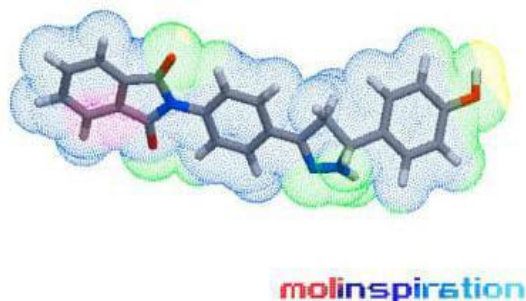
COMPOUND 1:



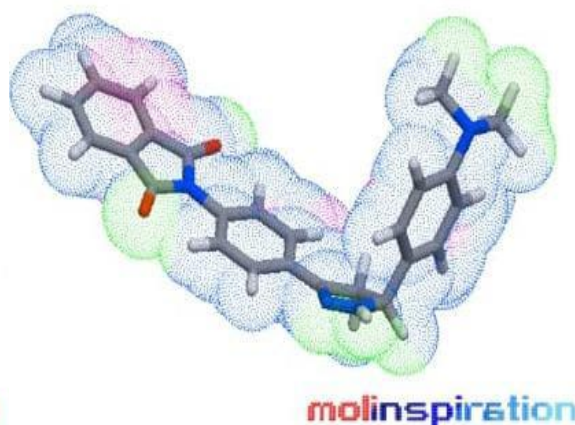
COMPOUND 2:



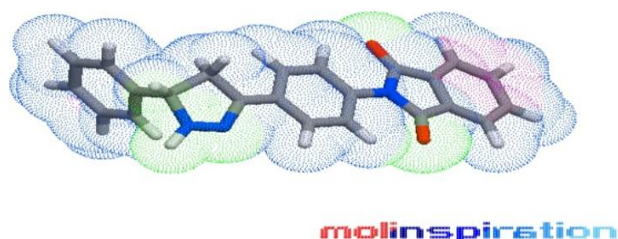
COMPOUND 3:



COMPOUND 4:



COMPOUND 5:



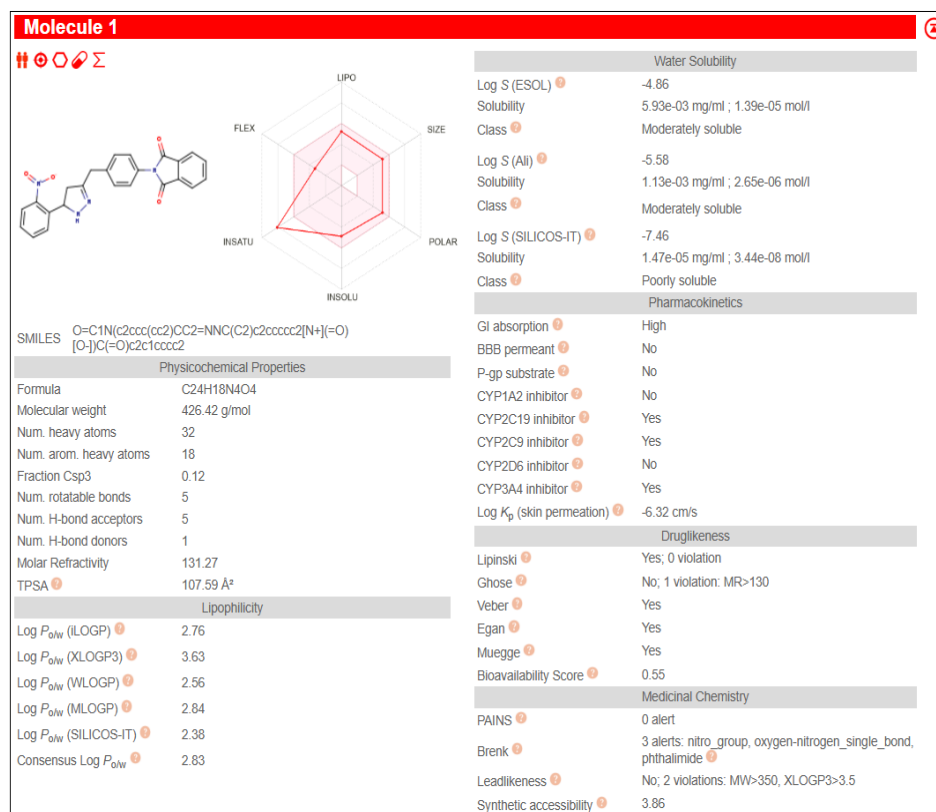
SWISS ADME

SWISS ADME is a web tool that gives free access to a pool of fast yet robust predictive models for physiochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules.

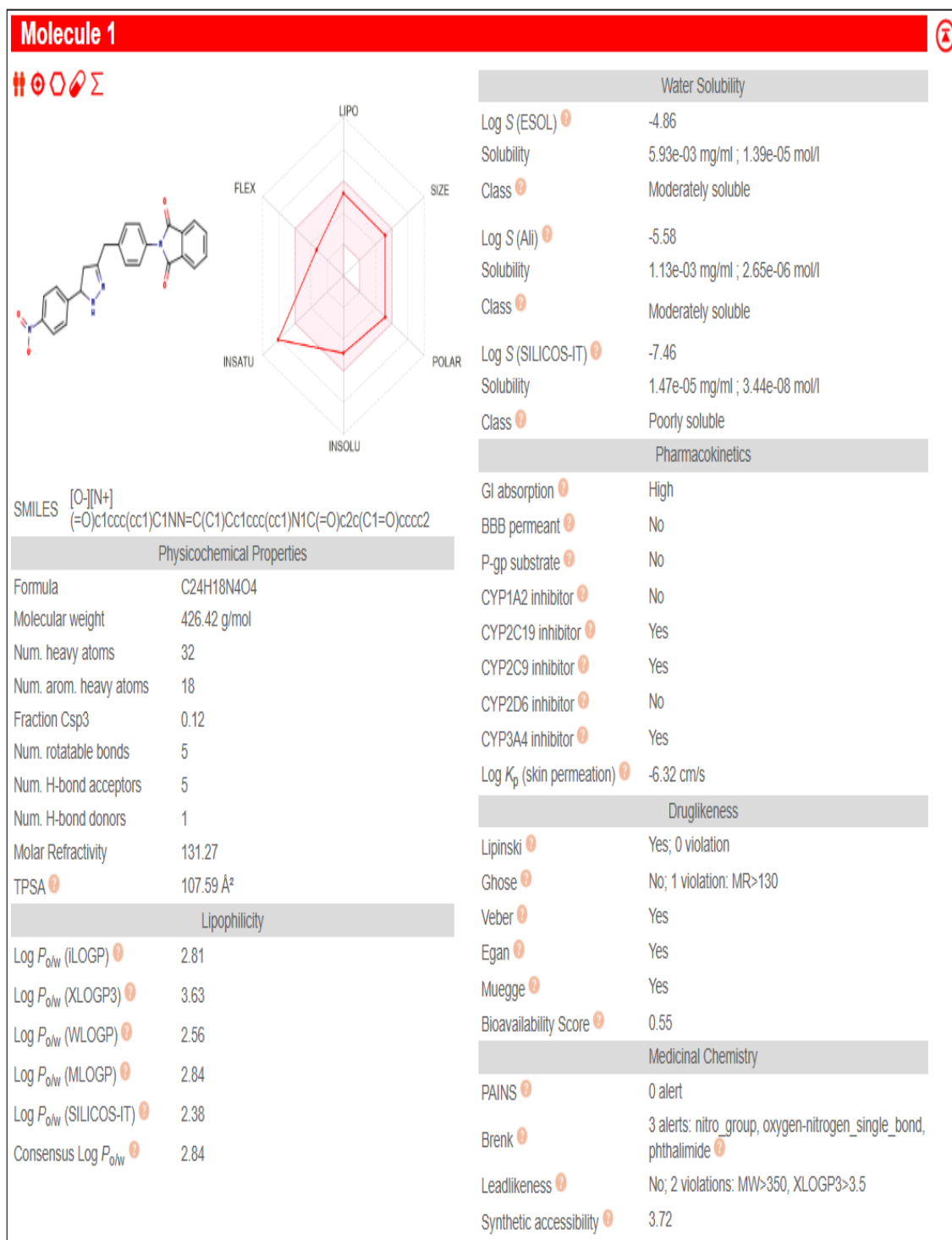
Six physiochemical properties are taken into account:

1. Lipophilicity,
2. Size,
3. Polarity,
4. Solubility,
5. Flexibility, 6. Saturation.

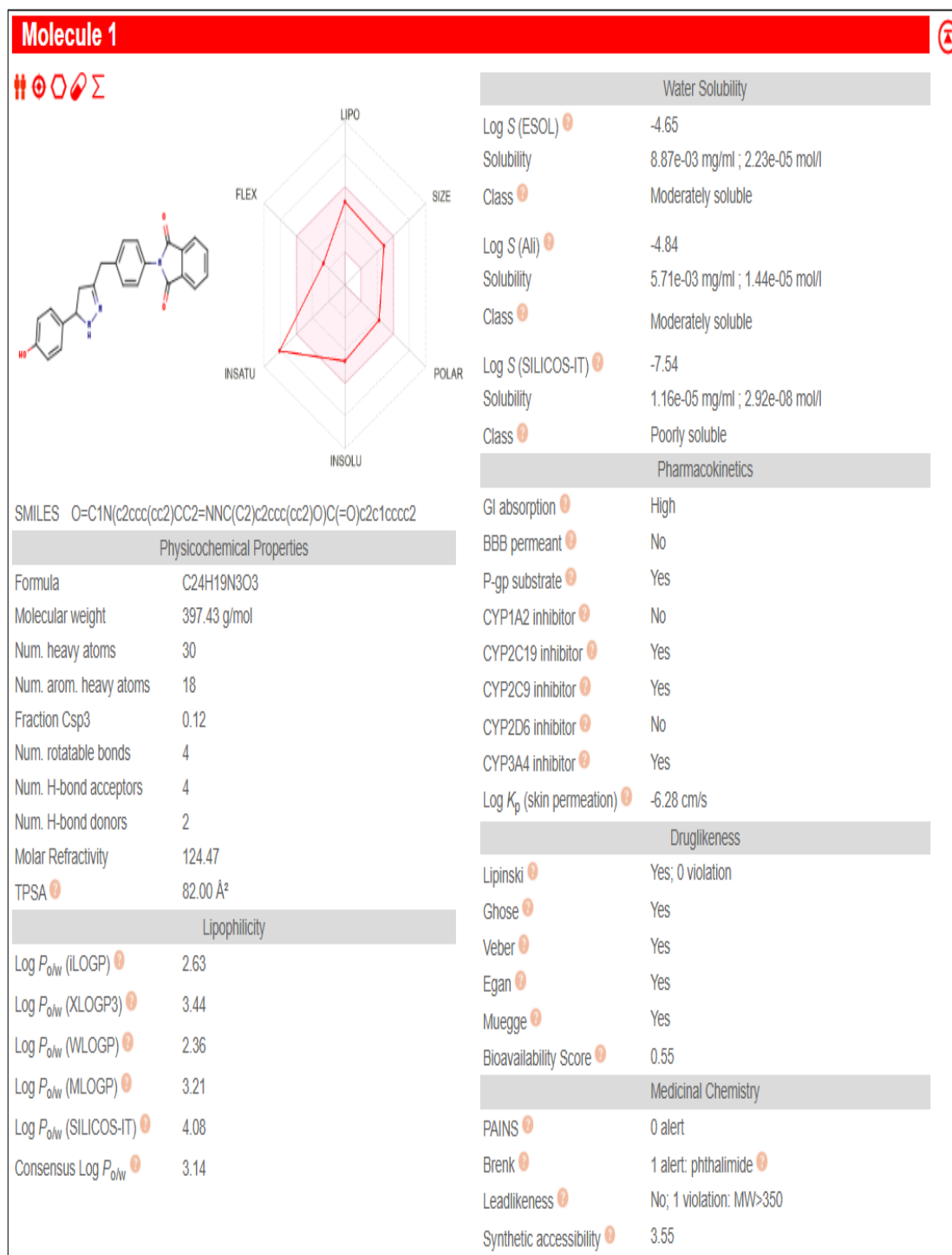
COMPOUND C1:



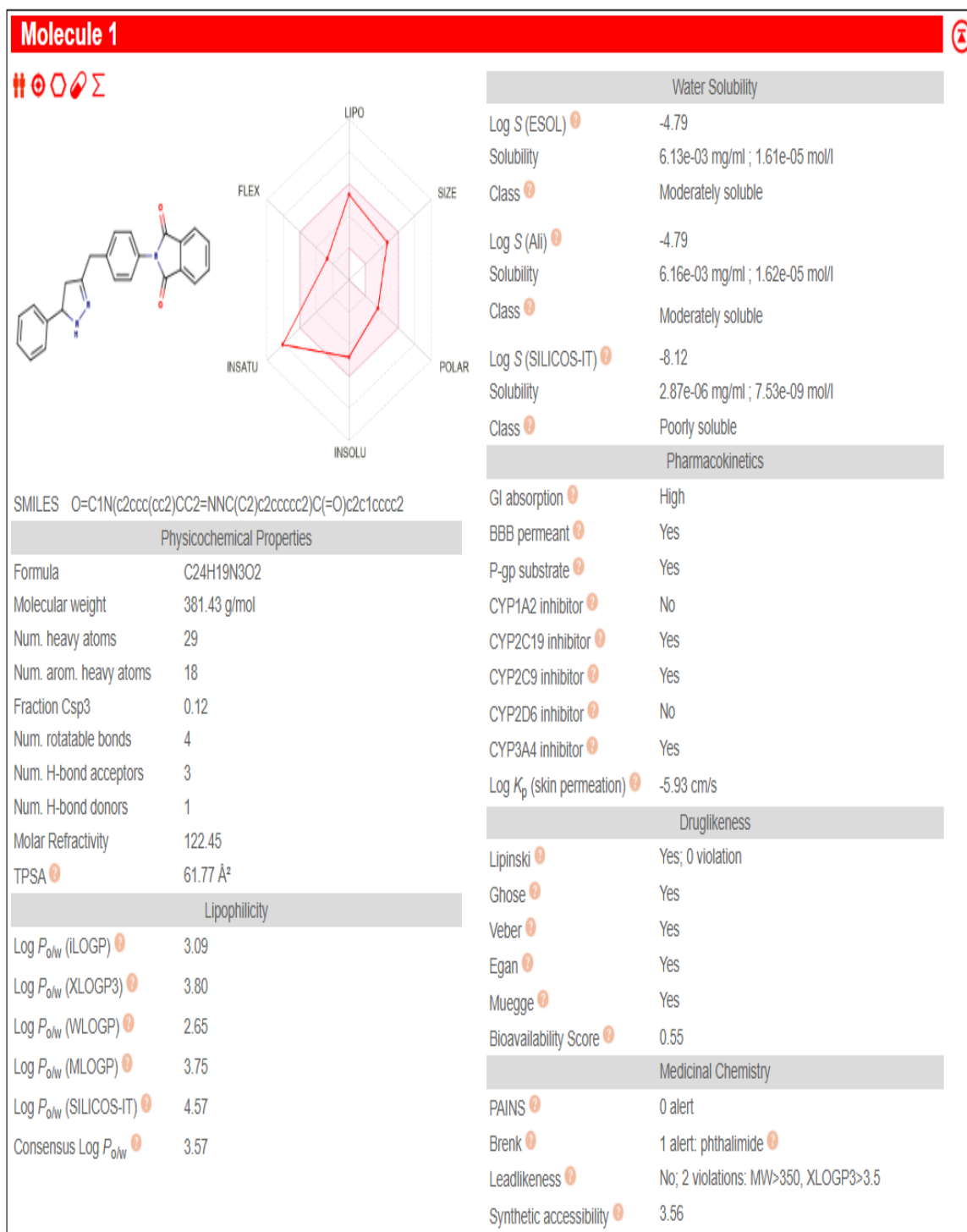
COMPOUND C2:



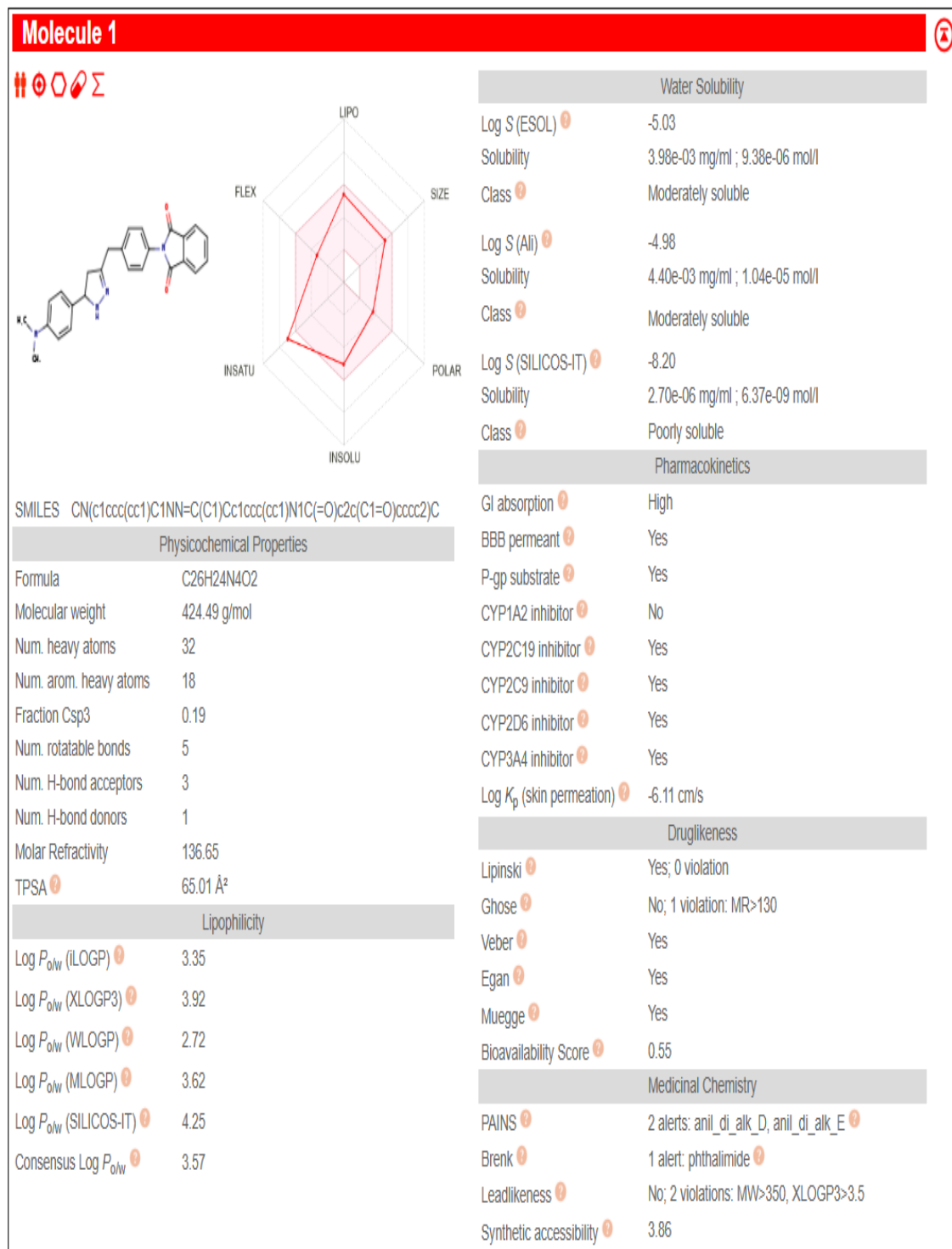
COMPOUND C3



COMPOUND C4:



COMPOUND C5:



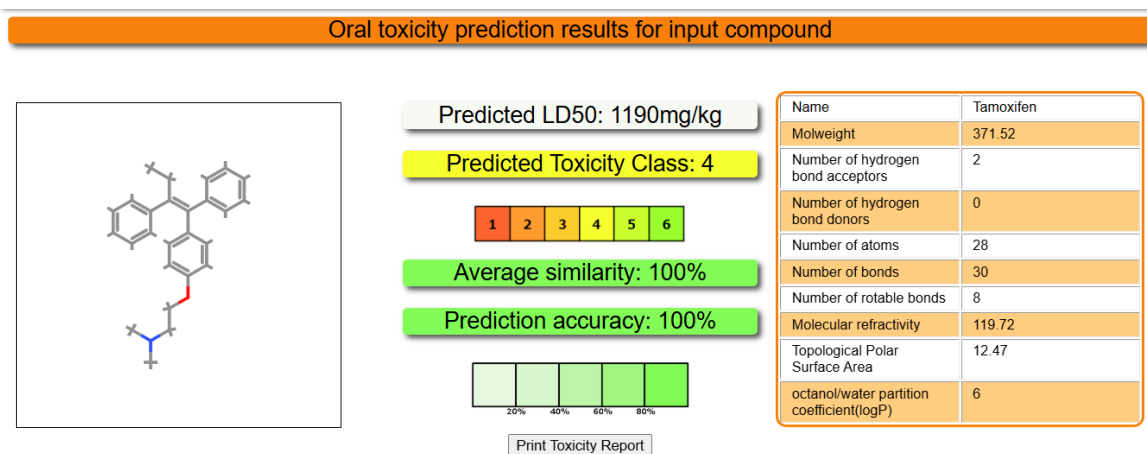
PROTOX 3.0

In-Silico Toxicity Assessment:

In silico toxicity assessment for these 10 compounds resulted by using PROTOX 3.0 based on online web tool. The web tool predicts the toxicity effect of chemicals, such as cardiotoxicity, immunotoxicity, androgen receptor, aromatase etc.,

On PROTOX 3.0, the user needs to specify either the name or canonical SMILES (*Simplified Molecular-Input Line Entry System*) string of the input compound to run a prediction on the server. The prediction results are also shown as a toxicity radar plot for active class prediction. The results will include a toxicity score and a classification (e.g., active or inactive).

COMPOUND C1:



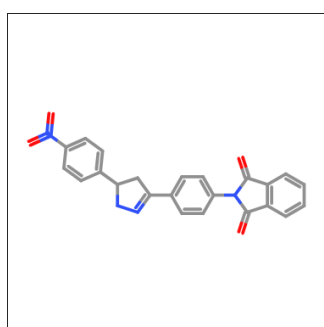
Comparison of input compound with dataset compounds

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
Toxicity end points	BBB-barrier	bbb	Inactive	1.0
Toxicity end points	Clinical toxicity	clinical	Inactive	0.56
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.88
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.78

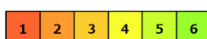
COMPOUND C2:

Oral toxicity prediction results for input compound



Predicted LD50: 1000mg/kg

Predicted Toxicity Class: 4



Average similarity: 62.9%

Prediction accuracy: 68.07%



Print Toxicity Report

Name	O=c2c1cccc1c(=O)n2c5
Molweight	412.4
Number of hydrogen bond acceptors	7
Number of hydrogen bond donors	1
Number of atoms	31
Number of bonds	35
Number of rotatable bonds	4
Molecular refractivity	126.46
Topological Polar Surface Area	107.59
octanol/water partition coefficient(logP)	4.19

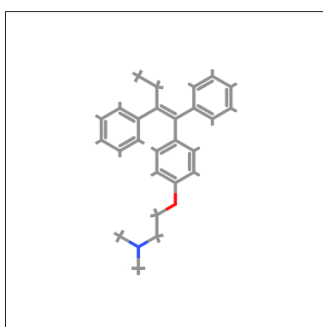
Comparison of input compound with dataset compounds

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Neurotoxicity	neuro	Inactive	0.54
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.94
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.86
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.89
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.81
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.83
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.85
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.68
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.81

COMPOUND C3:

Oral toxicity prediction results for input compound



Predicted LD50: 1190mg/kg

Predicted Toxicity Class: 4

1 2 3 4 5 6

Average similarity: 100%

Prediction accuracy: 100%

20% 40% 60% 80%

Print Toxicity Report

Name	Tamoxifen
Molweight	371.52
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	0
Number of atoms	28
Number of bonds	30
Number of rotatable bonds	8
Molecular refractivity	119.72
Topological Polar Surface Area	12.47
octanol/water partition coefficient(logP)	6

Comparison of input compound with dataset compounds

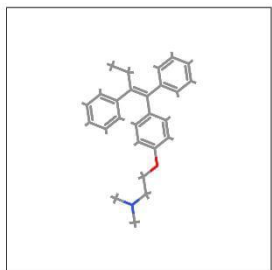
Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
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Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.96
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.78
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.98
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99

COMPOUND 4:

Oral toxicity prediction results for input compound



Predicted LD50: 1190mg/kg

Predicted Toxicity Class: 4

Average similarity: 100%

Prediction accuracy: 100%

Print Toxicity Report

Name	Tamoxifen
Molweight	371.52
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	0
Number of atoms	28
Number of bonds	30
Number of rotatable bonds	8
Molecular refractivity	119.72
Topological Polar Surface Area	12.47
octanol/water partition coefficient(logP)	6

Comparison of input compound with dataset compounds

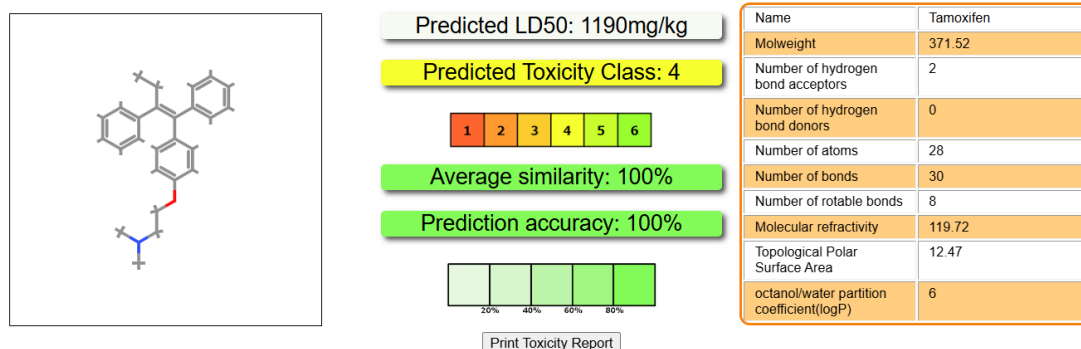
Toxicity Model Report

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Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
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Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.92
Molecular Initiating Events	Na ⁺ /I ⁻ symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.76

COMPOUND C5:

Oral toxicity prediction results for input compound



Comparison of input compound with dataset compounds

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
Toxicity end points	BBB-barrier	bbb	Inactive	1.0
Toxicity end points	Clinical toxicity	clinical	Inactive	0.56
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.92
Molecular Initiating Events	Na ⁺ /L- symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.76
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.87

MOLECULAR DOCKING STUDIES

Molecular docking plays a crucial role in structural molecular biology and computer-aided drug discovery. This technique aims to identify the primary binding orientation of a ligand to a protein with a known 3D structure. Effective docking methods efficiently explore complex spatial configurations and employ a scoring system to accurately rank potential binding modes. By leveraging docking, researchers can conduct virtual screenings of vast compound libraries, prioritize hits, and propose structural models of ligand-target interactions, thereby streamlining lead optimization. However, careful preparation of input structures is

equally vital as the docking process itself, and interpreting the results of stochastic searches can sometimes be challenging. This chapter provides an overview of the theoretical foundations and applications of molecular docking software, highlighting the usage of prominent docking tools.

Docking Principle

The two main components that are important for docking studies are:

- ✚ Secondary structure of our protein of interest.
- ✚ Library of ligands from suitable data base.

Docking tools are based on the search, algorithm and

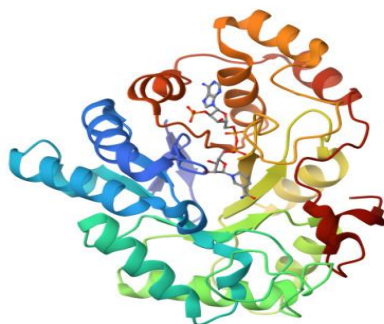
the scoring function. A search algorithm finds the best docking pose measured by the scoring function. A scoring function differentiates correct docking poses from incorrect ones.

The quality of any docking results depends on the reasonable starting structures of both the protein and the ligand. The protein and ligand structures require preparation before docking in order to achieve the best docking results.

PROTEIN PREPARATION

Anti-diabetic protein:

The protein target, obtained from the RCSB Protein Data Bank with the PDB accession code 1ads, functions as a docking receptor. The active site of the receptor was cleared of all sound ligands and water molecules.



PDB DOI: <https://doi.org/10.2210/pdb1ADS/pdb>

Classification: OXIDOREDUCTASE

Organism(s): Homo sapiens

Expression System: Escherichia coli

Mutation(s): No

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

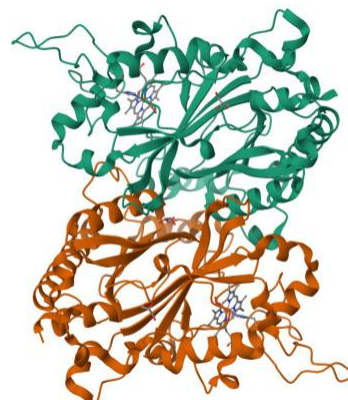
Resolution: 1.65 Å

R-Value Work: 0.200

R-Value Observed: 0.200

Anti microbial activity :

The protein target, obtained from the RCSB Protein Data Bank with the PDB accession code 4gs1 functions as a docking receptor. The active site of the receptor was cleared of all sound ligands and water molecules.



PDB DOI: <https://doi.org/10.2210/pdb4GS1/pdb>

Classification: OXIDOREDUCTASE

Organism(s): Thermobifida cellulosilytica

Expression System: Escherichia coli BL21(DE3)

Mutation(s): No

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 1.70 Å

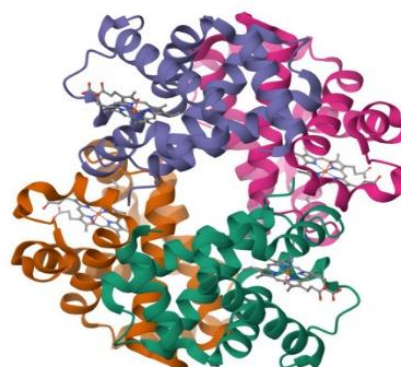
R-Value Free:0.174

R-Value Work:0.146

R-Value Observed: 0.147

Anti-oxidant protein:

The protein target, obtained from the RCSB Protein Data Bank with the PDB accession code 8puq, functions as a docking receptor. The active site of the receptor was cleared of all sound ligands and water molecules.



PDB

DOI: <https://doi.org/10.2210/pdb8PUQ/pdb>

Classification: OXYGEN TRANSPORT

Organism(s): Equus caballus

Mutation(s): No

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

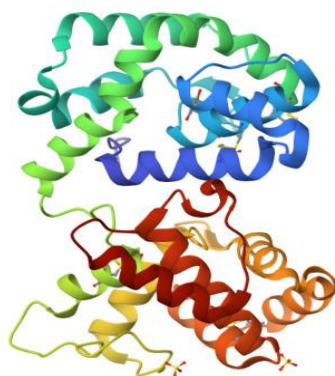
Resolution: 1.95 Å

R-Value Free: 0.174

R-Value Work: 0.125

Anti-inflammatory:

The protein target, obtained from the RCSB Protein Data Bank with the PDB accession code 3ngv, functions as a docking receptor. The active site of the receptor was cleared of all sound ligands and water molecule



PDB DOI: <https://doi.org/10.2210/pdb3NGV/pdb>

Classification: TRANSPORT PROTEIN

Organism(s): Anopheles stephensi

Expression System: Escherichia coli BL21(DE3)

Mutation(s): No

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 1.76 Å

R-Value Free: 0.204

R-Value Work: 0.158

R-Value Observed: 0.160

✚ the molecular docking investigations were conducted utilizing AutoDock Tools 42.7 (ADT), a freely available graphical user interface (GUT) designed for the Autolock Tool software.

✚ The compounds (1-10) were subjected to docking against the active site of proteins (POB ID: 1ADS, 4GS1, 8PUQ, and 3NGV)

<https://doi.org/10.2210/pdb1ADS/pdb>,
<https://doi.org/10.2210/pdb4GS1/pdb>
<https://doi.org/10.2210/pdb8PUQ/pdb>
<https://doi.org/10.2210/pdb3NGV/pdb>

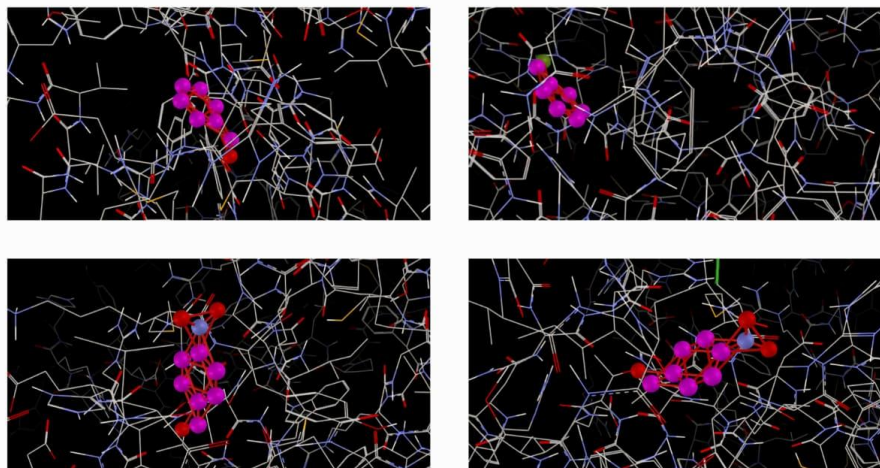
(using AutoDock tools using the standard method).

✚ The construction of the grid box involved the utilization of three dimensions, namely x, y, and z, with lengths of 120 x 120 x 120 units, respectively. The spacing between each grid point within the box was set at 0.575. Å. The dimensions of the central grid box are 18.427 Å, 56.689 Å, and -4.262 Å. A total of 25 distinct conformations were produced for each ligand and evaluated using the AutoDock Tool scoring functions.

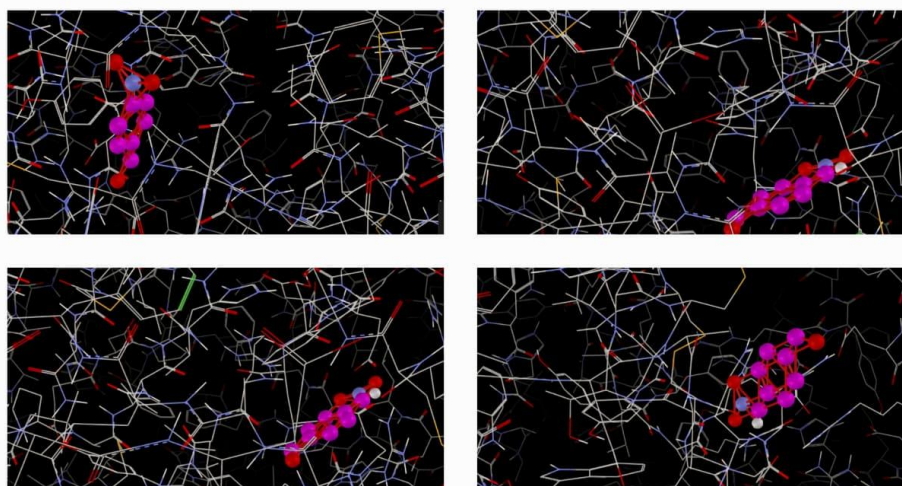
✚ The resulting data, including the binding energies, can be found in Table 1. The conformations were then arranged in order of their binding energies in Table.

✚ The post-docking evaluations were conducted using PyMOL. The PyMOL software was utilized to examine the interactions between the target receptor and ligands, with a focus on the conformations exhibiting the most favorable (or least unfavorable) free binding energy.

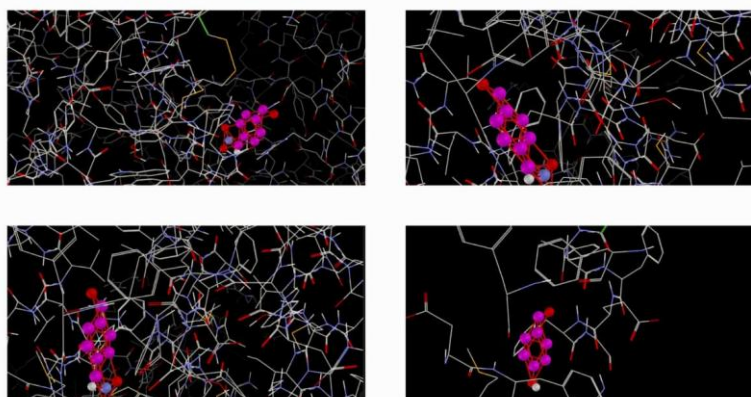
COMPOUND 1:



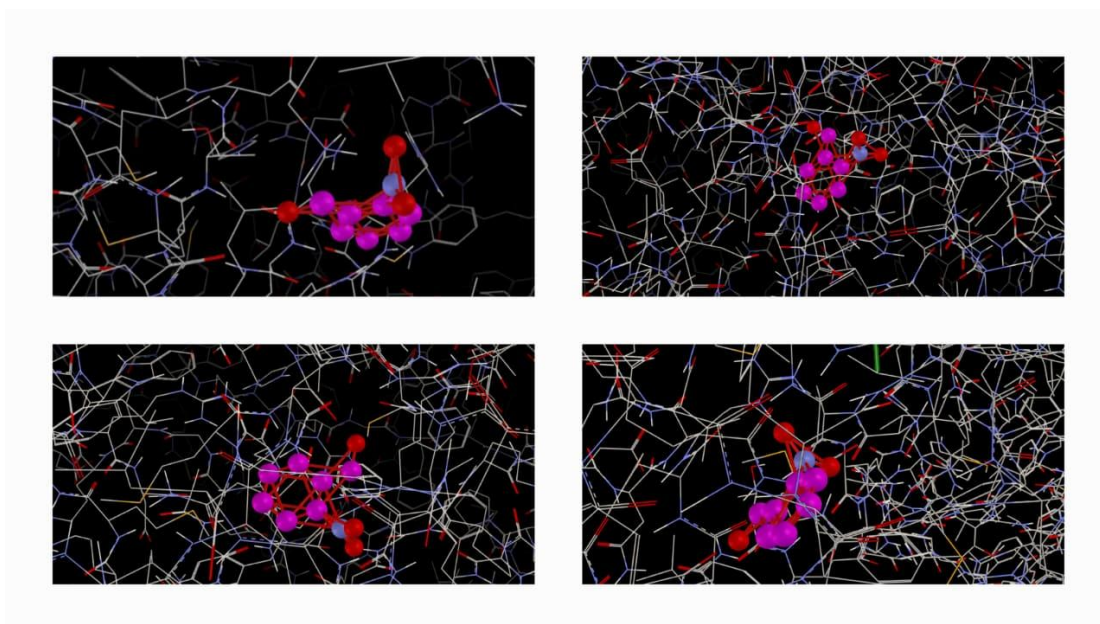
COMPOUND 2:



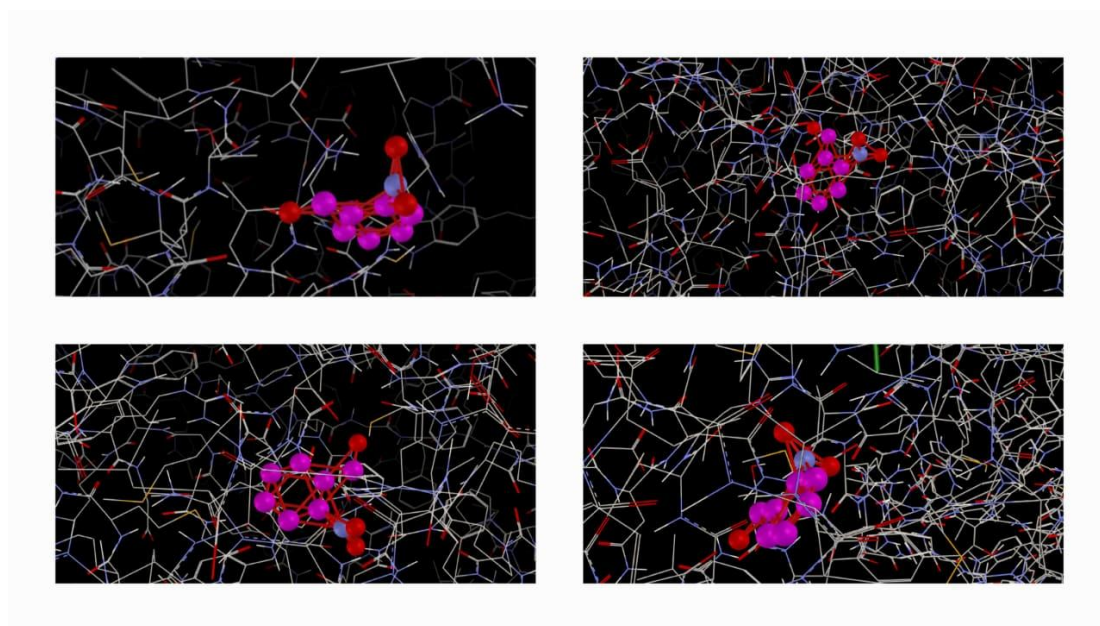
COMPOUND 3:



COMPOUND 4:



COMPOUND C5:



SYNTHESIS

COMMON SYNTHETIC PROCEDURE

Step 1 – Formation of Intermediate 1

Accurately weigh 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone. Dissolve in 30 mL of glacial acetic acid and reflux for 3 hours.

After completion, pour the reaction mixture onto 80 g of crushed ice, stir gently, and filter the precipitate to obtain Intermediate 1.

Step 2 – Formation of Intermediate 2

Take 5.305 g of Intermediate 1 and add 2.2 g of benzaldehyde, followed by 3 g NaOH in 40 mL ethanol. Reflux for 3 hours.

After reaction, add the mixture to 80 g of crushed ice, stir gently, and filter the precipitate to collect Intermediate 2.

Step 3 – Formation of Pyrazole Fused Product

Weigh 7.06 g of Intermediate 2, add 10 mL hydrazine hydrate and 3 g NaOH in 100 mL ethanol, and reflux for 4 hours.

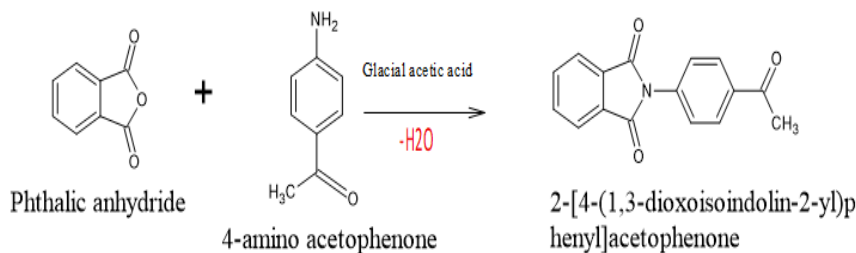
After completion, pour onto 80 g of crushed ice, stir gently, and filter the precipitate to obtain the final fused pyrazole derivative.

Step 4 – Final Isolation

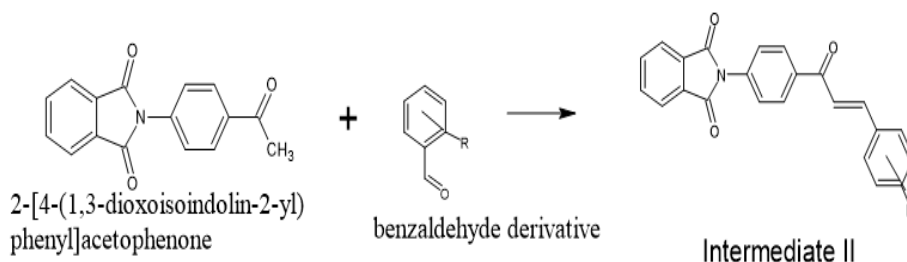
The filtered product is washed with cold water and dried to obtain the final compound.

SYNTHESIS OF COMPOUND

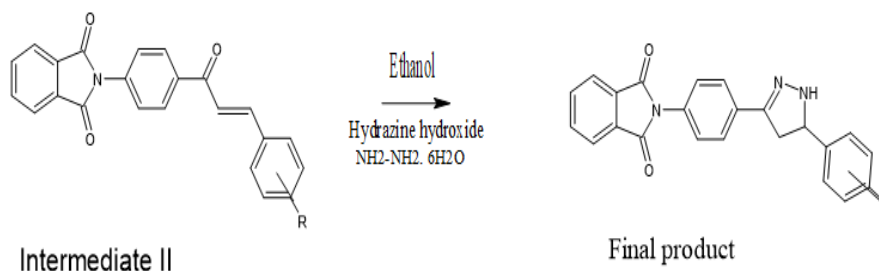
STEP 1



STEP 2



STEP 3



Synthesis procedure:
Compound 1

STEP 1

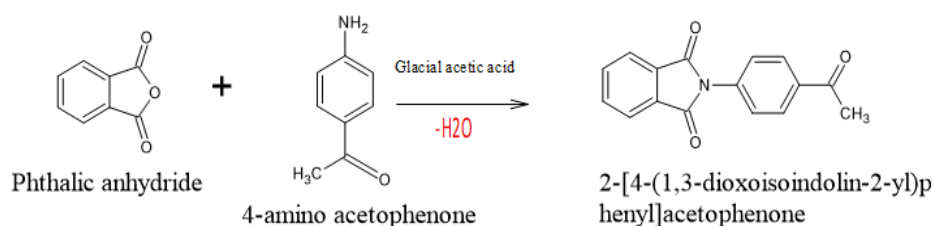
Accurately weighed 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone were mixed in 30 mL of glacial acetic acid, and the reaction mixture was refluxed for 3 hours. After completion, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 1:
IUPAC name: 2-(4-acetylphenyl)-isoindole-1,3-dione (MW 265.27 g/mol).

STEP 2

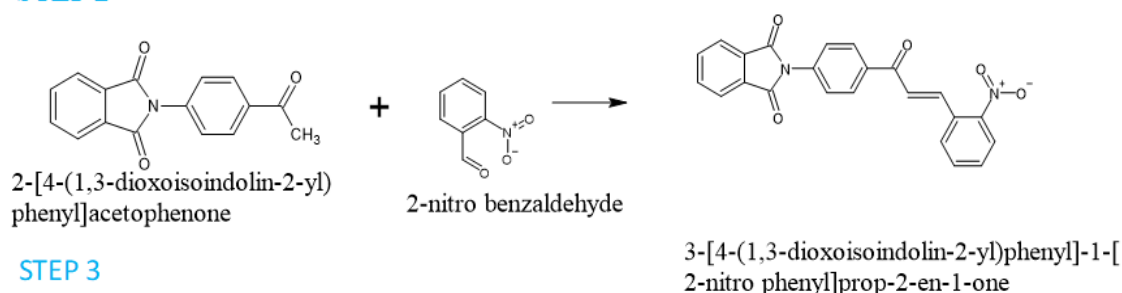
5.305 g of Intermediate 1 was added to 2.2 g of 2-nitro benzaldehyde, followed by 3 g of sodium hydroxide and 40 mL of ethanol, and the mixture was refluxed for 3 hours.

Compound 1

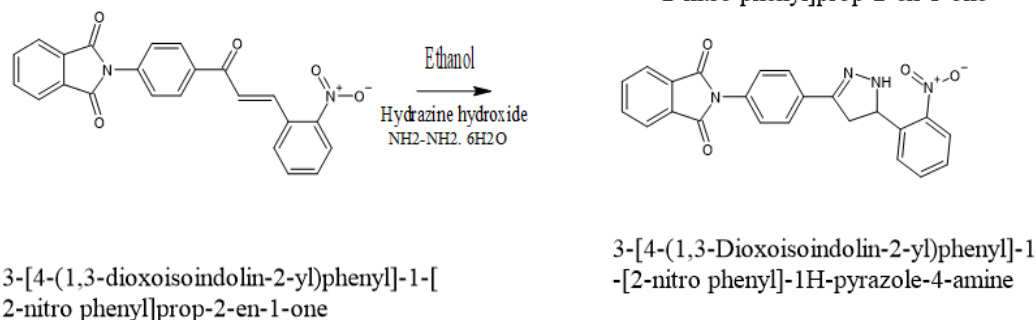
STEP 1



STEP 2



STEP 3



After the reaction, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 2.

IUPAC 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[2-nitro phenyl]prop-2-en-1-one

STEP 3

7.06 g of Intermediate 2 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[2-nitro phenyl]prop-2-en-1-one added to 10 mL of hydrazine hydrate, along with 3 g of sodium hydroxide and 100 mL of ethanol, and refluxed for 4 hours. After completion, the reaction mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain the final product.
IUPAC name: 3-[4-(1,3-Dioxoisoindolin-2-yl)phenyl]-1-[2-nitro phenyl]-1H-pyrazole-4-amine

Synthesis procedure:
Compound 2

STEP 1

Accurately weighed 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone were mixed in 30 mL of glacial acetic acid, and the reaction mixture was refluxed for 3 hours.

After completion, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 1:

IUPAC name: 2-(4-acetylphenyl)-isoindole-1,3-dione (MW 265.27 g/mol).

STEP 2

5.305 g of Intermediate 1 was added to 2.2 g of 4-nitro benzaldehyde, followed by 3 g of sodium hydroxide and 40 mL of ethanol, and the mixture was refluxed for 3 hours.

After the reaction, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 2.

IUPAC 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-nitro phenyl]prop-2-en-1-one

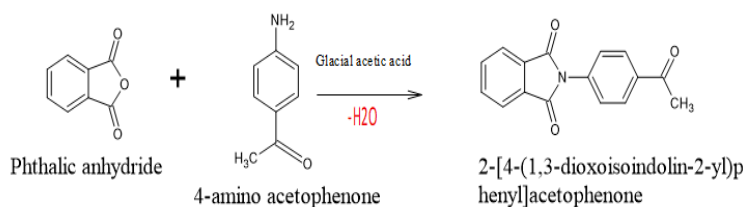
STEP 3

7.06 g of Intermediate 2 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-nitro phenyl]prop-2-en-1-one added to 10 mL of hydrazine hydrate, along with 3 g of sodium hydroxide and 100 mL of ethanol, and refluxed for 4 hours.

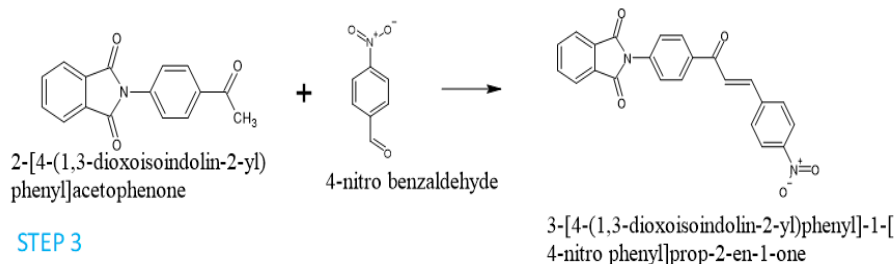
After completion, the reaction mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain the final product. IUPAC name: 3-[4-(1,3-Dioxoisoindolin-2-yl)phenyl]-1-[4-nitro phenyl]-1H-pyrazole-4-amine

Compound 2

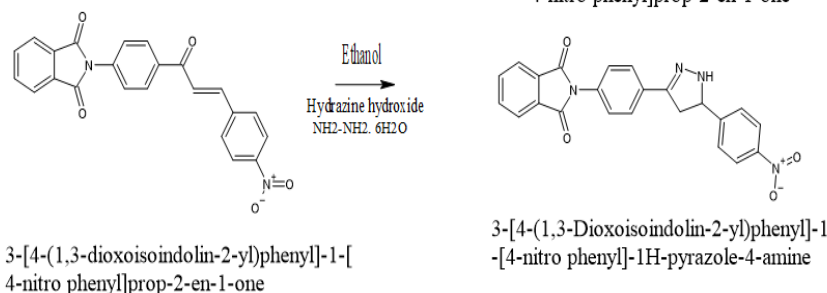
STEP 1



STEP 2



STEP 3



Synthesis procedure:
Compound 3

STEP 1

Accurately weighed 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone were mixed in 30 mL of glacial acetic acid, and the reaction mixture was refluxed for 3 hours.

After completion, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 1:

IUPAC name: 2-(4-acetylphenyl)-isoindole-1,3-dione (MW 265.27 g/mol).

STEP 2

5.305 g of Intermediate 1 was added to 2.2 g of 4-hydroxy benzaldehyde, followed by 3 g of sodium hydroxide and 40 mL of ethanol, and the mixture was refluxed for 3 hours.

After the reaction, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 2.

IUPAC 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]prop-2-en-1-one

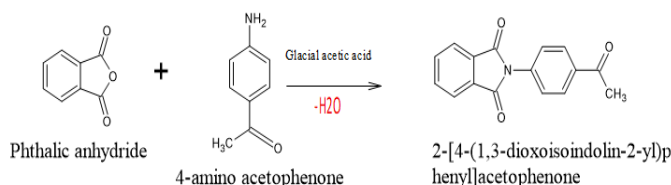
STEP 3

7.06 g of Intermediate 2 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]prop-2-en-1-one added to 10 mL of hydrazine hydrate, along with 3 g of sodium hydroxide and 100 mL of ethanol, and refluxed for 4 hours.

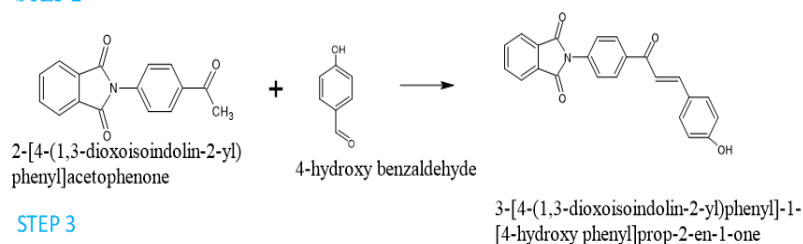
After completion, the reaction mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain the final product. IUPAC name: 3-[4-(1,3-Dioxoisoindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]-1H-pyrazole-4-amine

Compound 3

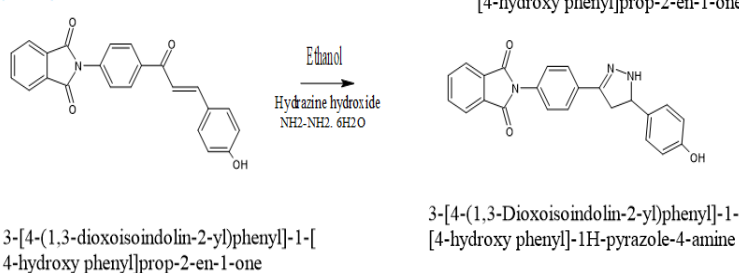
STEP 1



STEP 2



STEP 3



Synthesis procedure:
 Compound 4

STEP 1

Accurately weighed 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone were mixed in 30 mL of glacial acetic acid, and the reaction mixture was refluxed for 3 hours.

After completion, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 1:

IUPAC name: 2-(4-acetylphenyl)-isoindole-1,3-dione (MW 265.27 g/mol).

STEP 2

5.305 g of Intermediate 1 was added to 2.2 g of benzaldehyde, followed by 3 g of sodium hydroxide and 40 mL of ethanol, and the mixture was refluxed for 3 hours.

After the reaction, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 2.

IUPAC 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[phenyl]prop-2-en-1-one

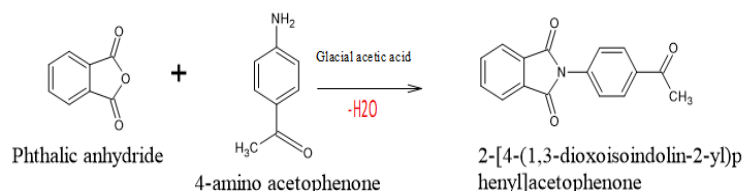
STEP 3

7.06 g of Intermediate 2 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[phenyl]prop-2-en-1-one added to 10 mL of hydrazine hydrate, along with 3 g of sodium hydroxide and 100 mL of ethanol, and refluxed for 4 hours.

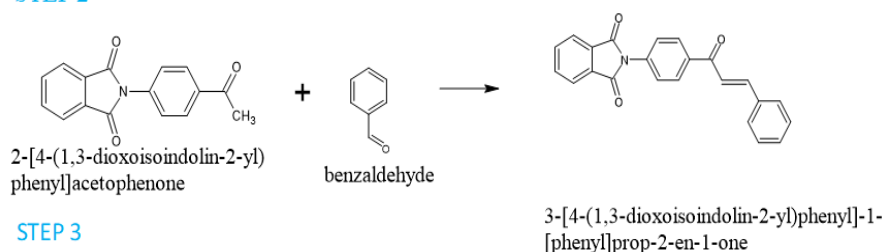
After completion, the reaction mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain the final product. IUPAC name: 3-[4-(1,3-Dioxoisoindolin-2-yl)phenyl]-1-[phenyl]-1H-pyrazole-4-amine

Compound 4

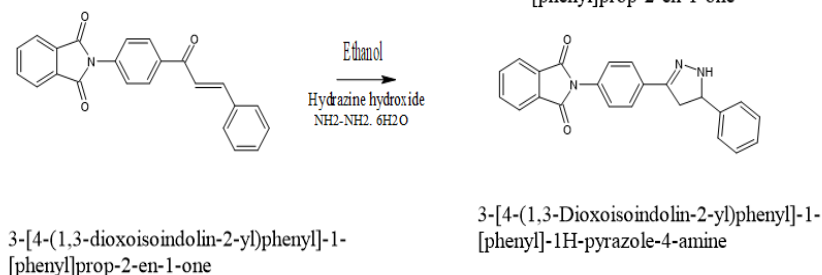
STEP 1



STEP 2



STEP 3



Synthesis procedure:

Compound 5

STEP 1

Accurately weighed 7.4 g of phthalic anhydride and 6.78 g of p-acetaminophenone were mixed in 30 mL of glacial acetic acid, and the reaction mixture was refluxed for 3 hours.

After completion, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 1:

IUPAC name: 2-(4-acetylphenyl)-isoindole-1,3-dione (MW 265.27 g/mol).

STEP 2

5.305 g of Intermediate 1 was added to 2.2 g of 4-dimethylamino benzaldehyde, followed by 3 g of sodium hydroxide and 40 mL of ethanol, and the mixture was refluxed for 3 hours.

After the reaction, the mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain Intermediate 2.

IUPAC 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-nitro phenyl]prop-2-en-1-one

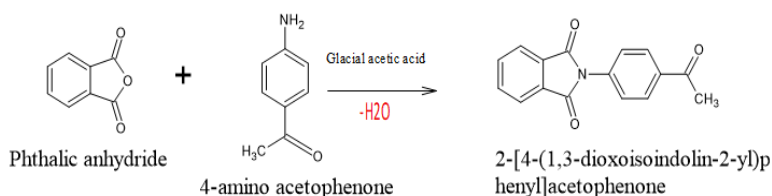
STEP 3

7.06 g of Intermediate 2 3-[4-(1,3-dioxoisoindolin-2-yl)phenyl]-1-[4-dimethyl amino phenyl]prop-2-en-1-one added to 10 mL of hydrazine hydrate, along with 3 g of sodium hydroxide and 100 mL of ethanol, and refluxed for 4 hours.

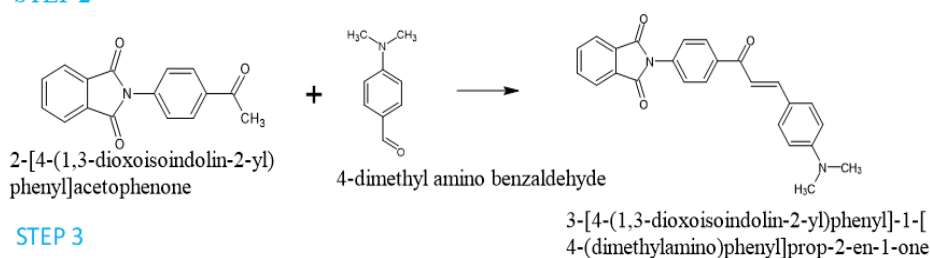
After completion, the reaction mixture was poured onto 80 g of crushed ice, stirred gently, and the precipitate was filtered to obtain the final product. IUPAC name: 3-[4-(1,3-Dioxoisoindolin-2-yl)phenyl]-1-[4-dimethyl amino phenyl]-1H-pyrazole-4-amine

Compound C5

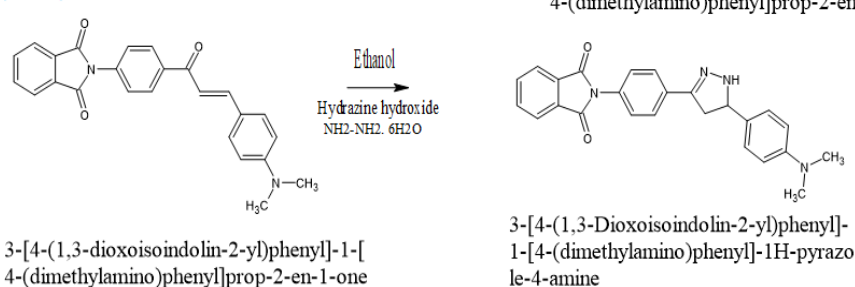
STEP 1



STEP 2



STEP 3



FINAL PRODUCT

CODE	IUPAC Name
COMPOUND C1	3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[2-nitro phenyl]-1H-pyrazole-4-amine
COMPOUND C2	3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-nitro phenyl]-1H-pyrazole-4-amine
COMPOUND C3	3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]-1H-pyrazole-4-amine
COMPOUND C4	3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[phenyl]-1H-pyrazole-4-amine
COMPOUND C5	: 3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-dimethyl amino phenyl]-1H-pyrazole-4-amine



CHARACTERIZATION

Spectroscopy is the branch of science that deals with the study of interaction of electromagnetic radiation with matter, spectroscopy is the most powerful tools available for the study of atomic and molecular structure of organic compound

IR SPECTROSCOPY:

IR spectrum were recorded by absorption of infrared radiation it causes changes in vibrational energy in the ground state. Using the KBr pellet press method and determined the functional group present in the compounds

UV-VISIBLE SPECTROSCOPY:

UV-Visible spectroscopy analysis revealed an absorption peak at 340 nm. This peak indicated the presence of a $\pi-\pi^*$ transition in the molecular structure. The analysis provided valuable insights into the compound's electronic transitions. The data supported the identification of the compound's functional groups.

UV-VISIBLE SPECTROPHOTOMETER :



BIOLOGICAL EVALUATION OF ANTI DIABETIC ACTIVITY

They are Various assays for antidiabetic activity in-vitro assays are,
 α -amylase inhibition
 α -glucosidase inhibition
Glucose uptake assay

A-AMYLASE INHIBITION ASSAY:

1. Principle of the Assay

The most common method uses DNSA (3,5-Dinitrosalicylic acid). Amylase breaks down starch into maltose. DNSA reacts with the resulting maltose to form a colored complex. If an inhibitor is present, less maltose is produced, leading to a lighter color.

Diabetic activity by α -amylase

Alpha-amylase is an enzyme that plays a crucial role in breaking down starch into simple Sugars like glucose and maltose. It is found in various organisms, including plants, animals, and microorganism. Alpha-amylase activity can be influenced by factors like temperature, pH and the presence of certain metal ions. It is also target for inhibitors, which can be used to manage conditions like diabetes by slowing down the digestion of carbohydrates.

Evaluation of in-vitro anti-diabetic activity by α -amylase inhibition assay Principle Inhibiting alpha-amylase slows the break down of starch in to glucose, thereby Reducing postprandial blood glucose spikes.

BIOLOGICAL EVALUATION OF ANTI INFLAMMATORY ACTIVITY

They are Various assays for anti-inflammatory activity in-vitro assays are,
protein denaturation inhibition assay
Membrane stabilization assay (HRBC method)
Lipoxygenase (LOX) inhibition
Enzyme inhibition assays
Nitric oxide (NO) inhibition assay

PROTEIN DENATURATION INHIBITION ASSAY

1. Preparation of Reagents

First, prepare your control and test solutions.

Control Solution: This usually consists of distilled water or a buffer like Phosphate Buffered Saline.

Test Solution: Use your plant extract or drug candidate dissolved in a suitable solvent at different concentrations.

Standard Drug: Typically, Diclofenac Sodium or Aspirin serves as a positive control.

Protein Source: Use a 1% to 5% aqueous solution of Bovine Serum Albumin (BSA).

BIOLOGICAL EVALUATION OF ANTI OXIDANT ACTIVITY

They are Various assays for anti-oxidant activity in-vitro assays are,

- Reducing power assay
- Hydrogen peroxide scavenging assay
- Superoxide radical scavenging assay
- Hydroxyl radical scavenging assay
- Nitric oxide scavenging assay
- Lipid peroxidation inhibition (TBARS) assay
- Metal chelating assay

HYDROGEN PEROXIDE SCAVENGING ASSAY

1. Preparing Reagents Phosphate Buffer: Create a 50 mM phosphate buffer with a pH of 7.4. Hydrogen Peroxide Solution: Mix up a 40 mM solution of H₂O₂ in the phosphate buffer. Standard/Samples: Get your plant extract or antioxidant (Ascorbic Acid) ready in different strengths (100, 200, 300, 400, 500 µg/mL).

2 .Hydrogen Peroxide (H₂O₂) Scavenging Assay

Principle

H₂O₂ absorbs strongly at 230 nm. Antioxidants decrease the absorbance by scavenging H₂O₂.

Lower absorbance = higher scavenging activity.

1. Reagents

a. Hydrogen Peroxide Solution (40 mM)

Dissolve 0.136 g of H₂O₂ (30% stock) in 100 mL phosphate buffer.

Prepare fresh due to instability.

b. Phosphate Buffer (0.1 M, pH 7.4)

c. Test Sample

Prepare concentrations: 100, 200, 300, 400 ,500 µg/mL in buffer.

Calculation Formula:

% of inhibition =

orbance of control – Absorbance of test

Abs

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

- This study, the molecular designing of the compounds were carried out by using different software.
- The lipinski's rule of five and some properties of the designed compound was calculated by using softwares such as chemsketch, molinspiration & swiss ADME.

TABLE NO.1

LIPINSKI'S RULE BY MOLINSPIRATION, CHEMSKETCH & swiss ADME Result:

Code	M.W	H-bond acceptor	H-bond donor	Log P	M.R (Cm ³ /mol)	No. of criteria
Rule	<500	<10	<5	<5	<150	Atleast-3
Compound C1	412.40	5	1	2.64	109.29	ALL
Compound C2	412.40	5	1	2.69	109.29	ALL
Compound C3	383.40	4	2	2.51	83.69	ALL
Compound C4	359.46	2	1	3.26	63.40	ALL
Compound C5	410.47	3	1	3.24	66.70	ALL

TABLE NO.2

CODE	SOLUBILITY (LOG S)	GI ABSORB	BBB PERMEANT	SYNTHETIC ACCESSIBILITY
COMPOUND C1	-4.89	HIGH	NO	3.60
COMPOUND C2	-4.89	HIGH	NO	3.51
COMPOUND C3	-4.69	HIGH	NO	3.28
COMPOUND C4	-4.88	HIGH	NO	4.00
COMPOUND C5	-5.06	HIGH	YES	3.68

In-Silico Toxicity Assessment Results:

The in-silico toxicity assessment results of the selected 5 ligand molecules were summarized in the Table 3. All the molecules were found to be non-toxic in the terms of cardiotoxicity, immunotoxicity, androgen receptor, aromatase etc., based on the in-silico toxicity assessment study.

TABLE NO. 3

CODE	CARDIOTOXICITY	IMMUNOTOXICITY	AROMATASE	CYTOCHROME CYP2E1
COMPOUND C1	INACTIVE	INACTIVE	INACTIVE	INACTIVE
COMPOUND C2	INACTIVE	INACTIVE	INACTIVE	INACTIVE
COMPOUND C3	INACTIVE	INACTIVE	INACTIVE	INACTIVE
COMPOUND C4	INACTIVE	INACTIVE	INACTIVE	INACTIVE
COMPOUND C5	INACTIVE	INACTIVE	INACTIVE	INACTIVE

S.NO	ACTIVITY	STANDARD COMPOUND	BINDING ENERGY (-Kcal/Mol)
01	ANTI-DIABETIC	DAPAGLIFZOLIN	-8.16
02	ANTI-INFLAMMATORY	IBUPROFEN	-8.67
03	ANTI-OXIDANT	DOXORUBICIN	-9.64
04	ANTI-MICROBIAL	AMOXICILLIN	-7.55

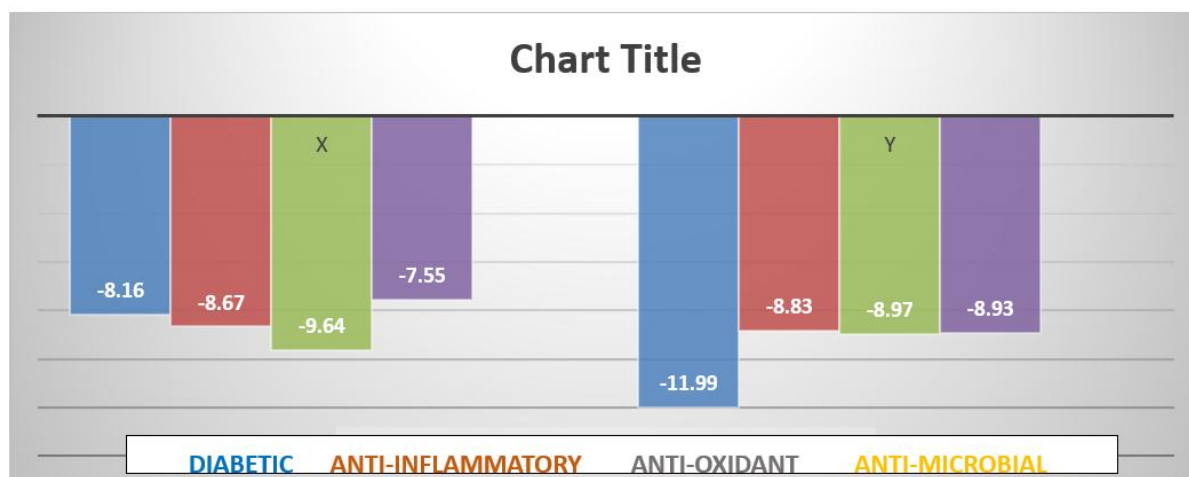
TABLE NO. 4

MOLECULAR DOCKING RESULTS

TABLE NO. 5

COMPOUND	BINDING ENERGY			
	DIABETIC	ANTI INFLAMMATORY	ANTI OXIDANT	ANTI MICROBIAL
C1	-8.93	-8.35	-6.97	-7.15
C2	-11.99	-7.1	-8.97	-8.56
C3	-10.38	-8.83	-7.1	-8.52
C4	-10.57	-8.74	-6.78	-8.79
C5	-9.93	-8.52	-7.65	-8.93

HIGH DOCKING SCORE:



PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

❖ The designed compounds (C1, C2, C3 & C4) are synthesized by the benzotriazole react with ethyl chloroacetate and potassium anhydrous in the presence of acetone to form the ethyl chloroacetate intermediate compound. It further treated with hydrazine hydrate in the presence of ethanol to form the hydrazine hydrate intermediate compound. It further reacts with the various types of aromatic aldehyde compound in presence of ethanol and it further react with chloramine T and ethanol for cyclization process.

TABLE NO. 6

CODE	Molecular formula	IUPAC Name
COMPOUND C1	C ₂₄ H ₁₈ N ₄ O ₄	3-[4-(1,3-Dioxoisindolin-2-yl)phenyl]-1-[2-nitro phenyl]-1H-pyrazole-4-amine
COMPOUND C2	C ₂₄ H ₁₈ N ₄ O ₄	3-[4-(1,3-Dioxoisindolin-2-yl)phenyl]-1-[4-nitro phenyl]-1H-pyrazole-4-amine
COMPOUND C3	C ₂₄ H ₁₉ N ₃ O ₂	3-[4-(1,3-Dioxoisindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]-1H-pyrazole-4-amine

COMPOUND C4	C ₂₃ H ₁₇ N ₃ O ₂	3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-phenyl-1H-pyrazole-4-amine
COMPOUND C5	C ₂₆ H ₂₄ N ₄ O ₂	: 3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-dimethyl amino phenyl]-1H-pyrazole-4-amine

TABLE NO. 7

CODE	APPEARANCE	YEILD (%)	MELTING POINT	SOLUBILITY
COMPOUND C1	Pale yellow powder	81.09%	249 °C	METHANOL & ACETONE
COMPOUND C2	Brown crystalline powder	82.04%	247 °C	METHANOL & ACETONE
COMPOUND C3	Brown crystalline powder	82.05%	255 °C	METHANOL & ACETONE
COMPOUND C4	Off white powder	80.03%	253 °C	METHANOL & ACETONE
COMPOUND C5	Deep yellow powder	83.27%	264°C	METHANOL & ACETONE

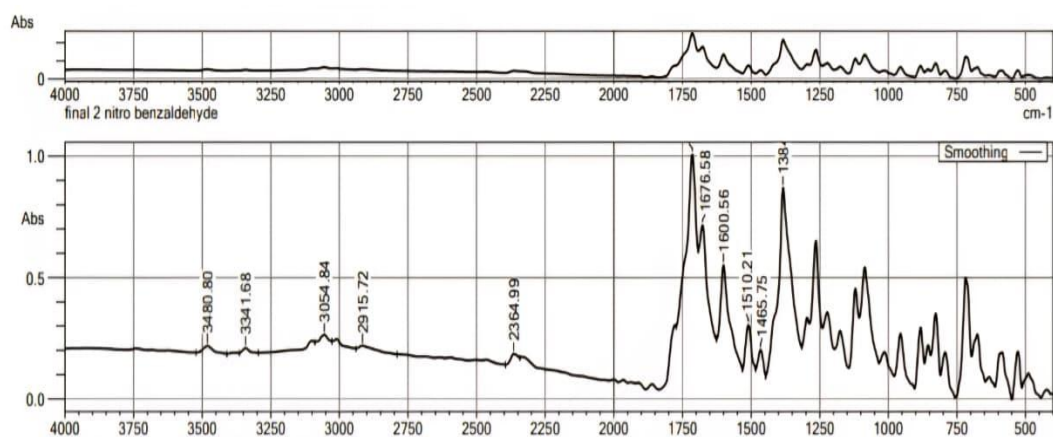
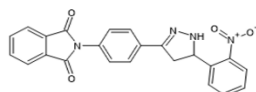
SPECTRAL DATA

The compounds were confirmed by the spectral analytical data such as following method.

○ INFRARED SPECTRUM:

Compound C1

3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-(2-nitrophenyl)-1H-pyrazole-4-amine



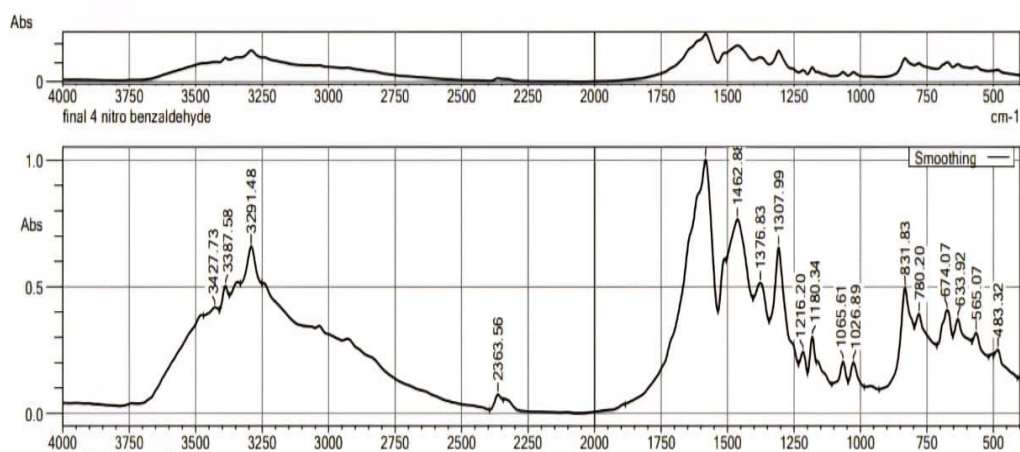
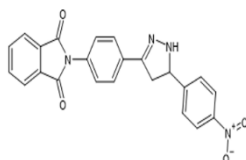
IR Interpretation Table for the Compound

Observed Peak (cm ⁻¹)	Functional Group Assignment	Interpretation / Justification
3340.80	N-H stretching (pyrazole NH)	Confirms presence of secondary NH in pyrazole ring
3054.84	Aromatic C-H stretch	Indicates multiple aromatic rings in the structure
2915.72	Aliphatic/aryl C-H stretch	Weak allowed band due to aromatic substitutions
2364.99	Atmospheric CO ₂	Non-structural; common in IR spectra
1676.58	C=O (imide carbonyl stretch)	Strong absorption characteristic of imide (1,3-dioxoisindolinone)
1600.56	Aromatic C=C stretching	Conjugated phenyl + pyrazole system vibrations
1510.21	NO ₂ asymmetric stretching	Confirms nitro substituent on aromatic ring
1465.75	NO ₂ symmetric stretching	Matches nitro group symmetric vibration
1380–1200 region	C-N, C-O, aromatic skeletal vibrations	Fingerprint region for heterocyclic + imide framework
750–700 region	Aromatic C-H out-of-plane bending	Confirms substituted benzene rings

TABLE NO. 8

Compound C2

3-[4-(1,3-Dioxoisindolin-2-yl)phenyl]-1-[4-nitro phenyl]-1H-pyrazole-4-amine

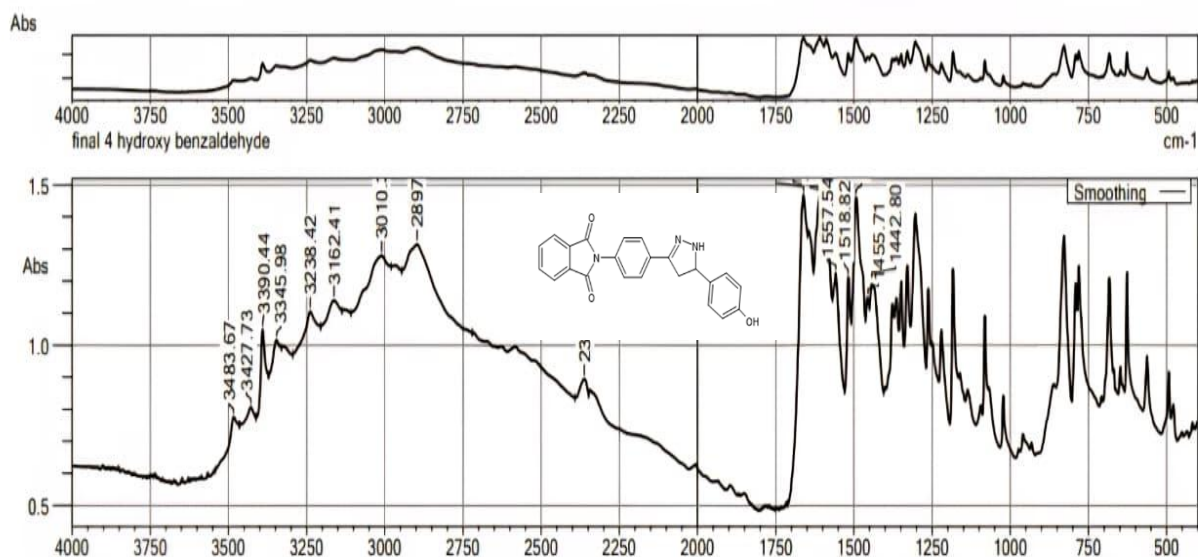


Observed Peak (cm ⁻¹)	Functional Group Assignment	Interpretation / Justification
3427.73, 3387.58 3291.48	N–H stretching (pyrazole NH)	Confirms presence of secondary NH in pyrazole ring
3145.12	Aromatic C–H stretch	Indicates multiple aromatic rings in the structure
2363.56	Atmospheric CO ₂	Non-structural; common in IR spectra
1580.54	NO ₂ asymmetric stretching	Confirms nitro substituent on aromatic ring
1465.75	NO ₂ symmetric stretching	Matches nitro group symmetric vibration
1380–1200 region	C–N, C–O, aromatic skeletal vibrations	Fingerprint region for heterocyclic + imide framework
750–700 region	Aromatic C–H out-of-plane bending	Confirms substituted benzene rings

TABLE NO. 9

Compound C3

3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-hydroxy phenyl]-1H-pyrazole-4-amine



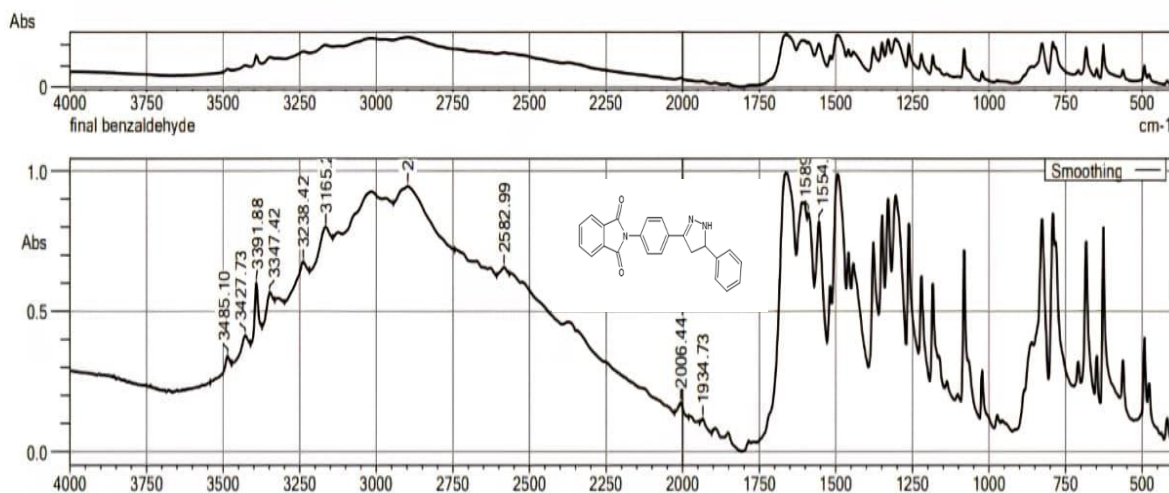
Peak Position (cm ⁻¹)	Intensity	Functional Group	Interpretation
3500–3200 (broad)	Medium	O–H stretching	Hydroxy phenyl present
3340–3200	Medium	N–H stretching	Imidazoliny NH

3050–3020	Medium	Aromatic C–H	Aryl rings
2900–2850	Weak	C–H stretching	Alkyl linker
1760–1700	Strong	Imide C=O	Confirms 1,3-dioxisoindolinone
1600–1500	Medium	Aromatic C=C	Conjugated system
1320–1220	Medium	C–O stretch	Phenolic functional group
1100–1000	Weak–Medium	C–N vibration	Heterocycle
900–750	Medium	Ar–H out-of-plane	Aromatic substitution

TABLE NO. 10

Compound C4

3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[phenyl]-1H-pyrazole-4-amine

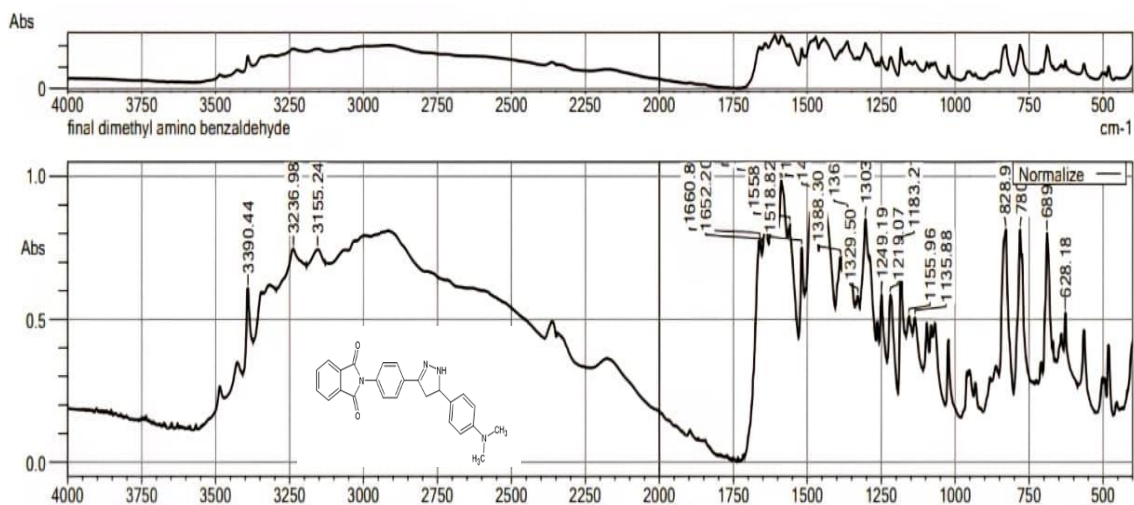


Peak Position (cm ⁻¹)	Intensity	Functional Group	Interpretation
3340–3200	Medium	N–H stretching	Imidazoliny NH
3050–3020	Medium	Aromatic C–H	Phenyl + phthalimide
2950–2850	Weak	Aliphatic C–H	Linker region
1760–1700	Strong	Imide C=O	Two strong carbonyl peaks
1600–1500	Medium	Aromatic C=C	Expected
1350–1200	Medium	C–N stretching	Heterocycle
900–750	Medium	Ar–H out-of-plane	Monosubstituted benzene ring

TABLE NO. 11

Compound C5

3-[4-(1,3-Dioxisoindolin-2-yl)phenyl]-1-[4-dimethyl amino phenyl]-1H-pyrazole-4-amine



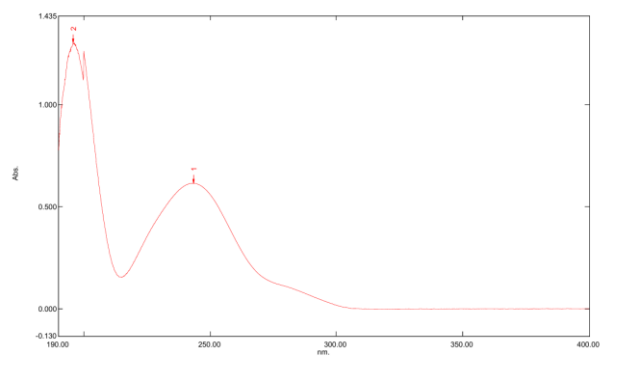
IR Integration Table – Compound C5

Peak Position (cm ⁻¹)	Intensity	Functional Group	Interpretation
3340–3200	Medium	N–H stretching	Imidazoliny NH present
3050–3020	Medium	Aromatic C–H stretching	Confirms aromatic rings
2950–2850	Medium	Aliphatic C–H stretching	Due to N(CH ₃) ₂ group
1760–1700	Strong	Imide C=O stretching	Isoindolin-1,3-dione carbonyls
1600–1500	Medium	Aromatic C=C stretching	Phenyl + heterocycle conjugation
1510–1460	Strong	C–N stretching	Dimethyl-aniline substituent
1350–1200	Medium	C–N / C–O stretching	Heterocyclic vibrations
900–750	Medium	Ar–H out-of-plane	Aromatic substitution

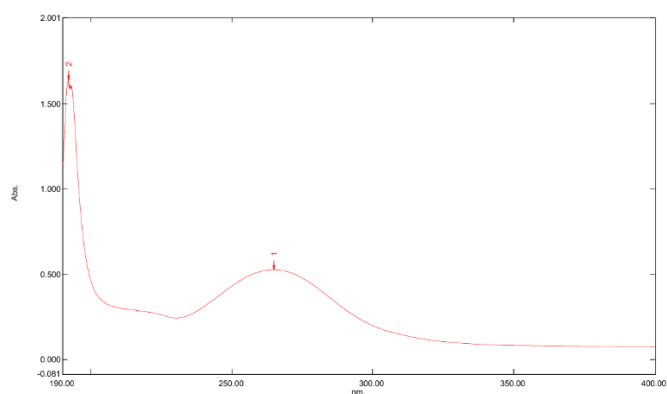
TABLE NO. 12

UV ABSORBANCE

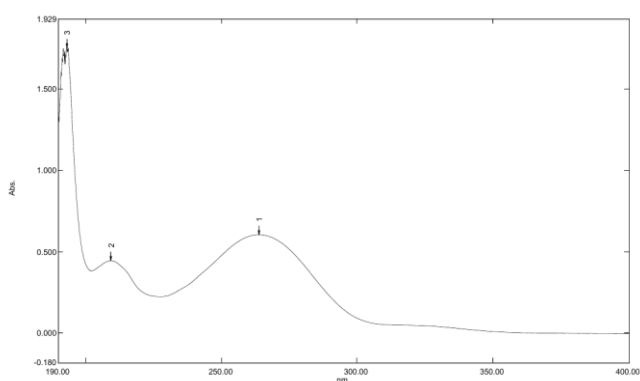
COMPOUND 1:



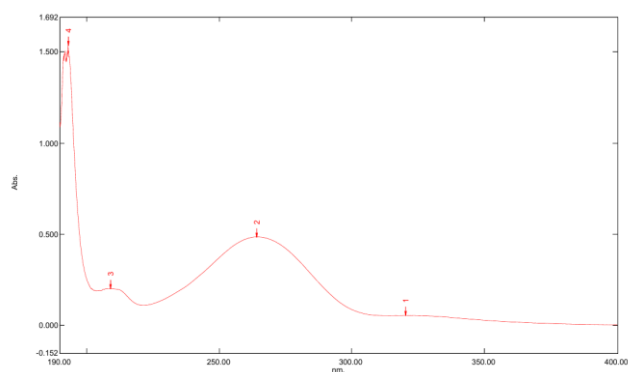
COMPOUND 2:



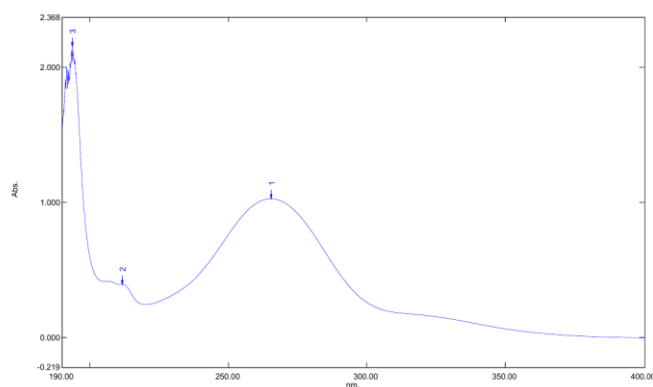
COMPOUND 3:



COMPOUND 4:



COMPOUND 5



COMPOUND	WAVELENGTH	MAXIMUM ABSORBANCE
C1	243.40	0.616
C2	264.90	0.528
C3	261.00	0.612
C4	264.10	0.485
C5	265.50	1.025

TABLE NO. 13

**BIOLOGICAL EVALUATION OF ANTI INFLAMMATORY:
 INHIBITION OF PROTEIN DENATURATION ASSAY**

Control: 0.548 absorbance

TABLE NO. 14

COMPOUND	CONC	ABS TEST 660 nm	% SCREENING
Diclofenac Na	50	0.156	71.53
C1	100	0.394	28.10
	200	0.335	38.86
	300	0.294	46.35
	400	0.245	55.29
	500	0.197	64.05
C2	100	0.38	30.65
	200	0.324	40.87
	300	0.284	48.17
	400	0.239	56.38
	500	0.197	64.05
C3	100	0.397	27.55
	200	0.348	36.49
	300	0.305	44.34
	400	0.245	55.29
	500	0.182	66.78
C4	100	0.376	31.38
	200	0.316	42.33
	300	0.275	49.81
	400	0.226	58.75
	500	0.174	68.24
C5	100	0.394	28.10
	200	0.304	44.52

	300	0.267	51.27
	400	0.268	51.09
	500	0.214	60.94

**BIOLOGICAL EVALUATION OF ANTI OXIDANT:
 HYDRGEN PEROXIDE ASSAY METHOD**

Control:0.852 absorbance

TABLE NO. 15

COMPOUND	CONC	ABS TEST 230 nm	% SCREENING
Ascorbic acid	50	0.350	58.92
C1	100	0.460	46.01
	200	0.420	50.70
	300	0.380	55.40
	400	0.350	58.92
	500	0.300	64.79
C2	100	0.440	48.36
	200	0.390	54.23
	300	0.350	58.92
	400	0.300	64.79
	500	0.250	70.66
C3	100	0.470	44.84
	200	0.430	49.53
	300	0.390	54.23
	400	0.340	60.09
	500	0.290	65.96
C4	100	0.50	41.31
	200	0.400	53.05
	300	0.360	57.75
	400	0.310	63.62
	500	0.260	69.48

C5	100	0.430	49.53
	200	0.380	55.40
	300	0.340	60.09
	400	0.290	65.96
	500	0.220	74.18

**BIOLOGICAL EVALUATION OF ANTI DIABETIC:
 INHIBITION OF ALPHA AMYLASE**

Control: 0.785 absorbance

TABLE NO. 16

COMPOUND	CONC	ABSORBANCE 540 nm	% INHIBITION
Voglibose	50	0.58	26.11%
C1	100	0.650	17.20%
	200	0.550	29.94%
	300	0.470	40.13%
	400	0.390	50.32%
	500	0.340	56.69%
C2	100	0.680	13.38%
	200	0.580	26.11%
	300	0.490	37.58%
	400	0.410	47.77%
	500	0.360	54.14%
C3	100	0.670	14.65%
	200	0.570	27.39%
	300	0.480	38.85%
	400	0.400	49.04%
	500	0.350	55.41%
C4	100	0.640	18.47%
	200	0.540	31.21%
	300	0.450	42.68%

	400	0.370	52.87%
	500	0.320	59.24%
C5	100	0.100	87.26%
	200	0.660	15.92%
	300	0.560	28.66%
	400	0.390	50.32%
	500	0.340	56.69%

SUMMARY AND CONCLUSION :

The present research focuses on the design, molecular docking, synthesis, spectroscopic characterization, and biological evaluation of a novel class of isoindole-1,3-dione fused with 1H-pyrazole derivatives. These hybrid scaffolds were conceptualized based on the pharmacophoric integration strategy, combining the isoindole-1,3-dione (phthalimide) core, known for anti-inflammatory, antimicrobial and anti diabetic properties, with 1H-pyrazole, a privileged heterocycle with significant enzyme-inhibitory and antioxidant potential.

MOLECULAR DESIGN AND DOCKING

The design of **isoindole-1,3-dione fused with 1H-pyrazole derivatives** was based on a **pharmacophore-hybridization approach**, integrating two biologically privileged scaffolds:

- **Isoindole-1,3-dione (phthalimide)**
 - Known for anti-inflammatory, anticancer, and enzyme-modulating activities.
 - Contains two imide carbonyls capable of forming stable hydrogen bonds with key amino acid residues.
- **1H-Pyrazole ring**
 - Exhibits strong binding affinity due to its **N–N heterocycle**, π – π stacking potential, and H-bond donor/acceptor capacity.

By fusing these two moieties, the design aimed to enhance:

- Target selectivity
- Binding affinity
- Metabolic stability
- Overall pharmacological profile

Electron-withdrawing and electron-donating substituents were strategically introduced at the aromatic ring to modulate **Lipinski parameters**, **electronic density**, and **steric orientation**, thereby

improving receptor affinity.

Five isoindole-1,3-dione fused 1H-pyrazole derivatives (C1–C5) were evaluated through molecular docking against four biological targets corresponding to **antidiabetic**, **anti-inflammatory**, **antioxidant**, and **antimicrobial** activities. Across all targets, the compounds showed favorable binding energies, indicating good theoretical affinity.

Antidiabetic

Target
C2 (–11.99 kcal/mol) exhibited the strongest affinity, followed by C4 (–10.57 kcal/mol) and C3 (–10.38 kcal/mol). These values indicate stable binding within the α -amylase active site, suggesting strong inhibitory potential.

Anti-inflammatory

Target
The best interaction was observed for C3 (–8.83 kcal/mol), closely followed by C4 (–8.74 kcal/mol) and C5 (–8.52 kcal/mol). These values indicate efficient stabilization within the COX-2 active pocket.

Antioxidant

Target
The highest binding affinity was demonstrated by C2 (–8.97 kcal/mol), indicating strong interaction with antioxidant-related proteins, suggesting enhanced radical-scavenging potential.

Antimicrobial

Target
C5 (–8.93 kcal/mol) and C4 (–8.79 kcal/mol) showed the most potent binding, indicating strong affinity towards microbial enzyme/protein targets. Overall, **C2**, **C3**, **C4**, and **C5** displayed strong multi-target docking profiles, while C1 showed moderate but consistent activity across all targets.

Characterization:

The synthesized compounds are characterization done by determined the physical data such as molecular formula, IUPAC name, physical appearance, percentage yield, Rf-value, melting point, solubility and spectral data such as IR-spectrum, NMR-spectrum, UV-Visible spectrum confirmed the structure of the compound.

SYNTHESIS:

The designed compounds (C1–C5) were synthesized through a three-step procedure. First, phthalic anhydride and p-acetaminophenone were condensed in glacial acetic acid to form Intermediate 1: 2-(4-acetylphenyl)-isoindole-1,3-dione. This was followed by an aldol condensation with 2-nitrobenzaldehyde in basic ethanol to yield Intermediate 2, a chalcone derivative. Finally, cyclization with hydrazine hydrate produced the target isoindole-1,3-dione fused 1H-pyrazole derivatives, completing the designed molecular framework.

BIOLOGICAL INVITRO EVALUATION:

The synthesized isoindole-1,3-dione fused 1H-pyrazole derivatives (C1–C5) were evaluated for anti-inflammatory, antioxidant, and antidiabetic activities using standard in vitro models.

Anti-inflammatory Activity (Protein Denaturation Assay)

All compounds showed dose-dependent protection against protein denaturation. C4 and C3 exhibited the highest activity, reaching 68.24% and 66.78% inhibition at 500 µg/mL, approaching the standard diclofenac (71.53%). C2 and C1 also displayed moderate to good inhibition.

Antioxidant Activity (Hydrogen Peroxide Assay)

All derivatives demonstrated significant H₂O₂ scavenging ability. C2 and C5 showed the strongest antioxidant effect, reaching 70.66% and 74.18% inhibition, comparable to ascorbic acid (58.92% at 50 µg/mL). Activity increased consistently with concentration across all compounds.

Antidiabetic Activity (α-Amylase Inhibition)

The compounds showed progressive inhibition of α-amylase with increasing concentrations. C4 produced the highest inhibition (59.24% at 500 µg/mL), followed by C1, C2, and C3, all showing moderate activity. C5 showed inconsistent activity but reached 56.69% at higher doses.

Overall, the results confirm that the synthesized derivatives possess notable anti-inflammatory, antioxidant, and antidiabetic properties, with C4, C3, C2, and C5 emerging as the most promising

candidates.

CONCLUSION:

The present study successfully designed, synthesized, characterized, and biologically evaluated a new series of isoindole-1,3-dione fused 1H-pyrazole derivatives. The molecular design and docking studies confirmed that the fused heterocyclic framework possesses strong theoretical affinity toward anti-inflammatory, antioxidant, antidiabetic, and antimicrobial targets, validating the rational design strategy.

The three-step synthetic pathway efficiently yielded the target compounds, and all intermediates and final structures were confirmed through appropriate characterization techniques. Biological screening demonstrated that the derivatives exhibited significant multi-target activity:

- Anti-inflammatory: C4 and C3 showed the highest inhibition, approaching the standard diclofenac.
- Antioxidant: C2 and C5 displayed strong hydrogen peroxide scavenging activity, comparable to ascorbic acid.
- Antidiabetic: C4 exhibited the highest α-amylase inhibition among the synthesized compounds.

Overall, the combined computational, synthetic, and biological findings indicate that these fused isoindole-pyrazole derivatives represent promising lead molecules with potential for further optimization and development as multifunctional therapeutic agents. The study provides a strong foundation for future in vivo studies, SAR exploration, and drug development efforts.

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