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Ulcerogenic Activity in Pyrazolopyrimidine Derivatives in Lab and It's in Silico Toxicity: An Update on Novel Research

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Abstract:
Toxicity is a measure of any unfavourable or negative impact of substances. Toxicity endpoints, such as carcinogenicity or genotoxicity, are certain sorts of unfavourable effects that can be quantitative (e.g., LD50: deadly dosage to 50% of tested individuals)1 or qualitative, such as binary (e.g., toxic or nontoxic) or ordinary (e.g., low, moderate, or high toxicity). Toxicity studies seek to discover the adverse effects of chemicals on humans, animals, plants, or the environment via acute (single dosage) or repeated exposures (multiple doses). The toxicity of chemicals is determined by a number of factors, including the route of exposure (e.g., oral, dermal, or inhalation), the dose (amount of the chemical), the frequency of exposure (e.g., single versus multiple exposure), the duration of exposure (e.g., 96 h), ADME properties (absorption, distribution, metabolism, and excretion/elimination), biological properties (e.g., age, gender), and chemical properties.

Keywords: Pharmacokinetics, toxicity, Ulcerogenic activity.

INTRODUCTION:

Peptic ulcer is a benign lesion of the stomach or duodenal mucosa that develops when the mucosal epithelium is exposed to acid and pepsin. Stress, smoking, dietary deficiencies, and the use of nonsteroidal anti-inflammatory medicines (NSAIDs) can all raise the risk of developing a stomach ulcer. (*Belaiche et al*., 2002).

In silico study: The process of developing new drugs takes a significant amount of time and resources. Theoretical investigations play a critical role in mitigating these effects since they reveal indicators of prospective therapeutic uses. Several authors state that it is not enough for a compound to have high biological activity and low toxicity to be tested as a drug; it must also meet the ADME pharmacokinetics parameters (absorption, distribution, metabolism, and excretion), which determine the compound's access and concentration in the therapeutic target, as well as its subsequent elimination by the organism. Many medication candidates are abandoned

because their pharmacokinetics is undesirable. In silico investigations based on derived physicochemical standards can be used to validate the ADME parameters. These specifications place a premium on lipophilicity, water solubility, molecular size, and flexibility.[1]

Prior examination of these characteristics significantly minimises the time required for the clinical phase pharmacokinetic investigation. Several investigations connecting physicochemical standards to ADME characteristics were conducted in the 1990s. The most widely used study was by Lipinski et al., who demonstrated a link between pharmacokinetics and physicochemical characteristics.[2]

Acute toxicity is defined as the unfavourable consequence (s) that occurs immediately or within a short time period following single or repeated administrations of such chemical within 24 hours. Any consequence that causes functional impairments in organs and/or biochemical lesions, which might change the functioning of the organism in general or particular organs, is considered an undesired (or undesirable) consequence. Acute toxicity studies, on the other hand, tend to establish the dosage dependent undesired (or bad) effect(s) that may occur, and this includes all information that is necessary in the evaluation of acute toxicity, including death. The measurement of the lethal dosage (LD50) (the dosage that kills 50% of the test animal population) is currently utilised as a significant criterion in evaluating acute toxicity as well as a starting process for screening chemical and pharmacological compounds for toxicity. Other biological consequences, as well as the timing of start, duration, and degree of recovery in surviving animals, are essential in acute toxicity assessments. Acute toxicity research only provides data on the LD50, therapeutic index, and degree of safety of a pharmacological substance. The toxicity evaluation of pharmacological drugs is a critical technique that is normally performed before they are authorised to be sold on the market. In contrast, numerous approaches for assessing acute toxicity have been devised and implemented. However, most of these approaches have flaws, and it is therefore critical to find a better approach, which may need the use of fewer animals if feasible. The goal of this work is to present a new approach for measuring acute toxicity that, if used, should give more accurate and reproducible results with fewer animals.[3]

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Animal models have been utilised for toxicity testing for a long time. However, developments in high throughput screening have made in vitro toxicity studies feasible. In silico toxicology (computational toxicology) is a sort of toxicity assessment that makes use of computational resources (methods, algorithms, software, data, and so on) to organise, analyse, model, simulate, depict, or forecast the toxicity of substances. It is linked to in silico pharmacology, which analyses the beneficial or unfavourable effects of medications using computational techniques for medicinal reasons.[5]

Computational approaches are intended to supplement in vitro and in vivo toxicity studies in order to possibly eliminate the need for animal testing, lower the cost and time required for toxicity studies, and enhance toxicity prediction and safety evaluation. Furthermore, computational approaches have the distinct benefit of being able to evaluate the toxicity of substances even before they are created. A wide range of computational methods are used in in silico toxicology (Figure (Figure1):1): (A) databases for storing data about chemicals, their toxicity, and chemical properties; (B) software for generating molecular descriptors; (C) simulation tools for systems biology and molecular dynamics; (D) toxicity prediction modelling methods; (E) modelling tools such as statistical packages and software for generating prediction models; and (F) expert systems that include prebuilt models in wetland environments.[6]

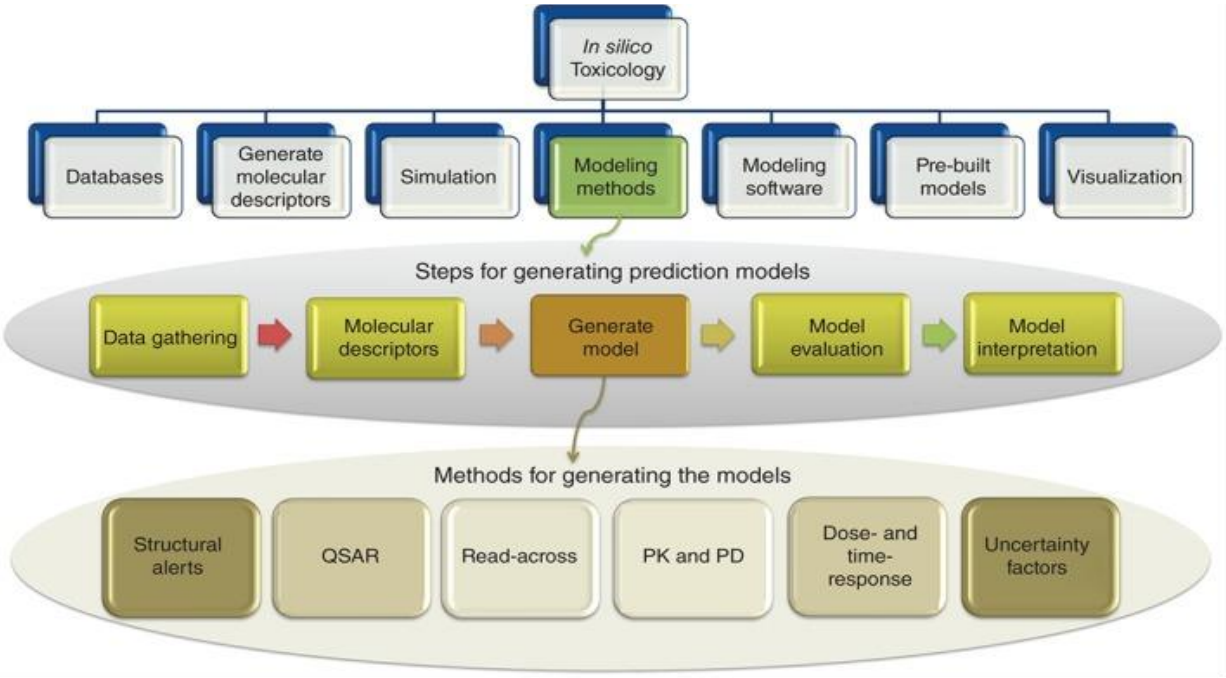

Fig 1.1. In silico toxicology tools, prediction model generation procedures, and prediction model types

The goal of this research is to provide a comprehensive overview of existing modelling methods and algorithms for toxicity prediction (element D above), with a special (but not exclusive) focus on computational tools that can implement these methods (element E), and expert systems that

deploy the prediction models (element F). Because of the nature of this rapidly evolving subject, this study cannot offer a thorough analysis of all seven in silico components described above. To learn more about toxicity databases, molecular descriptor generating software, toxicology simulation tools, statistical modelling packages, expert systems, and visualisation tools, the reader is advised to consult current literature. In general, when constructing prediction models, modelling procedures contain five essential phases (Figure (Figure1):1): (1) Collecting biological data including connections between chemicals and toxicity endpoints, (2) determining molecular descriptors of the chemicals, (3) developing a prediction model, (4) assessing the model's accuracy, and (5) interpreting the model

The scope of this evaluation is limited to the third phase, which is the generation of prediction models. We are interested in applying computational approaches to predict the toxicity of various substances such as pharmaceuticals, other compounds, mixtures, and nanomaterials, both quantitatively and qualitatively. There are several approaches for solving such issues, and each technique has its own set of strengths, limits, area of application, and interpretive specificity. The objective is to identify the most efficient solution to the situation at hand. However, all five of the preceding processes are interconnected. As a result, we address the remaining phases as needed. This review was broken into four major components. We begin by explaining and debating the available in silico approaches. Then, we'll go over two types of chemicals in more detail: mixes and nanomaterials. Following that, we make advice on how to create and use toxicity prediction models. Finally, we present an overview of toxicology in the twenty-first century.[7]

The area of in silico toxicology has been evolving at a rapid pace, with new approaches being introduced, established ones being improved, and some being abandoned. Unfortunately, a method that works well for one sort of toxicity endpoint or chemical may not perform well (or at all) for another. When utilised appropriately, in silico methods may be quite helpful in determining the toxicity of substances. As a result, in order to ensure accurate and effective application of in silico models, it is necessary to (1) understand the methods' strengths, limitations, scope of application, and interpretation; (2) select the most effective method for the problem at hand; and (3) customise these methods for each problem as needed. Users of toxicity prediction models may only take these three steps if the data and techniques used to construct the model are transparent, the application domains are well defined, the model outputs are well described, and the models are simplified.[8]

The safety testing paradigm is changing away from substantial animal testing and toward the use of computer models and mechanistic in vitro data. However, no validated or scientifically recognised in silico or in vitro tests that predict acute oral mammalian toxicity are currently available. Although revisions to prior acute testing standards reduced the use of animals, determining acute toxicity for regulatory reasons still necessitates in vivo testing. Alternative nonanimal techniques might include in silico quantitative structure-activity relationship (QSAR) models (Diaza et al., 2015) or other types of read-across (European Chemicals Agency, 2012), such as mechanistic tests based on in vitro data. Estimates of acute oral toxicity are frequently

required for substances before any testing, including in vitro testing. This is especially true when dealing with novel or experimental R&D chemicals. The sensitivity of various current in silico QSAR models for predicting acute oral rat toxicity was low (Diaza et al., 2015), particularly for more lethal Globally Harmonized System (GHS) 1 or 2 chemicals (manuscript in preparation).

Because of the diversity of chemical classes, acute toxicity mechanisms, and the effects of bioavailability and metabolism, existing QSAR models for this endpoint are solely statistical and, as such, have little use for forecasting acute oral toxicity. Because adequate QSAR models are not available, alternative approaches must rely on read-across, a datagap filling technique in which endpoint information from one chemical is used to predict the same endpoint for another chemical that is thought to be similar in terms of mode-of-action, toxicokinetics, metabolism, and so on, in relation to that endpoint (European Chemicals Agency, 2012). Although there is some growing guidelines for doing read-across, there is no universally acknowledged technique, and most studies are done on a case-by-case basis utilising nonstandardized methodologies that need extensive knowledge. Although classic read-across relies on existing data for the same endpoint for structurally related drugs, a broader definition incorporates data from any combination of different chemicals, routes, species, or research types, including mechanistic in vitro data. Because many compounds may undergo metabolic transformation or hydrolysis, a good read-across evaluation should take into account both the parent and analogues of metabolic products. A number of papers suggest that read across between species, pathways, or in vitro data is possible (Patlewicz et al., 2013a, b; Schu u rmann et al., 2011). In vitro basal cytotoxicity, for example, has been proposed as a method of predicting acute oral toxicity, but limitations include a lack of concordance for compounds that are poorly bioavailable or highly metabolised, as well as a lack of activity for compounds with toxicity mechanisms that are not applicable in vitro.[9]

Data sources for acute toxicity. Data on rat oral median lethal dose (LD50) were gathered from a variety of sources, including the public literature (Zhu et al., 2009), the European Chemicals Agency (ECHA), the OECD e-ChemPortal (eChemPortal), and ChemID Plus (ChemIDPlus). Data for fish 96-h half-maximum lethal concentration (LC50) and daphnia 48-h LC50 were obtained from the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), Aquatic Japan, and the US-EPA ecotoxicology database (ECOTOX), which are all available in the OECD QSAR Toolbox v3.2 (http://www.oecd.org/chemicalsafety/risk-assessment (eChemPortal). Additional data from the public literature were also obtained for fish 96-h LC50 (Lammer et al., 2009; Schirmer et al., 2008; Vittozzi and De Angelis, 1991) and daphnia 48-h LC50 (Lammer et al., 2009; Schirmer et al., 2008; Vittozzi and De Angelis, 1991). (Cassotti et al., 2014; Guilhermino et al., 2000). For all three species, only Klimisch-reliability score 1–2 data (Klimisch et al., 1997) were acquired from ECHA. Similarly, ChemID Plus was used to acquire rat intravenous LD50 data for ToxCast Phase II compounds (ChemIDPlus). Database creation. For all endpoints, numerous data sources were included in many situations, including the Chemical Abstract Service (CAS) number and name. Several protocols in Pipeline Pilot 8.5 (http://accelrys.com/products/pipeline-pilot/) were written to select single high-quality endpoint

data and to have the correct and single substance identity CAS, name, and Simplified Molecular Input Line Entry System (SMILES) representation for unique compounds. In the case of rat LD50, the lowest value corresponding to the maximum toxicity was preserved, as did the lowest LD50 value of range-finding investigations where a single LD50 value could not be recovered. Pimephales promelas (fathead) > Oncorhynchus mykiss (trout) > Poecilia reticulata 96-h LC50 data were picked in the order of importance for Pimephales promelas (fathead) > Poecilia reticulata (guppy). Similarly, for daphnia, the priority ranking was Daphnia magna > Daphnia pulex > Daphnia spinulata. This priority decision was based on the discovery that the bulk of the data belonged to the top-ranked species when compared to the others. The priority of data ranking between multiple endpoints for daphnia was LC50> EC50> IC50. This priority scale was used because death (LC50) is a more well-defined outcome than immobilisation (EC50) or incipient concentration (IC50).

Methodology:

Purified Pyrazolopyrimidine derivatives were produced in yields ranging from 45 to 95%. Scheme 1 illustrates the synthetic pathway. Thin layer chromatography was employed to complete the reaction and to determine the purity of the chemicals produced, with silica gel as the stationary phase and Toulene: ethyl acetate: formic acid as the solvent system (4:2:1), and the results were viewed using an ultraviolet visualising cabinet.

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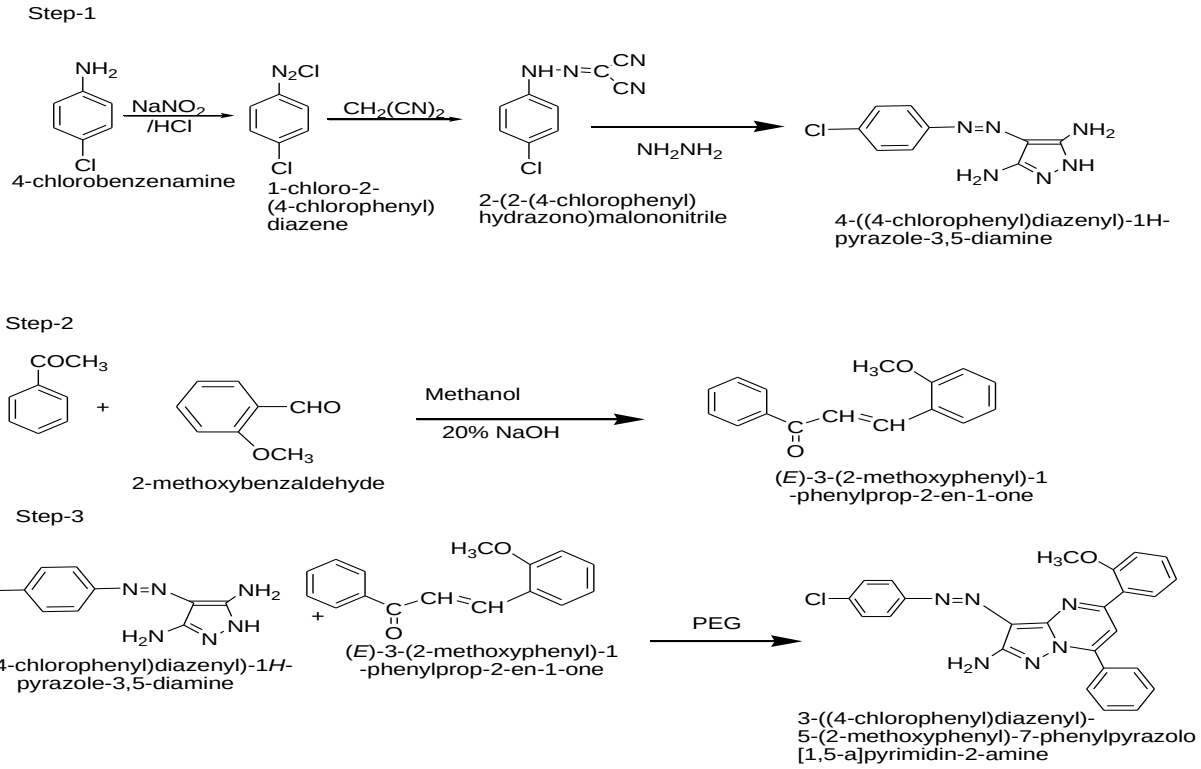
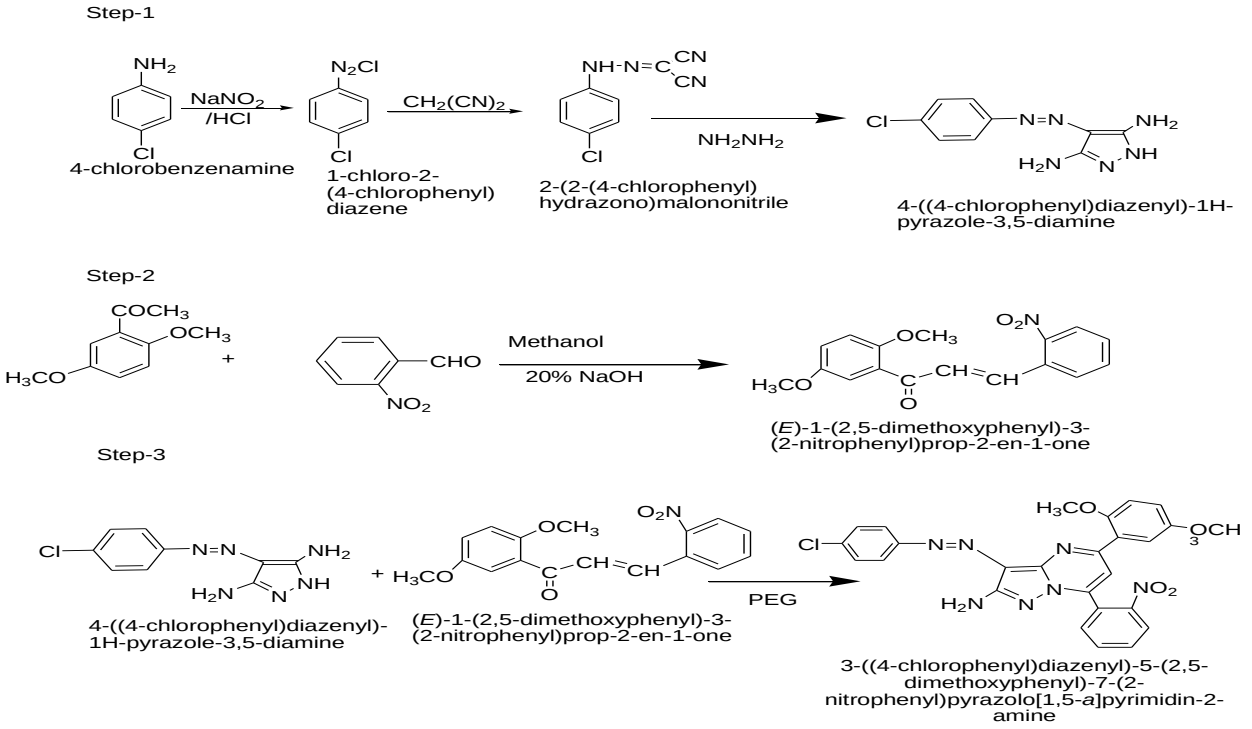
Procedure: A Mixture of α, β- unsaturated carbonyl compounds (chalcone) (1mmol), substituted pyrazole (1mmol) and 1-2 pellets of NaOH in polyethylene glycol (PEG-400) (20ml). For the duration of the interval, the reaction mixture was heated. TLC was used to monitor the reaction's development. Following completion of the reaction, the reaction mixture was extracted using 220mL diethyl ether. Drying the mixed organic layers over anhydrous Na2SO4 and evaporating the solvent under decreased pressure. The crude product was recrystallized using the appropriate solvent to get the finished product (PP1- PP3).

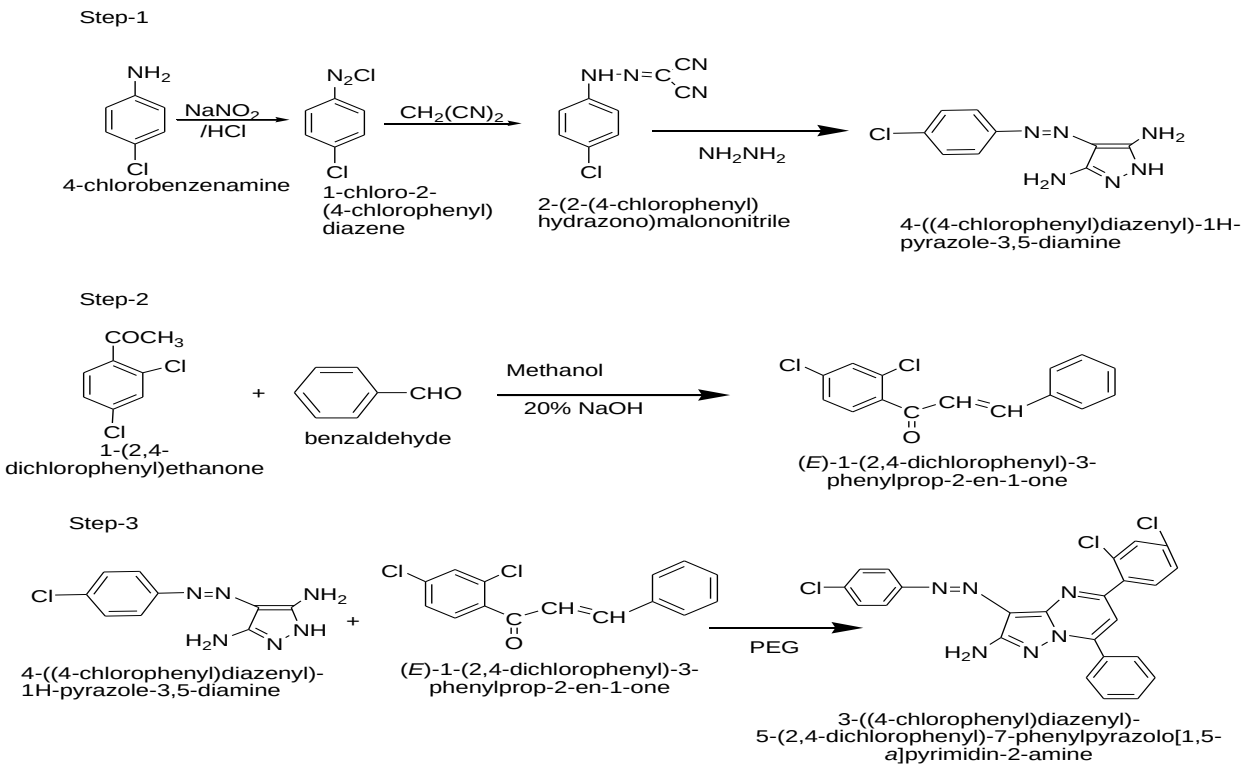
PP_1:


Step-1
NaNO2 /HCl
CH2(CN)2
NH2NH2
4-chlorobenzenamine
1-chloro-2-(4-chlorophenyl) diazene
2-(2-(4-chlorophenyl) hydrazono)malononitrile
4-((4-chlorophenyl)diazenyl)-1H-pyrazole-3,5-diamine
Step-2
Methanol
20% NaOH
2-methoxybenzaldehyde
(E)-3-(2-methoxyphenyl)-1-phenylprop-2-en-1-one
Step-3
PEG
4-((4-chlorophenyl)diazenyl)-1H-pyrazole-3,5-diamine
(E)-3-(2-methoxyphenyl)-1-phenylprop-2-en-1-one
3-((4-chlorophenyl)diazenyl)-5-(2-methoxyphenyl)-7-phenylpyrazolo[1,5-a]pyrimidin-2-amine

PP_2:


Step-1
NaNO2 /HCl
CH2(CN)2
NH2NH2
4-chlorobenzenamine
1-chloro-2-(4-chlorophenyl) diazene
2-(2-(4-chlorophenyl) hydrazono)malononitrile
4-((4-chlorophenyl)diazenyl)-1H-pyrazole-3,5-diamine
Step-2
Methanol
20% NaOH
(E)-1-(2,5-dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one
Step-3
PEG
4-((4-chlorophenyl)diazenyl)-1H-pyrazole-3,5-diamine
(E)-1-(2,5-dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one
3-((4-chlorophenyl)diazenyl)-5-(2,5-dimethoxyphenyl)-7-(2-nitrophenyl)pyrazolo[1,5-a]pyrimidin-2-amine

PP_3:



Toxicity:

Using a notional single oral dosage of 300 mg/kg in a 250 g rat, a one-compartment pharmacokinetic model was used to predict absorption characteristics, systemic bioavailability, and chemical levels in the blood. Suspension was used as the dosage formulation type. The Advanced Compartmental Absorption and Transit model is used in GastroPlus to estimate passive absorption through the gut and accounts for soluble and insoluble components of the supplied dosage. Predictions of bioavailability were made by incorporating metabolism due to three main CYP enzymes (2C, 2D, and 3A) in the rat liver. These QSAR predictions of metabolic clearance (enzyme kinetics -Km and Vmax based on recombinant CYP enzymes) were developed using the drugs' SMILES representations using (ADMET Predictor v7.0). Because no models exist to account for the effect of hydrolysis on carboxylic acid esters [CC(¼O)OC] (aliphatic and aromatic) and amides [NHC(¼O)C] (containing lactams and cyclic-diamides), they were identified using a SMARTS query and highlighted by creating procedures in Pipeline Pilot. The ADMET predictor estimates whether a molecule is a substrate for one phase-2 conjugation enzyme, UGT. The new design molecules also tested for their possible toxicity against following parameters:

- Human Ether-a-go-go-Related Gene Inhibition: The Human Ether-a-go-go-related Gene (hERG) Potassium Channel represents an Unusual Target for Protease-mediated Damage. This is responsible for cardiac arrhythmias and sudden death (PubMed ID: 16787254).

- AMES toxicity: The Ames test (Salmonella typhimurium reverse mutation assay) is a bacterial short-term test for identification of carcinogens using mutagenicity in bacteria as an endpoint (J.G. Hengstler, F. Oesch, in Encyclopedia of Genetics, 2001).
- Carcinogenesis: Test for causing cancer due to the molecule[10]
- Fish toxicity
- Tetrahymena toxicity
- Honey Bee toxicity
- Biodegradation
- Acute oral toxicity
- Rat acute toxicity

The complete table about toxicity screening of PP1-PP3 is available as following. The molecules can be further estimated for their utility for further study.

Toxicity Interpretation:

Compounds	Probability (Acute Oral Toxicity)	Low (<0.6); Mild (>=0.6 to <0.7); High (>=0.70)
P1	0.4106	LOW
P2	0.6895	**Mild**
P3	0.6034	Mild

Ulcerogenic activity

Experimental procedure : The Cioli et al. Method was used to perform the acute ulcerogenic test. The albino rats were separated into 14 groups, each with six individuals. Group I functioned as the normal control group (Received 0.5 ml of CMC as Vehicle). Group II was in charge of ulcer control (they were given Diclofenac Sodium 30mg/Kg). Group III served as the treatment control (received synthetic compounds at 150 mg/kg by oral administration). All animals were tested 24 hours before the test chemicals were administered. Following the medication treatment, the rats were fed a regular meal for 17 hours before being slaughtered. The stomach was removed and opened along the Greater Curvature, rinsed with distilled water, and gently cleansed with saline. A magnifying lens was used to inspect the mucosal injury (10X).

Ulcerogenic activity of synthesized compounds.

Compound	No of ulcer spots (mean ± sem)
(Standard)	2. 33 ± 0.42
PP-1	1.5 ± 0.23**
PP-2	1.7± 0.19***
PP-3	1.5 ± 0.22**

Method:

Ulcerogenic activity by Cioli *et al* method ; Albino rats; number of animals per group: 6; route of administration: oral; standard: Diclofenac sodium (50mg/kg.); test compound 150 mg/kg. ****p*≤ *0.001* statistically significant; Statistical analysis was performed by one way─ANOVA followed by Tukey's 't' test. All the values were expressed as **Mean ± Sem** and*p*≤ *0.001* indicates the level of statistical significance compared with standard Diclofenac sodium.

Result and Discussion:

The various biological actions are included in it which includes anti-viral, anti-bacterial, anti-tubercular, anti microbial, anti protozoal, anti hypertensive, antihistaminic, pain killers or analgesics, and anti inflammatory etc. an important pharmacophore is there in the pyrazolopyrimidine moiety which with the nucleic acid synthesis and function is seen to interact. In large amount of alkaloids, antimicrobial, antibiotics and drugs the nucleus of pyrimidine is is present. Purines and pyrimidines are simply fused pyrimidines which by themselves are active from biological point of view and for some naturally occurring substrates they are very essential components such as nucleic acids. Pyrimethamine and Trimethoprim are some of the diamino pyrimidines which are strong anti malarial medicines and along with sulphonamides they are used in combination for better outcomes. This is also an antibacteriostatic which is potent.

In Ulcerogenic activity compound **PP3** found to have low ulcer spots when compared with standard drug. *In Silico* Toxicity shows PP3 have low toxicity due to low LD_{50} Value.Compounds PP1-PP3 were evaluated for their ulcerogenic potential in rats according to the method reported by Cioli et al. The results indicated low ulcerogenic potential of the tested compounds (severity index 1.5-0.22). The lowest reduction in ulcerogenic potential (severity index 1.5) was observed for compound PP3 The other tested compounds, PP1, also exhibited a better gastrointestinal safety profile compared to the standard drug diclofenac sodium No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there were no observable changes. In the present study mortality was not observed and the tested compounds were well tolerated by the experimental animals up to 1000 mg kg–1.

Conclusion:

The Pyrazolopyrimidine moiety containing chemotherapeutic includes one of the most important suphadiazine. On the other hand, for the treatment of tuberculosis the nitrogen containing a lot of heterocycles are used such as Pyrazinamide and Clofazimine, Isoniazid etc. The structural precedence is offered by these compounds which along with analogues of Pyrazolopyrimidine as well as chalcone can end up in the generation of new therapeutics for tuberculosis. On the development of newer six member heterocyclic derivatives like pyrimidine and pyridine having antimycobacterial properties is the main focus of our research.

The new and improved artificial applicability as well as action of these heterocycles biologically are considered helpful for the pharmacists to plan and put into practice better and improved ways to find out and discover new series of drugs.

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