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Unveiling Innovative Purine Dione Derivatives: A Comprehensive Study for Advancing Medicinal Applications

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Abstract

The purpose of this study is to investigate the novel purine Dione derivatives, with a particular emphasis on the primary chemical, pentoxifylline. This research goes into the field of medicinal chemistry. Utilizing cutting-edge analytical methods including nuclear magnetic resonance (NMR), mass spectroscopy, and infrared spectroscopy, the inquiry includes the design, synthesis, and characterization of these new derivatives. One of the most important aspects of the research is the pharmacological evaluation of these compounds against a wide variety of microbial strains, such as e. Coli, m. Luteus, s. Typhi, s. Aureus, and candida albicans, to determine whether or not they have the potential to act as antimicrobial agents. These purine Dione compounds are positioned as possible candidates for advanced medicine as a result of the studies, which reveal promising characteristics. To create the platform for further study in the field of medication development, the complete synthesis and structural analysis provide a foundational understanding of these molecules. This research shed light on the value of articulating newly created compounds for medical interest, but it also highlights the potential significance of these molecules in the creation of novel treatments. This research makes a significant contribution to the field of medicinal chemistry by providing useful insights into the synthesis, structural characteristics, and prospective pharmaceutical applications of purine Dione derivatives.

Keywords: Characterization, Purine derivatives, Spectroscopy, Synthesis.

Introduction

Purine ring systems are among the most prevalent heterocycles in nature, acting as building blocks for a wide range of compounds with significant biological functions, including nucleosides and nucleotides. A handful of these later heterocycles demonstrate an extensive range of very important biological activities. (1) Additionally, because the fused purine class's basic structure resembles that of purine alkaloids that exist naturally, it is believed that they are ideal targets. (2, 3) As a result, many purine derivatives and analogues have been created and synthesized for use in medicine. These chemical families have been investigated for their potential as phosphodiesterase inhibitors (4), antimicrobial agents (5-8), asthma medicines (9, 10), antitumor and anticancer amplifiers (11, 12). A number of 9-deazaxanthines are also potential strong adenosine antagonists that may be employed as medications to treat asthma. These medications also can inhibit phosphoenolpyruvate carboxykinase (PEPCK), which has antidiabetic properties (13).

Further evidence that anilides are important binding blocks in nature and chemical synthesis comes from the observation that they are effective when used therapeutically to treat asthma (14) and as anti-HIV-1 medications (15). Adenosine receptor agonistic mechanisms of action have been demonstrated to be useful in treating erectile dysfunction, septic shock, and atherosclerosis in certain anti-arthritis pharmaceuticals, while others have been converted into amide prodrugs (16, 17).

In light of this context, we have persisted in our investigation into the synthesis of new heterocyclic molecules containing purine-2,6-Dione moieties that are 1,8-disubstituted and the screening of their biological activity (18-20). In this work, we report

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on the synthesis of new purine-2,6-Diones that are 1,8-disubstituted and discuss their anti-inflammatory chracteristics. It should come as no surprise that 6-dimethylaminopurine and isopentenyladenine, two purine scaffold containing CDK inhibitors, were among the first to be reported (21) because the purine ring is included in the structure of ATP itself. Positions C-2, C-6, and N-9 trisubstituted purines were the most effective CDK inhibitors (22). It was demonstrated that at micromolar dosages, olomoucine specifically inhibited the CDK1, CDK2, and CDK5 kinases.

Generally, xanthine [1H-purine-2,6(3H,7H)- Diones] and it is derivatives are purine-based nitrogenous compounds, it is structure consists of pyrimidine Dione fused with imidazole ring (23). However, Xanthine derivatives are commonly known for their broad range of biological activities such as PDEs inhibitors, Alzheimer's disease, asthma, antidepressants, anxiolytics, cancer, diabetics, analgesics, parkinsonism, diuretics, and others (24, 25).

Pentoxifylline is a synthetic Methylxanthine derivative. It is considered a nonselective inhibitor of PDEs especially isozymes PDE3 and PDE4 that is primarily present in inflammatory cells (26). It is the main indication in the treatment of intermittent claudication primarily due to its role in reducing blood viscosity and increasing red blood cell deformability (27). PTX activity and its role in anticancer treatment are currently receiving considerable attention (28). PTX when used in combination with chemotherapy and radiotherapy showed synergistic activity with an increase in the effectiveness of therapy (29, 30).

Purine Dione derivatives have garnered substantial attention in the field of medicinal chemistry due to their versatile pharmacological properties and potential therapeutic applications. This paper explores the innovative design and articulation of newly formulated purine Dione derivatives, focusing on their significance in the context of medicinal interest. The synthesis and modification of purine Dione derivatives present an exciting avenue for researchers to develop novel drugs and therapies that can target various diseases. In this paper, we aim to elucidate the goals and objectives of our research, as well as the organizational structure of our discussion, which will serve as a guide for understanding the comprehensive exploration of these newly designed compounds.

Purine Dione derivatives have shown potential benefits in various medical applications, supported by both theoretical and experimental data. Research has demonstrated the design, synthesis, and evaluation of purine/pteridine-based derivatives as dual inhibitors of EGFR and BRAFV600E, showing high potential for drug development, particularly in the treatment of neoplasms, microbial infections, and chronic inflammatory disorders (31). Additionally, studies have identified purine-2,6-Dione derivatives as potential SARS-CoV-2 main protease inhibitors, indicating their possible application in antiviral therapy (32). Furthermore, purine-2,6-Dione derivatives have been evaluated for their analgesic, anti-inflammatory, and anti-remodeling activities, suggesting their potential in pain management and inflammatory conditions (33, 34). Moreover, the development of novel purine-2,6-Dione derivatives as 5-HT1A receptor partial agonists has implications for the treatment of depression and anxiety disorders (35). These findings collectively support the potential of purine Dione derivatives in expanding medical applications, including cancer treatment, antiviral therapy, pain management, and mental health disorders. Several purine Dione derivatives act as potent inhibitors of Phosphodiesterase (PDEs), enzymes responsible for breaking down cyclic adenosine monophosphate (cAMP). Increased cAMP levels suppress inflammation, making PDE inhibitors potential anti-inflammatory agents. Studies on butane hydrazide derivatives of purine-2,6-Dione demonstrated significant anti-TNF-α effects in rats with LPS-induced inflammation, highlighting their therapeutic potential (36).

The study focuses on purine Dione derivatives due to their promising potential in advancing medicinal applications. These derivatives exhibit unique chemical structures and versatile properties that make them attractive candidates for drug development. Researchers are intrigued by the prospect of unveiling innovative purine Dione derivatives and exploring their pharmacological activities. The comprehensive nature of the study aims to unravel the molecular intricacies of these

derivatives, assess their bioactivity, and understand their potential therapeutic applications. By delving into the synthesis, characterization, and biological evaluation of purine Dione derivatives, the research seeks to contribute valuable insights that could pave the way for the development of novel drugs with enhanced efficacy and reduced side effects, thereby advancing the field of medicinal chemistry. Purine Dione derivatives, including notable compounds like Pentoxifylline, are characterized by their diverse therapeutic indications, anti-inflammatory properties, and potential in emerging fields such as antimicrobial and anticancer research. In contrast, other medical chemicals encompass a wide array of structures and mechanisms, with established safety profiles and efficacy in various clinical settings. Table 1 compares aims to highlight key distinctions in chemical structure, mode of action, and developmental stages, providing insights into the unique attributes of purine Dione derivatives in the realm of medicinal applications.

At the molecular level, purine Dione derivatives engage in intricate interactions that can profoundly influence biological processes (37). These derivatives often contain structural elements that facilitate diverse interactions, such as hydrogen bonding, π-π stacking, and hydrophobic interactions. These forces contribute to the formation of molecular assemblies, impacting the overall structure, stability, and functionality of purine Dione compounds.

Table 1: Comparison between purine Dione derivatives and other chemicals that are currently in use for medical purposes

Criteria	Purine Dione Derivatives	Other Medical Chemicals
Chemical Structure	Derived from purine nucleus, may include Pentoxifylline, novel modifications	Diverse structures including small molecules, biologics, peptides, etc.
Therapeutic Indications	Anti-inflammatory, vasodilatory, antimicrobial, anticancer	Varied: Pain management, cardiovascular diseases, infectious diseases, oncology, etc.
Mechanism of Action	Inhibition of phosphodiesterase's, anti-inflammatory effects, modulation of cellular signaling	Diverse mechanisms: Enzyme inhibition, receptor activation/blockade, gene expression modulation, etc.
Selectivity	Structure-based design for targeted binding, tailored selectivity	Varies, from highly selective to broad-spectrum, depending on the drug
Efficacy	Potential efficacy in specific applications (e.g., anti-inflammatory, antimicrobial)	Established efficacy for various medical conditions, often backed by extensive clinical data
Safety Profiles	Limited clinical data, ongoing optimization, potential for unknown side effects	Established safety profiles for many drugs, with known side effects and adverse reactions
Development Stage	Early-stage to Phase II clinical trials for diverse applications	Primarily late-stage development or marketed for established indications
Advantages	Novel scaffold, potential for broad applicability, tailored selectivity	Extensive clinical data, established safety profiles, proven efficacy
Disadvantages	Limited clinical data, ongoing optimization, potential for unknown side effects	Established side effects, limited target range for some drugs, potential for resistance emergence
Examples	Pentoxifylline, anti-SARS-CoV-2 derivatives, TRPA1 antagonists, KLK3 inhibitors	Aspirin, statins, antibiotics, antihypertensives, monoclonal antibodies, etc.

Hydrogen bonding is a crucial form of interaction in purine Dione derivatives (38). The carbonyl groups present in these compounds can participate in hydrogen bonding with other functional groups. In a biological context, these interactions may occur within the same molecule or between different molecules, influencing the overall
conformation and stability of the derivatives. Such interactions are vital for the three-dimensional structure of biomolecules, affecting their biological activity (39).

The presence of aromatic rings in purine Dione derivatives allows for π-π stacking interactions. This stacking can occur between adjacent aromatic rings within the same molecule or between molecules. In biological processes, π-π stacking interactions play a role in molecular recognition, influencing the binding of purine Dione derivatives to biomolecules like proteins or nucleic acids (40). This can affect the regulation of enzymatic activity, signal transduction, or gene expression.

The hydrophobic nature of certain regions in purine Dione derivatives leads to hydrophobic interactions (41). In a biological context, these interactions are relevant for the binding of these derivatives to hydrophobic pockets in proteins or cell membranes. Such interactions can influence the pharmacokinetics and cellular uptake of purine Dione-based drugs, impacting their efficacy and potential side effects. Purine Dione derivatives can be explored for their efficacy in treating inflammatory conditions, such as rheumatoid arthritis or vascular diseases, owing to their ability to modulate immune responses and improve blood flow.

Material and methods

Pentoxifylline was obtained from Sigma-Aldrich (USA), the aldehyde derivatives from Pharmaceutical Factory (India). The remaining chemical are obtained from commercial sources and all used without extra purification. The newly synthesized chalcones C1 and C2 were obtained from the Department of Pharmaceutical Chemistry.

Bacterial strains and culture media

Chalcones was tested against clinical isolates of MRSA and one laboratory control strain of methicillin-resistant Escherichia coli ATCC 4230, Micrococcus luteus ATCC 9345, Salmonella Typhi ATCC 14028, Staphylococcus aureus ATCC 6538, Candida albicans ATCC 14053 as positive control.

A stock solution of 10 mg of each synthesised product was made using dimethyl sulfoxide (DMSO) as the solvent. The synthetic compounds were tested for antibacterial activity using disc diffusion and micro broth dilution, which both determine minimum inhibitory concentrations (MIC) (42).

Antibacterial activity

Antibacterial activity against Gram-negative and Gram-positive bacterial strains was assessed utilizing the disc diffusion assay with the synthesized compounds (43). The diagnostic laboratory at KAUH supplied the bacterial cultures. Pure colonies obtained from newly cultivated bacteria were transferred from the plates to a sterile normal saline solution, where they underwent vortexing, to attain uniform bacterial suspensions. The suspensions were plated on Mueller-Hinton agar (MHA) once the turbidity value had been reduced to 0.5 McFarland standard units. Plates were covered with sterile filter paper discs that measured 6 mm in diameter. A solution of 10 mg/mL dissolved in DMSO was used to impregnate 20 µL of the tested compounds onto the sterile discs (44). Sterile distilled water was employed as the negative control and Amoxicillin was utilised as the positive control. Following this, the dishes were incubated for 24 hours at 37°C. The zones of inhibition were quantified in millimeters. The MIC for bacteria was ascertained through the measurement of absorbance on microtiter plates at 570 nm (45).

Antifungal activity

The fungus was grown for 24 hours at 35 0C on Sabouraud dextrose agar, but the mould fungi needed 5 days on a potato dextrose agar slant. A sterile loop was used to transfer pure Candida species colonies into a sterile normal saline tube (46). After being covered, the mould colonies were again suspended in 1 milliliter of sterile distilled water that contained 0.1% Tween 20. Using a hemocytometer, the suspension was adjusted at a concentration of 2-5.0 106 conidia/mL. By further diluting the material by a factor of 10, final working inoculums of 2-5.0 105 conidia/mL were obtained. MHA that had been laced with 2% glucose was given the inoculum (47). Sterilized 6 mm discs impregnated with a 20 L test substance (10 mg/mL

concentration) were used to cover the plate. During the 48-hour incubation period at 35 0C, sterile distilled water and the common antifungal medication Nystatin were both used as positive controls. The inhibitory zone with a millimeter width was noted. To determine the MIC for fungus, microtiter plate absorbance at 530 nm was utilized (48).

Anticancer activity

Using the MTT assay, the synthetic compounds' anticancer activity was assessed. T47D human breast cancer cells were seeded at a density of 1x104 cells per well and grown for 24 hours in phenol red-free RPMI 1640 medium (10% FBS) (49). The synthesised compounds were then applied for a whole day with doxorubicin as a positive control (at seven different concentrations). After adding a 0.5% MTT solution to the well as a 1/10 volume of media, the well was incubated for another 4 hours at 37 0C/5% CO2. After thorough pipetting to spread the produced blue formazan, each well was filled with a volume of stop solution (0.04 N HCl in isopropanol), and absorbance was measured at 570 nm (peak) and 630 nm (bottom). The experiment was done three times (50).

Experimental

Chemical synthesis

The synthesis of target compounds [1, 2] and their intermediates [1-5] was accomplished following procedures illustrated below in Figure 1. Table 2 shows the synthesis of chalcone derivatives through the reaction of pentoxifylline with aldehyde, with a total of ten different synthesis methods being explored. But here we studied only two Chalcones C1 and C2. We will study the remaining in the next study.

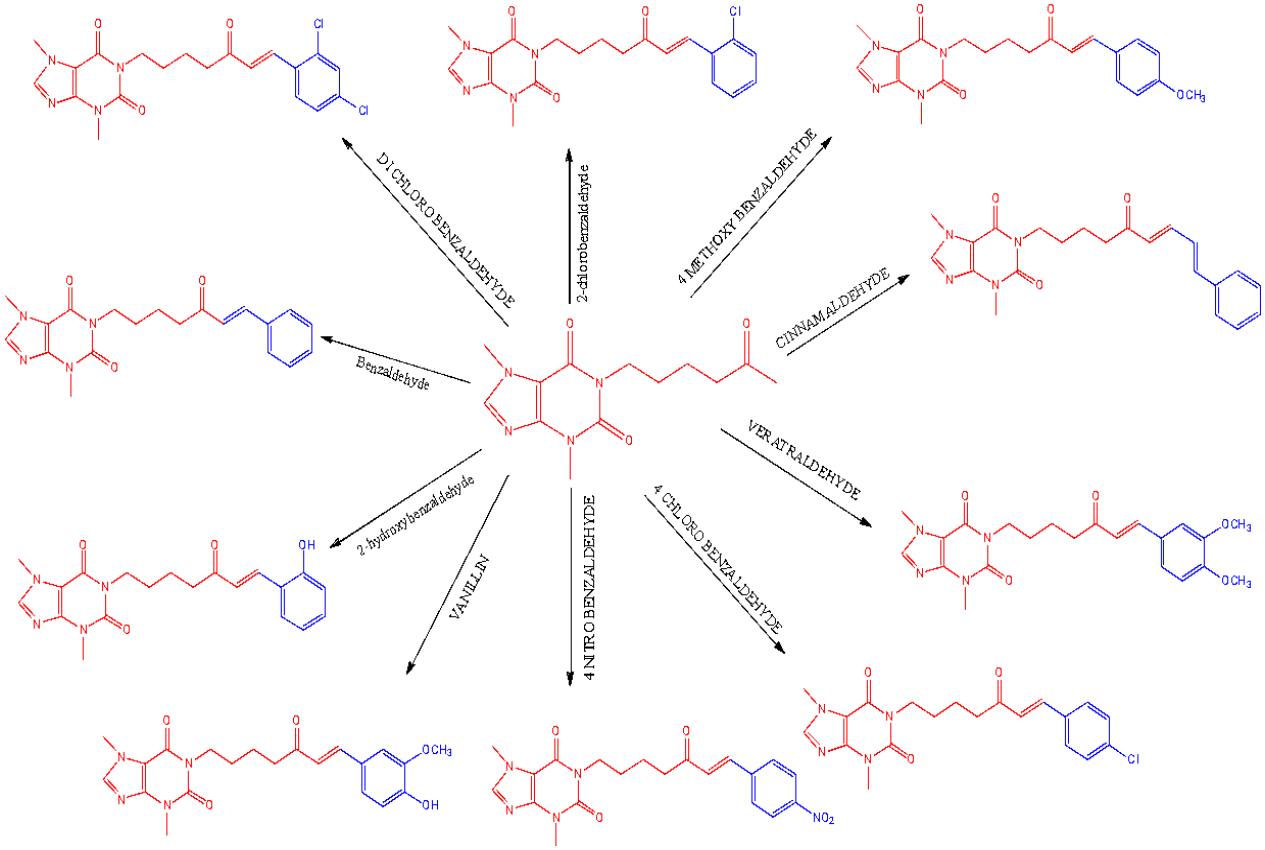


Figure 1: Target compounds

Table 2: Pentoxifylline reaction with 10 aldehydes

Sr. No.	Reaction	Chalcone Code
1.	Pentoxifylline + 2 Chloro Benzaldehyde (A1)	C1
2.	Pentoxifylline + 4 Methoxy Benzaldehyde (A2)	C2
3.	Pentoxifylline + Cinnamaldehyde (A3)	C3
4.	Pentoxifylline + Veratraldehyde (A4)	C4
5.	Pentoxifylline + 4 Chloro Benzaldehyde (A5)	C5
6.	Pentoxifylline + 4 Nitro Benzaldehyde (A6)	C6
7.	Pentoxifylline + Vanillin (A7)	C7
8.	Pentoxifylline + Salicylaldehyde(A8)	C8
9.	Pentoxifylline + Benzaldehyde (A9)	C9
10.	Pentoxifylline+ 2,4 Di-Chloro Benzaldehyde (A10)	C10

Spectroscopy Methods

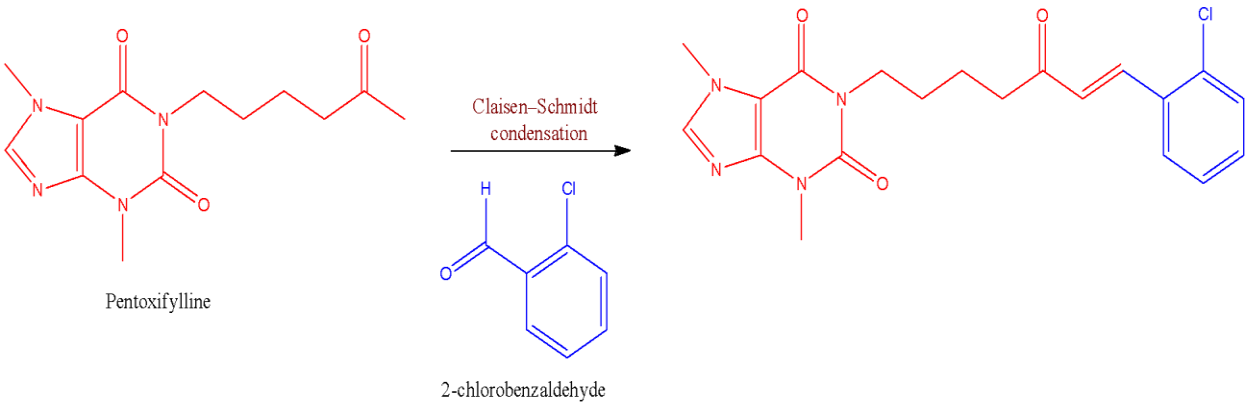
Nuclear Magnetic Resonance (NMR) spectroscopy, Mass spectroscopy, and Infrared (IR) spectroscopy were employed to characterize the newly designed purine Dione derivative, Pentoxifylline, for its medicinal interest. For NMR spectroscopy, ^{1}H and ^{13}C NMR spectra were recorded in a suitable solvent, and chemical shifts were referenced to an internal standard. Mass spectroscopy determines the molecular weight and fragmentation patterns of Chalcone. Infrared spectroscopy (IR) was performed to identify functional groups by analyzing the absorption bands using a suitable IR spectrometer. These spectroscopic techniques collectively provided insights into the chemical structure, purity, and functional groups present in Chalcone, ensuring its suitability for medicinal applications.

Result and Discussion

Claisen-Schmidt condensation served as the basis for the overall synthetic approach used to produce the chalcone derivatives.

C1 Synthesis

In this reaction, the reagents pentoxifylline and benzaldehyde are reacted in the presence of sodium hydroxide (NaOH) as a catalyst. Methanol is used as the solvent for the reaction, creating a suitable medium for the chemical transformation. The reaction time is set at 25 minutes, signifying the duration for which the reactants are allowed to interact. Additionally, the Thin-Layer Chromatography (TLC) system used for analysis utilizes a mobile phase composed of n-hexane and ethyl acetate in a 2:3 ratio, facilitating the separation and visualization of different components of the reaction mixture based on their affinities for the mobile and stationary phases (scheme 1).



Scheme 1: Preparation of chalcone of Purine Dione and 2-Chloro benzaldehyde

Mass Spectroscopy

In mass spectroscopy, the highest observed peak in the mass spectrum has an m/z (mass-to-charge ratio) value of 401.05. This value represents the most abundant ion detected in the analyzed sample. Mass spectroscopy is a powerful analytical technique used to determine the mass and composition of molecules by measuring their m/z values. The highest peak with an m/z of 401.05 suggests the presence of a specific molecular species or fragment with that particular mass-to-charge ratio, which can provide valuable information for identifying and characterizing the compound under investigation (Figure 2).

IR Spectroscopy

The table presents data from IR spectroscopy, indicating the position of absorption bands in wave numbers (cm^{-1}) and their corresponding functional groups. The absorption band at 3063 cm^{-1} represents the stretching vibration of C-H bonds, indicative of an aromatic functional group. At 2950 cm^{-1}, the asymmetric stretching vibration of C-H bonds, characteristic of methyl groups (-CH_3), is observed. The absorption band at 1323 cm^{-1} signifies the stretching vibration of the C=N bond, suggesting the presence of the purine ring. The band at 1550 cm^{-1} corresponds to the stretching vibration of C=C bonds, characteristic of aromatic compounds. The absorption band at 1708 cm^{-1} is indicative of the carbonyl (C=O) functional group. The band at 3597 cm^{-1} suggests the presence of the O=C-N functional group in an amide linkage. Lastly, the absorption band at 651 cm^{-1} represents the stretching vibration of C-Cl bonds, indicating the presence of a chloro group (Table 3 and Figure 3 A and B).

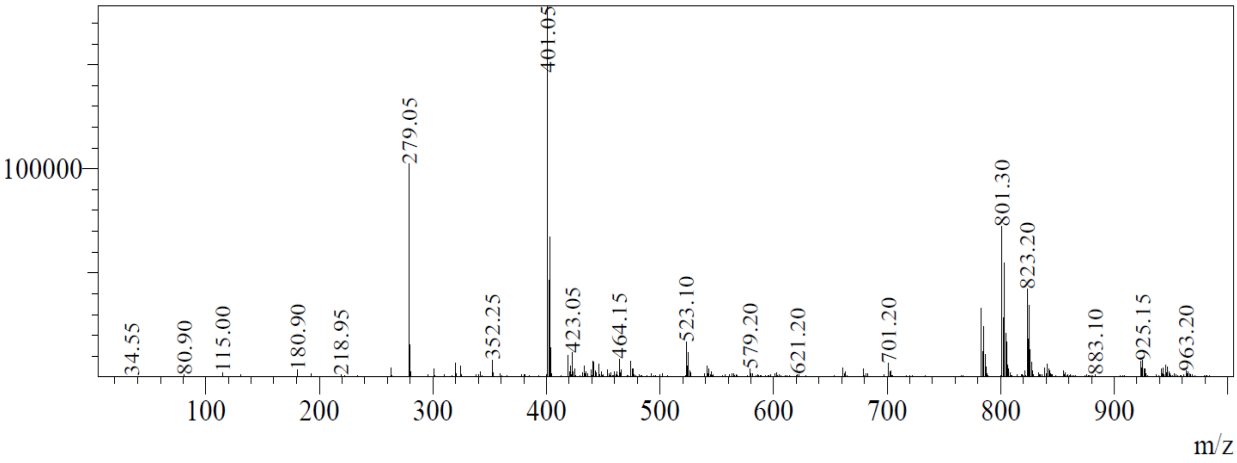


Figure 2: Mass spectrum of C1 compound

Table 3: Spectral data of IR of C1 Compound

Position of absorption band wave number cm^1	Band and its mode vibration	Functional group
3063 cm^{-1}	C-H str.	Aromatic
2950 cm^{-1}	Asym. C-H str.	-CH3 (Methyl)
1323 cm^{-1}	C=N Str.	Purine ring
1550 cm^{-1}	C=C str.	Aromatic
1708 cm^{-1}	C=O str.	carbonyl
3597 cm^{-1}	O=C-N str.	Amide
651 cm^{-1}	C-Cl str	chloro

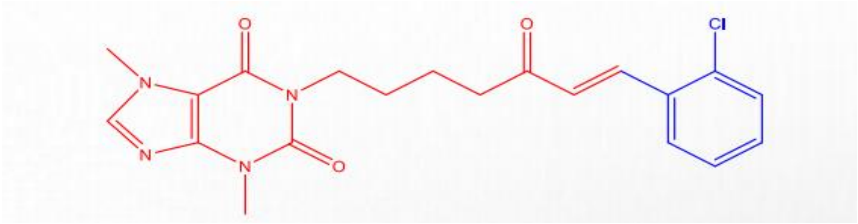


Figure 3A: Chalcone of Purine Dione and 2-Chlorobenzaldehyde

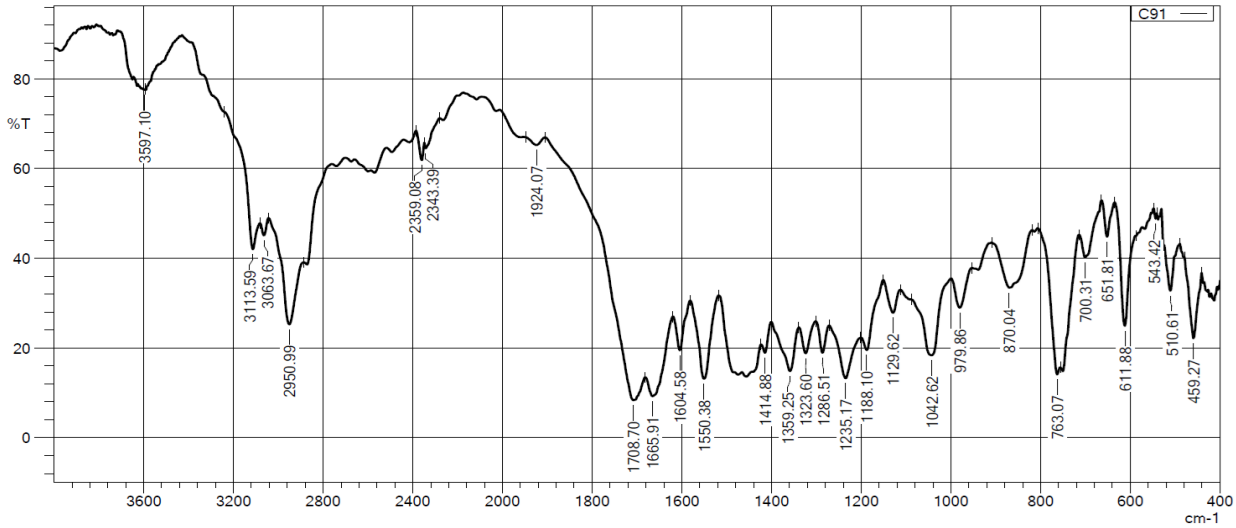


Fig. 3B: IR spectrum of C1 compound

^{1}H NMR Spectroscopy

This table provides information obtained from ^{1}H NMR spectroscopy, detailing signal positions (in δ ppm), the number of protons responsible for each signal, their multiplicity, and the structural inferences they imply. The signals in the range of 1.59-1.65 ppm, with four protons, appear as doublets, indicating the presence of -CH_2- groups. At 2.89 ppm, two protons contribute to a singlet, signifying another -CH_2- group. A range of signals between 3.06 and 3.84 ppm, with six protons, are singlets, representing the -CH_3 groups of an N-CH_3 moiety. In the range of 4.28-5.06 ppm, two protons contribute to a singlet, indicating the presence of a -CH_2- group in an N-CH_2 structure. At 6.70 and 7.78 ppm, two protons appear as doublets, suggesting the presence of C-H groups in a chalcone structure. The signals between 7.27 and 7.48 ppm, with four protons, exhibit multiplet patterns, indicative of C-H groups in an aromatic ring. Lastly, the signal at 7.59 ppm, with one proton, is a singlet, representing the -C-H group of a C=N bond (Table 4 and Figure 4).

Table 4: Spectral data of ^{1}HNMRof C1 compound

Signal Position (δ ppm)	Number of proton (s)	Multiplicity	Inference
1 .59 - 1 .65	4 H	doublet	-CH 2 –
2 .89	2 H	singlet	-CH 2 –
3 .06 - 3 .84	6 H	Singlet	-CH 3 of – N -CH 3
4 .28 - 5 .06	2 H	singlet	-CH 2 -of – N -CH 2
6 .70 and 7 .78	2 H	Doublet and doublet	C - H of Chalcone
7 .27 - 7 .48	4 H	Multiplate	C - H of aromatic ring
7 .59	1 H	Singlet	- C - H of C=N

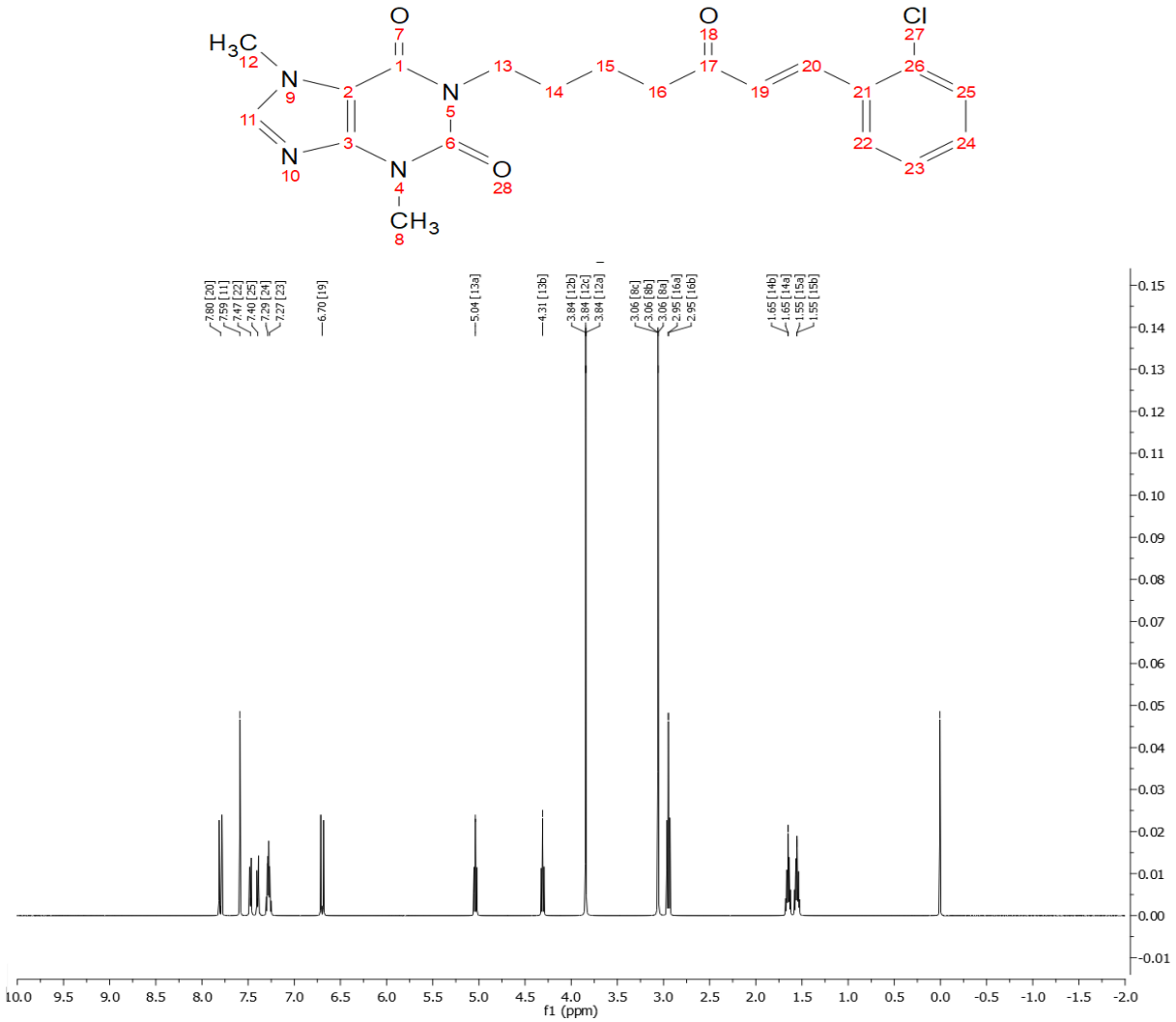


Figure 4: [1]HNMRspectrum of C1 compound

Table 5: Spectral data of [13]CNMR of C1 Compound

Signal Position (δ ppm)	Position	Inference
34.96, 39.38	34.96, 39.38	N-CH3
23.74, 26.96	14 and 15	C-CH2
39.71, 45.48	13 and 16	CH
59.19	6	N CH2 N
128-133	21-26	Aromatic C
125.35 and142.87	19 and 20	Chalcone
116.76	2	C=C-N
150.75 and 146	3 and 11	N-C-N
151.82, 203.30	1 and 17	C=O

[13]CNMR Spectroscopy

This table provides valuable information obtained from [13]C NMR spectroscopy. It presents signal positions (in δ ppm) and their corresponding structural inferences. The signals at 34.96 and 39.38 ppm suggest the presence of N-CH_3 groups, while those at 23.74 and 26.96 ppm correspond to C-CH2 groups at positions 14 and 15. Signals at 39.71 and

45.48 ppm indicate the presence of non-methyl CH groups at positions 13 and 16, and the signal at 59.19 ppm represents a nitrogen-bound CH_2 group (N-CH_2-N). The range between 128-133 ppm signifies aromatic carbons at positions 21-26, and the signals at 125.35 and 142.87 ppm suggest the presence of chalcone structures at positions 19 and 20. The signal at 116.76 ppm is indicative of a C=C-N bond, and signals at 150.75 and 146 ppm indicate nitrogen-carbon-nitrogen (N-C-N) linkages at positions 3 and 11. Finally, signals at 151.82 and 203.30 ppm represent carbonyl groups (C=O) at positions 1 and 17 (Table 5 and Figure 5)

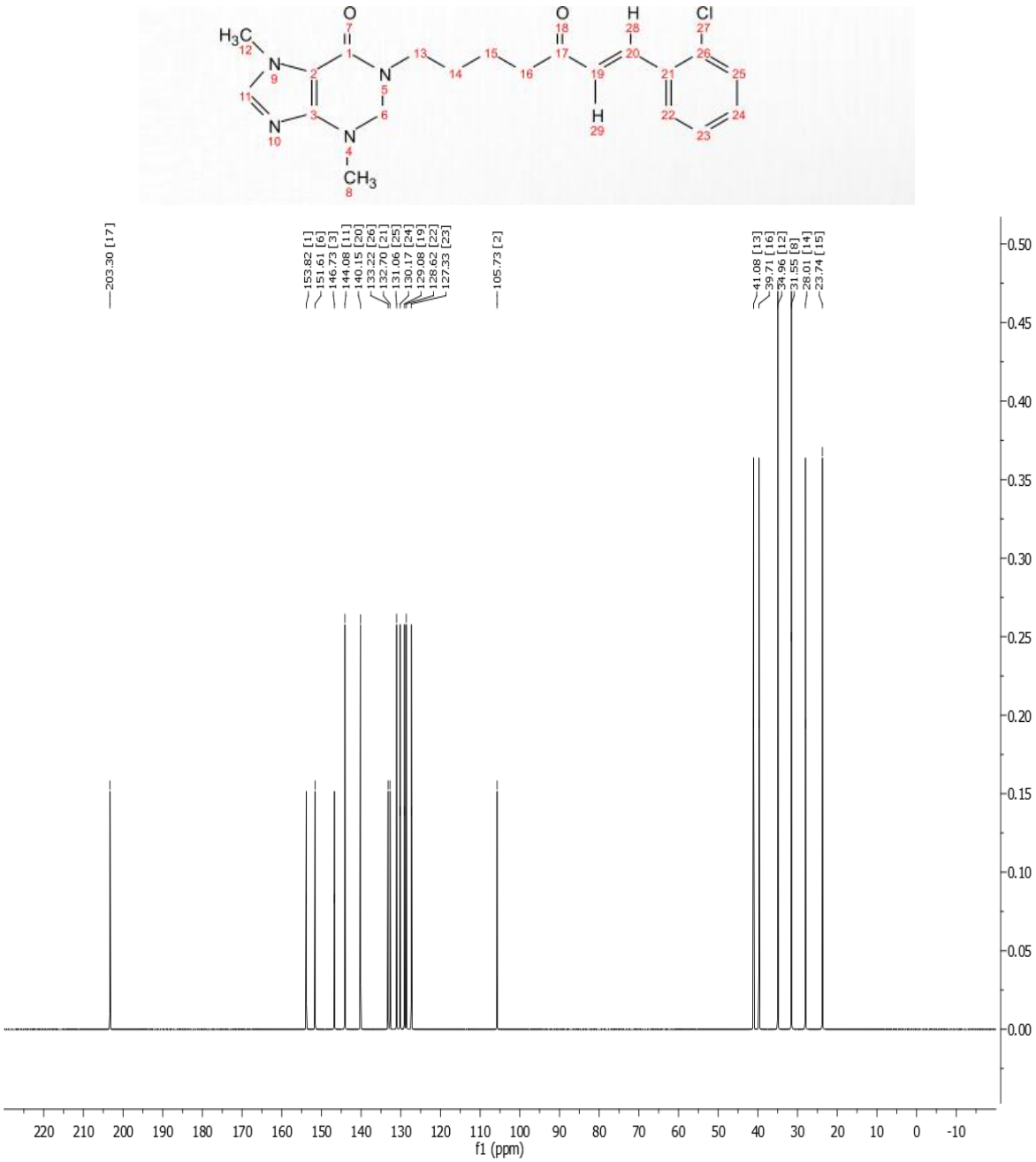


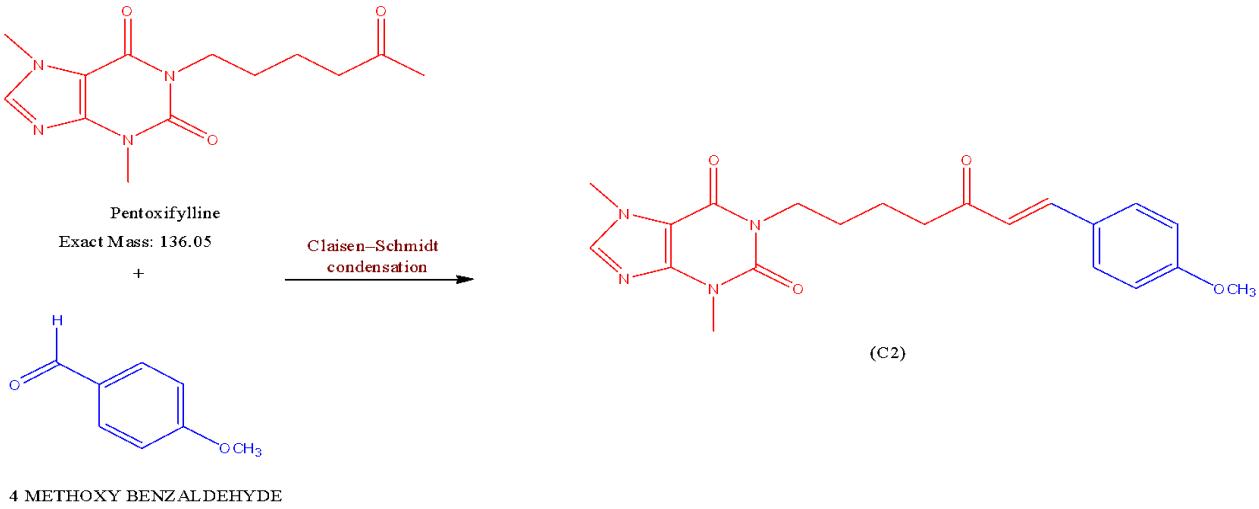
Figure 5: ^{13}CNMR spectrum of C1 compound

C2 Chalcone

The reaction involves the reagents pentoxifylline and 4-methoxybenzaldehyde, and it is catalyzed by sodium hydroxide (NaOH) in a solvent of methanol. The reaction time is set at 30 minutes, indicating the duration for which the reagents are allowed to react. Additionally, the Thin-Layer Chromatography (TLC) system used for analyzing the reaction progress or products consists of a mobile phase composed of n-hexane and ethyl acetate in a ratio of 2:3. This system aids in separating and visualizing different components of the reaction mixture based on their relative affinities for the mobile and stationary phases. These experimental parameters provide essential information for conducting and monitoring the chemical reaction and subsequent analysis through TLC (scheme 2).

Mass spectroscopy

In mass spectroscopy, the observed m/z (mass-to-charge ratio) high peak value is 397.10. This value represents the heaviest ion detected in the mass spectrum, providing insight into the molecular mass or charge of the compound under analysis. Mass spectroscopy is a powerful analytical technique that helps determine the mass and composition of molecules by measuring their m/z values and is widely used in various fields, including chemistry and biochemistry.



Scheme 2: Preparation of chalcone of Purine Dione and 4-Methoxy benzaldehyde

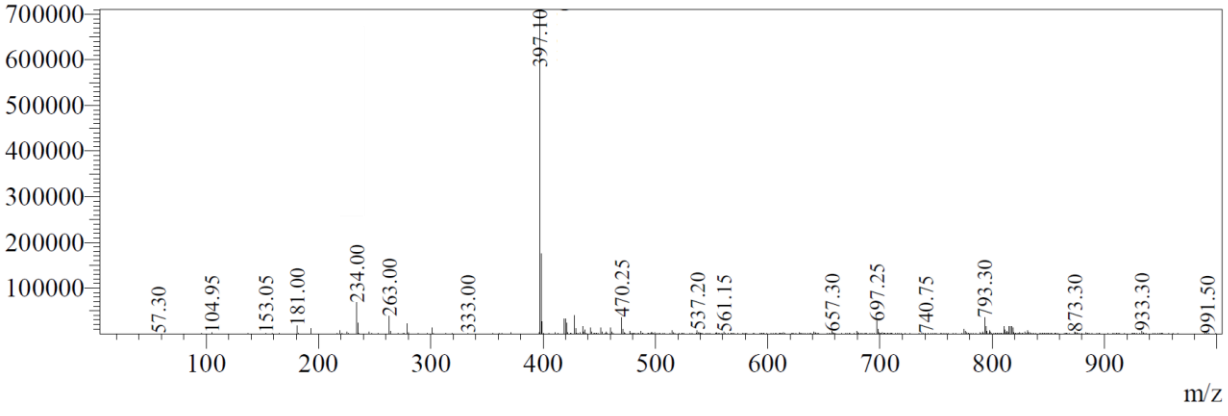


Fig. 6: ^{13}CNMR spectrum of C1 compound

IR Spectroscopy

The table presents the results of IR spectroscopy, indicating the position of absorption bands in wave numbers (cm^{-1}) and their corresponding functional groups. The absorption band at 3100 cm^{-1} is attributed to the stretching vibration of C-H bonds, indicating the presence of aliphatic hydrocarbons. The band at 2952 cm^{-1} represents the asymmetric stretching vibration of C-H bonds in methyl groups (CH_3), confirming the presence of methyl functional groups in the compound. The absorption band at 1320 cm^{-1} is associated with the stretching vibration of the C=N bond, indicating the presence of the purine ring. The band at 1600 cm^{-1} corresponds to the stretching vibration of C=C bonds, characteristic of aromatic compounds. The absorption band at 1700 cm^{-1} is indicative of the carbonyl (C=O) functional group. The band at 3600 cm^{-1} suggests the presence of the O=C-N functional group in an amide linkage. Finally, the band at 2852 cm^{-1} represents the stretching vibration of C-OCH_3 bonds, confirming the presence of a methoxy (OCH_3) functional group (Table 6 and Figure 6).

Table 6: Spectral data of IR of C1 Compound

Position of absorption band wave number cm^{-1}	Band and its mode vibration	Functional group
3100cm^{-1}	C-H str.	C-H str.
2952cm^{-1}	Asym. C-H str	CH3 (Methyl)
1320cm^{-1}	C=N Str	Purine ring
1600cm^{-1}	C=C str.	Aromatic
1700cm^{-1}	C=O str.	carbonyl
3600cm^{-1}	O=C-N str	Amide
2852cm^{-1}	C-OCH3 str.	methoxy

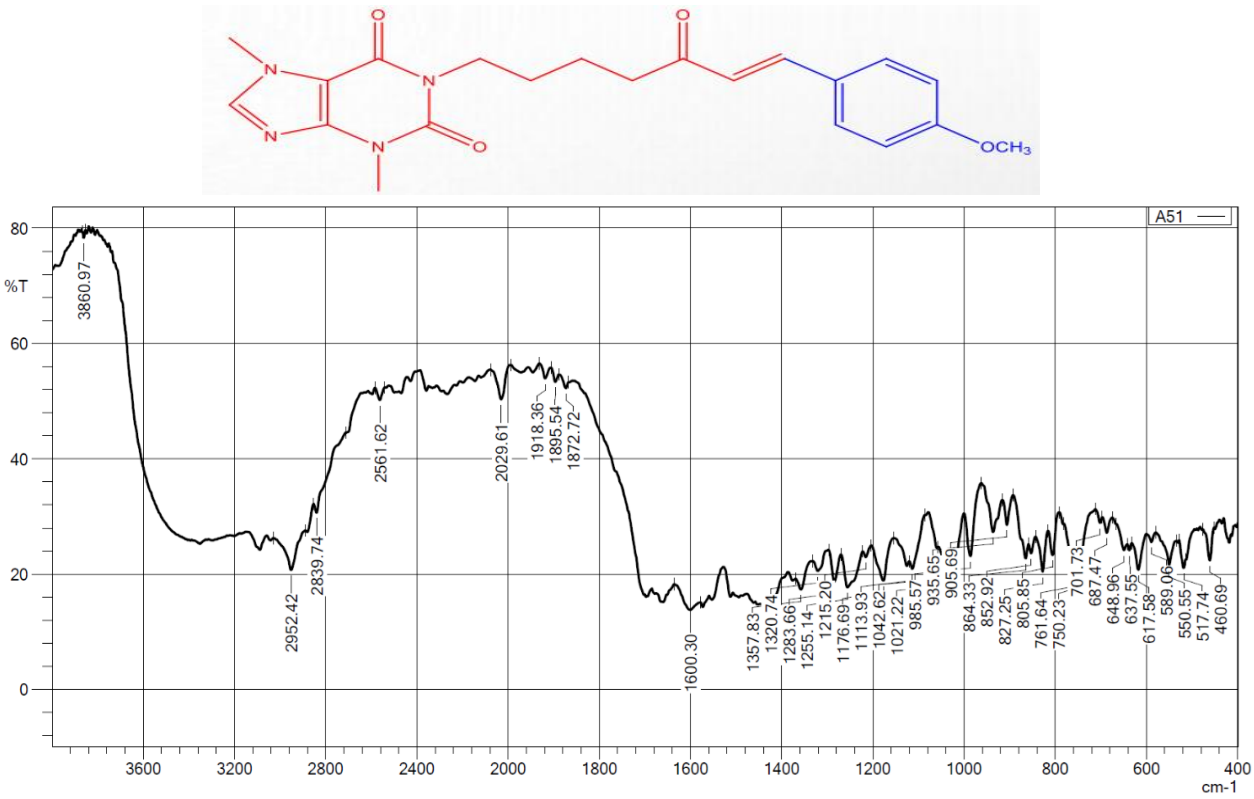


Figure 6: IR spectrum of C1 compound

Table 7: Spectral data of ^{1}HNMR of C1 compound

Signal Position (δ ppm)	Number of proton (s)	Multiplicity	Inference
1 .53 - 1 .77	4 H	Doublet	-CH 2 –
2 .92 , 5 .21	4 H	Singlet	-CH 2 –
3 .05	3 H	Singlet	-CH 3 of – N -CH 3
3 .81 , 3 .84	6 H	Singlet	-CH 3 -of – N -CH 3
7 .05 and 7 .29	2 H	Double anddoublet	C - H of Chalcone
7 .00 , 7 .51	4 H	Multiplate	aromatic ring
7 .57	1 H	Singlet	- C - H of C=N

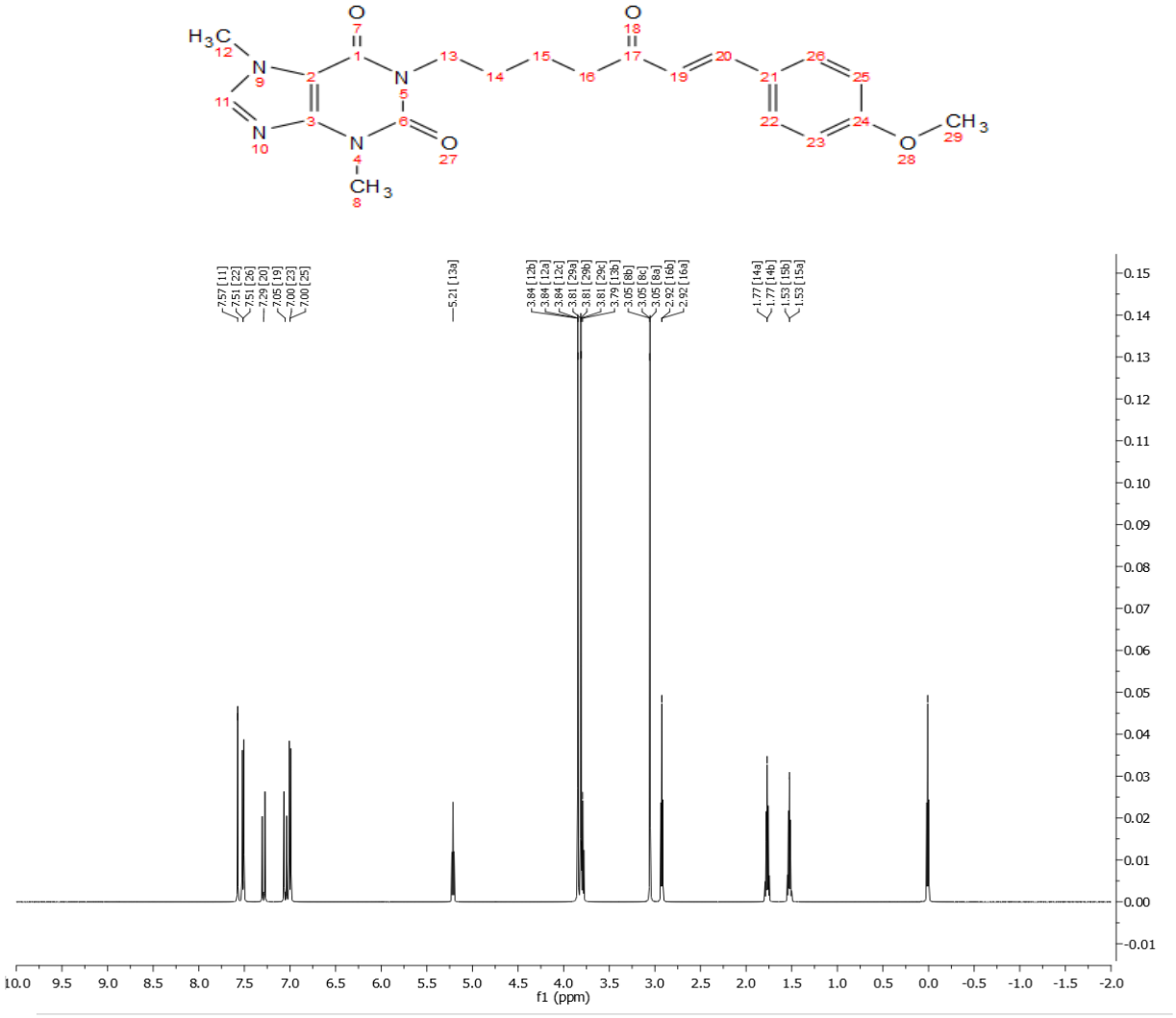


Figure 7: ^{1}HNMR spectrum of C1 compound

^{1}HNMR Spectroscopy

This table provides insights from ^{1}H NMR spectroscopy, presenting signal positions (in δ ppm), and the number of protons contributing to each signal, their multiplicity, and structural inferences. Signals in the range of 1.53-1.77 ppm with four protons are doublets, indicating the presence of -CH_2- groups. The signals at 2.92 and 5.21 ppm, each with four protons, are singlets, suggesting another -CH_2- group. The singlet at 3.05 ppm with three protons corresponds to the -CH_3 group of an N-CH_3

moiety. Two singlets at 3.81 and 3.84 ppm with six protons are indicative of -CH_3 groups from another N-CH_3. Signals at 7.05 and 7.29 ppm, each with two protons, are a doublet and a doublet, respectively, and represent the C-H groups of a chalcone structure. The aromatic ring is identified by signals at 7.00 and 7.51 ppm with four protons, displaying multiple patterns. Finally, the singlet at 7.57 ppm with one proton corresponds to the -C-H group of a C=N bond. This table aids in the structural elucidation of the compound by providing information on the number of protons, their multiplicity, and their chemical environments, allowing for the identification of specific functional groups and their positions within the molecule based on their unique chemical shifts in the ^{1}H NMR spectrum (Table 7 and Figure 7).

^{13}CNMR Spectroscopy

This table presents the results of ^{13}C NMR spectroscopy, displaying signal positions (in δ ppm) and their corresponding structural inferences. The signal positions at 23.74 and 28.15 ppm suggest the presence of methylene (-CH_2) groups at positions 15 and 14, while positions 31.55 and 34.96 ppm indicate N-methyl (-CH_3) groups at positions 8 and 12. The signals at 39.71 and 41.08 ppm correspond to non-methyl CH groups at positions 16 and 13, and 56.04 ppm signifies an oxygen-bound methyl group (O-CH_3) at position 29. The table also identifies characteristic signals for carbon-carbon double bonds (C=C) at 105.73 ppm and aromatic carbons at 114.57 ppm (positions 23 and 25) and between 128-129 ppm (positions 21, 22, and 26). Signals at 126.35 and 142 ppm suggest the presence of chalcone structures at positions 19 and 20. Lastly, signals at 147-147.5 ppm are indicative of nitrogen-carbon-nitrogen (N-C-N) linkages at positions 3 and 11, and positions 151, 153, and 203 ppm correspond to carbonyl groups (C=O) at positions 1, 6, and 17. This table provides critical information for the structural characterization of the compound, allowing for the identification of various functional groups and their positions in the molecule based on their unique chemical shifts in the ^{13}C NMR spectrum (Table 8 and Figure 8).

Biological activity

The table presents the results of antimicrobial susceptibility testing for various compounds (C1 and C2), ampicillin trihydrate, and fluconazole against different bacterial and fungal strains (Escherichia coli, Micrococcus luteus, Salmonella Typhi, Staphylococcus aureus) and Candida albicans. The values in the table represent the minimum inhibitory concentrations (M_{IC}) of these compounds required to inhibit the growth of the respective microorganisms. For example, C1 inhibits the growth of Escherichia coli at an M_{IC} of 640, Micrococcus luteus at an M_{IC} of 1280, and so on. These M_{IC} values indicate the relative effectiveness of each compound against the tested microorganisms, with lower values suggesting higher potency. Ampicillin trihydrate and fluconazole serve as reference antibiotics for comparison, with their M_{IC} values listed as well. Candida albicans is sensitive to fluconazole with an M_{IC} of 5, while the other compounds are not effective against it (Table 9 and Figure 9).

Table 8: Spectral data of ^{13}C NMR of C2 Compound

Signal Position (δ ppm)	position	Inference
23.74 and 28.15	15 and14	C -CH2
31.55 and 34.96	8 and 12	N -CH 3
39.71 and 41.08	16 and 13	CH
56.04	29	O -CH 3
105.73	2	C=C - N
114.57	23 and 25	Aromatic C
128 -129	21, 22 and 26	Aromatic C
126.35 and 142	19 and 20	Chalcone
147 -147.5	3 and 11	N - C - N
151,153,203	1, 6 and 17	C=O

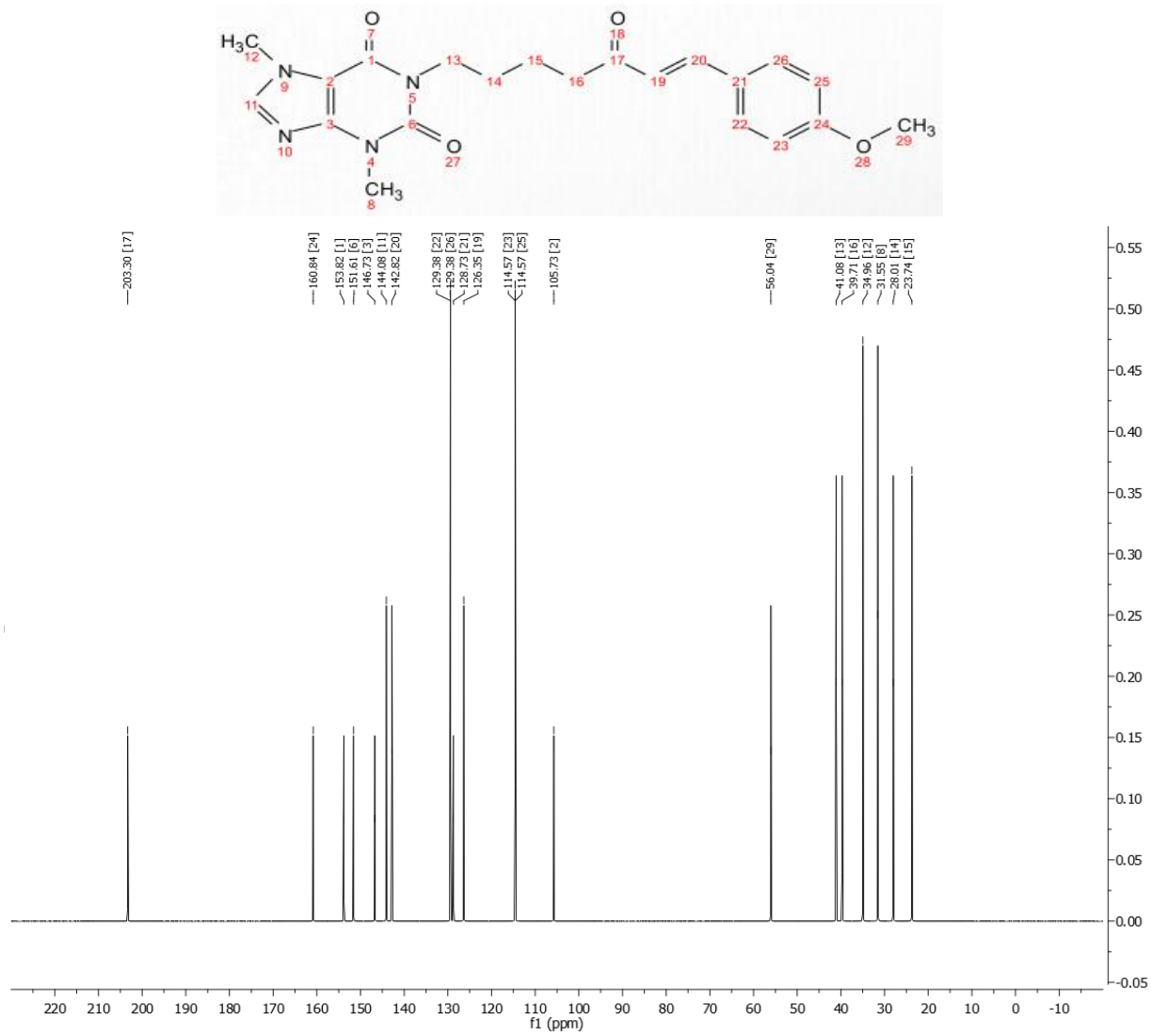


Figure 8: ^{13}C NMR spectrum of C2 Compound

Table 9: Minimal inhibitory concentration of biological activity

Compounds	***Escherichia coli* ATCC 4230**	***Micrococcus luteus* ATCC 9345**	***Salmonella Typhi* ATCC 14028**	***Staphylococcus aureus* ATCC 6538**	***Candida albicans* ATCC 14053**
C1	640	1280	160	320	80
C2	160	640	160	160	1280
Ampicillin trihydrate	20	10	10	10	-
Fluconazole	-	-	-	-	5
Fluconazole	-	-	-	-	5

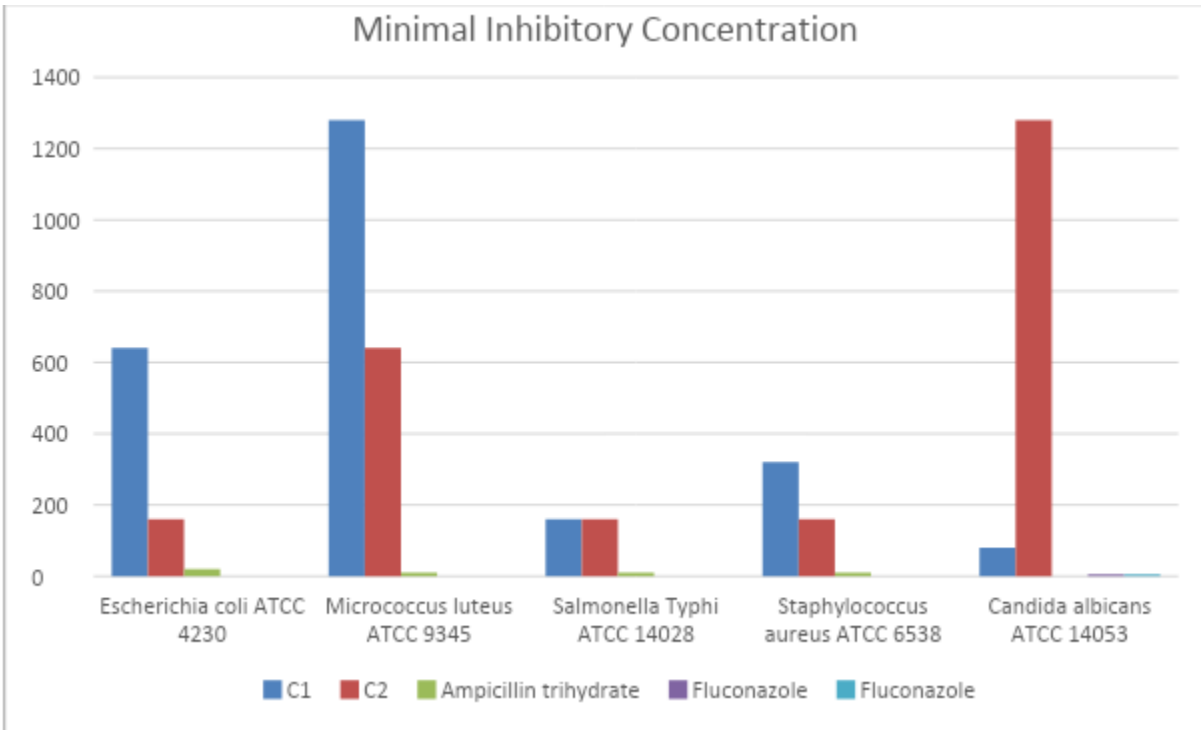


Figure 9: Minimal inhibitory concentration of biological activity

Conclusion

In conclusion, the articulation of newly designed purine Dione derivatives, with a specific focus on Pentoxifylline, has revealed promising prospects for medicinal interest. This research successfully synthesized and characterized these compounds, elucidating their structural attributes and potential pharmacological applications. The pharmacological evaluation against various microbial strains demonstrated their antimicrobial potential, highlighting their utility in combating infectious diseases. The outcomes of this study emphasize the significance of these newly designed purine Dione derivatives in medicinal chemistry and provide a foundation for further exploration in drug development. These findings underscore the potential of Pentoxifylline and related compounds as candidates for future therapeutic agents, underscoring their importance in the ever-evolving landscape of pharmaceutical research.

Abbreviations

Nil

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Author contributions

Conceptualization and Supervision – Dr. Hardik Bhatt, Dr. Gayatri Prasad.
Investigation, writing, and editing- Sanjay Bamaniya and Jitubhai Morabiya.
All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

This work was completely self-investigated and all generated data by self-diagnosis are from the Google Scholar literature survey Given that all the figures are drawn by the author, further no need for any approval.

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References

1. Petrucci R, Feroci M, Mattiello L, and Chiarotto I. Xanthine Scaffold: Available Synthesis Routes to Deliver Diversity by Derivatization. Mini-Reviews in Organic Chemistry. 2021; 18(1): 27-42.
2. Zaki RM, Kamal El-Dean AM, Radwan SM, Abd ul-Malik MA. Efficient synthesis, reactions, and biological activities of new thieno and furopyrazolo [3, 4-b] pyrazines and their related heterocycles. Journal of the Chinese Chemical Society. 2020 Apr;67(4):658-73.
3. Jan MS, Ahmad S, Hussain F, Ahmad A, Mahmood F, Rashid U, Ullah F, Ayaz M, Sadiq A. Design, synthesis, in-vitro, in-vivo and in-silico studies of pyrrolidine-2, 5-Dione derivatives as multitarget anti-inflammatory agents. European Journal of Medicinal Chemistry. 2020 Jan 15;186:111863.
4. Kapri A, Pant S, Gupta N, Nain S. Recent advances in the biological significance of xanthine and its derivatives: a review. Pharmaceutical Chemistry Journal. 2022 Jul;56(4):461-74.
5. Divekar K, Rekha S, Vedigounder M, Shivaprakash H. Synthesis, Characterization and Evaluation of some newer Pyrimidine derivatives as Anti-inflammatory Agents. Research Journal of Pharmacy and Technology. 2021;14(5):2529-34.
6. Yousaf M, Zahoor AF, Faiz S, Javed S, Irfan M. Recent synthetic approaches towards biologically potent derivatives/analogues of theophylline. Journal of Heterocyclic Chemistry. 2018 Nov;55(11):2447-79.
7. Faghih Z, Emami L, Zomoridian K, Sabet R, Bargebid R, Mansourian A, Zeinali B, Rostami Z, Khabnadideh S. Aryloxy Alkyl theophylline derivatives as antifungal agents: design, synthesis, biological evaluation and computational studies. Chemistry Select. 2022 Jul 7;7(25):e202201618.
8. Gumber D, Yadav D, Yadav R, Kachler S, Klotz KN. Bronchospasmolytic activity and adenosine receptor binding of some newer 1, 3-dipropyl-8-phenyl substituted xanthine derivatives. Chemical biology & drug design. 2020 Jun;95(6):600-9.
9. Chandrasekaran B, Samarneh S, Jaber AM, Kassab G, Agrawal N. Therapeutic potentials of A2B adenosine receptor ligands: current status and perspectives. Current Pharmaceutical Design. 2019 Jul 1;25(25):2741-71.
10. Müller CE, Baqi Y, Hinz S, Namasivayam V. Medicinal chemistry of A 2B adenosine receptors. The adenosine receptors. 2018:137-68.
11. Li S, Yuan S, Zhao Q, Wang B, Wang X, Li K. Quercetin enhances chemotherapeutic effect of doxorubicin against human breast cancer cells while reducing toxic side effects of it. Biomedicine & Pharmacotherapy. 2018 Apr 1;100:441-7.
12. Peng T, Gong J, Jin Y, Zhou Y, Tong R, Wei X, Bai L, Shi J. Inhibitors of phosphodiesterase as cancer therapeutics. European journal of medicinal chemistry. 2018 Apr 25;150:742-56.
13. Seenappa V, Joshi MB, Satyamoorthy K. Intricate regulation of phosphoenolpyruvate carboxykinase (PEPCK) isoforms in normal physiology and disease. Current Molecular Medicine. 2019 May 1;19(4):247-72.
14. Jana R, Begam HM, Dinda E. The emergence of the C–H functionalization strategy in medicinal chemistry and drug discovery. Chemical Communications. 2021;57(83):10842-66.
15. Peng P, Chen H, Zhu Y, Wang Z, Li J, Luo RH, Wang J, Chen L, Yang LM, Jiang H, Xie X. Structure-based design of 1-heteroaryl-1, 3-propanediamine derivatives as a novel series of CC-chemokine receptor 5 antagonists. Journal of medicinal chemistry. 2018 Sep 20;61(21):9621-36.
16. Betti M, Catarzi D, Varano F, Falsini M, Varani K, Vincenzi F, Dal Ben D, Lambertucci C, Colotta V. The aminopyridine-3, 5-dicarbonitrile core for the design of new non-nucleoside-like agonists of the human adenosine A2B receptor. European Journal of Medicinal Chemistry. 2018 Apr 25;150:127-39.
17. van Rensburg HD, Legoabe LJ, Terre'Blanche G, Aucamp J. Synthesis and evaluation of methoxy substituted 2-benzoyl-1-benzofuran derivatives as lead compounds for the development adenosine A1 and/or A2A receptor antagonists. Bioorganic Chemistry. 2020 Jan 1;94:103459.
18. Vincenzi F, Pasquini S, Borea PA, Varani K. Targeting adenosine receptors: a potential pharmacological avenue for acute and chronic pain. International Journal of Molecular Sciences. 2020 Nov 18;21(22):8710.
19. Mohamed AR, El Kerdawy AM, George RF, Georgey HH, Gawad NM. Design, synthesis and in silico insights of new 7, 8-disubstituted-1, 3-dimethyl-1H-purine-2, 6 (3H, 7H)-Dione derivatives with potent anticancer and multi-kinase inhibitory activities. Bioorganic Chemistry. 2021 Feb 1;107:104569.
20. Khaliullin F, Shabalina Y. Thietanyl Protection in the Synthesis of 8-Substituted 1-Benzyl-3-methyl-3, 7-dihydro-1H-purine-2, 6-Diones. Current Organic Synthesis. 2020 Nov 1;17(7):535-9.
21. Kapadiya K, Kavadia K, Gohel J, Khunt R. Regioselective synthesis of triazolo [3, 4-e] purine derivatives and their anti-cancer activity against NCI-60 cell-lines. Folia Medica. 2021 Apr 30;63(2):213-20.
22. Shi Z, Tian L, Qiang T, Li J, Xing Y, Ren X, Liu C, Liang C. From structure modification to drug launch: A systematic review of the ongoing development of cyclin-dependent kinase inhibitors for multiple cancer therapy. Journal of Medicinal Chemistry. 2022 Apr 29;65(9):6390-418.
23. Monteiro J, Alves MG, Oliveira PF, Silva BM. Pharmacological potential of methylxanthines: Retrospective analysis and future expectations. Critical reviews in food science and nutrition. 2019 Sep 8;59(16):2597-625.
24. Singh N, Shreshtha AK, Thakur MS, Patra S. Xanthine scaffold: scope and potential in drug development. Heliyon. 2018 Oct 1;4(10).
25. Peng J, Li Y, Zhou Y, Zhang L, Liu X, Zuo Z. Pharmacophore modeling, molecular docking and

molecular dynamics studies on natural products database to discover novel skeleton as non-purine xanthine oxidase inhibitors. Journal of Receptors and Signal Transduction. 2018 May 4;38(3):246-55.
26. Donate-Correa J, Sanchez-Niño MD, González-Luis A, Ferri C, Martín-Olivera A, Martín-Núñez E, Fernandez-Fernandez B, Tagua VG, Mora-Fernández C, Ortiz A, Navarro-González JF. Repurposing drugs for highly prevalent diseases: pentoxifylline, an old drug and a new opportunity for diabetic kidney disease. Clinical Kidney Journal. 2022 Dec;15(12):2200-13.
27. Donate-Correa J, Tagua VG, Ferri C, Martín-Núñez E, Hernández-Carballo C, Ureña-Torres P, Ruiz-Ortega M, Ortiz A, Mora-Fernández C, Navarro-González JF. Pentoxifylline for renal protection in diabetic kidney disease. A model of old drugs for new horizons. Journal of clinical medicine. 2019 Feb 27;8(3):287.
28. HANNA JS, KHAN AK, ESSA HJ. Synthesis, Molecular Docking, and Cytotoxic Evaluation of Some Novel 1H-Pyrazole Derivatives from Pentoxifylline. International Journal of Pharmaceutical Research (09752366). 2020 Apr 1;12(2).
29. Abou-Zied, HA, Youssif BG, Mohamed MF, Hayallah AM, and Abdel-Aziz M. EGFR inhibitors and apoptotic inducers: Design, synthesis, anticancer activity and docking studies of novel xanthine derivatives carrying chalcone moiety as hybrid molecules. Bioorganic chemistry. 2019;89:102997.
30. Yu Y, Xu S, You H, Zhang Y, Yang B, Sun X, Yang L, Chen Y, Fu S, Wu J. In vivo synergistic anti-tumor effect of paclitaxel nanoparticles combined with radiotherapy on human cervical carcinoma. Drug Delivery. 2017 Jan 1;24(1):75-82.
31. El-Kalyoubi SA, Gomaa HA, Abdelhafez EM, Ramadan M, Agili F, Youssif BG. Design, Synthesis, and Anti-Proliferative Action of Purine/Pteridine-Based Derivatives as Dual Inhibitors of EGFR and BRAFV600E. Pharmaceuticals. 2023 May 8;16(5):716.
32. Nada H, Elkamhawy A, Lee K. Identification of 1H-purine-2, 6-Dione derivative as a potential SARS-CoV-2 main protease inhibitor: molecular docking, dynamic simulations, and energy calculations. PeerJ. 2022 Oct 7;10:e14120.
33. Zygmunt M, Ślusarczyk M, Jankowska A, Świerczek A, Bryła A, Mogilski S, Kazek G, Sapa J, Wyska E, Chłoń-Rzepa G. Evaluation of analgesic and anti-inflammatory activity of purine-2, 6-Dione-based TRPA1 antagonists with PDE4/7 inhibitory activity. Pharmacological Reports. 2022 Oct;74(5):982-97.
34. Wójcik-Pszczoła K, Chłoń-Rzepa G, Jankowska A, Ellen E, Świerczek A, Pociecha K, Koczurkiewicz P, Piska K, Gawędzka A, Wyska E, Knapik-Czajka M. Novel phosphodiesterases inhibitors from the group of purine-2, 6-Dione derivatives as potent modulators of airway smooth muscle cell remodelling. European Journal of Pharmacology. 2019 Dec 15;865:172779.
35. Partyka A, Zagórska A, Kotańska M, Walczak M, Jastrzębska-Więsek M, Knutelska J, Bednarski M, Głuch-Lutwin M, Mordyl B, Janiszewska P, Wesołowska A. Antidepressant-like activity and safety profile evaluation of 1 H-imidazo [2, 1-f] purine-2, 4 (3H, 8H)-Dione derivatives as 5-HT1A receptor partial agonists. Plos one. 2020 Aug 7;15(8):e0237196.
36. Chłoń-Rzepa G, Jankowska A, Ślusarczyk M, Świerczek A, Pociecha K, Wyska E, Bucki A, Gawalska A, Kołaczkowski M, Pawłowski M. Novel butanehydrazide derivatives of purine-2, 6-Dione as dual PDE4/7 inhibitors with potential anti-inflammatory activity: design, synthesis and biological evaluation. European journal of medicinal chemistry. 2018 Feb 25;146:381-94.
37. Arjmand F, Afsan Z, Sharma S, Parveen S, Yousuf I, Sartaj S, Siddique HR, Tabassum S. Recent advances in metallodrug-like molecules targeting non-coding RNAs in cancer chemotherapy. Coordination Chemistry Reviews. 2019 May 15;387:47-59.
38. Osifová Z, Šála M, Dračínský M. Hydrogen-Bonding Interactions of 8-Substituted Purine Derivatives. ACS omega. 2023 Jul 1;8(28):25538-48.
39. Shukla R, Bandopadhyay P, Sathe M, Chopra D. Quantitative investigation on the intermolecular interactions present in 8-(4-ethoxyphenyl)-1, 3-dimethyl-3, 7-dihydro-1H-purine-2, 6-Dione with insight from interaction energies, energy framework, electrostatic potential map and fingerprint analysis. Journal of Chemical Sciences. 2020 Dec;132:1-7.
40. Kansız S, Azam M, Dege N, Ermiş N, Al-Resayes SI, Alam M. Supramolecular assembly in designing co-crystals of fumaric acid and pyrimidine/picolinate derivatives. Green Chemistry Letters and Reviews. 2022 Jul 3;15(3):825-36.
41. Eissa IH, Yousef RG, Elkaeed EB, Alsfouk AA, Husein DZ, Ibrahim IM, Alesawy MS, Elkady H, Metwaly AM. Anticancer derivative of the natural alkaloid, theobromine, inhibiting EGFR protein: Computer-aided drug discovery approach. Plos one. 2023 Mar 9;18(3):e0282586.
42. Narwal S, Kumar S, Verma PK. Synthesis and biological activity of new chalcone scaffolds as prospective antimicrobial agents. Research on Chemical Intermediates. 2021 Apr;47(4):1625-41.
43. Govekar A, Sonal SG. Determination of Minimum Inhibitory Concentration by Broth Dilution Method-A Review. BTRA Scan. 2022 Apr 1;51(2).
44. Siddiqa A, Tajammal A, Irfan A, Azam M, Munawar MA, Hardy RS, Basra MA. Synthesis, molecular docking, bio-evaluation and quantitative structure activity relationship of new chalcone derivatives as antioxidants. Journal of Molecular Structure. 2023 Apr 5;1277:134814.
45. Li Y, Sun B, Zhai J, Fu L, Zhang S, Zhang J, Liu H, Xie W, Deng H, Chen Z, Sang F. Synthesis and antibacterial activity of four natural chalcones and their derivatives. Tetrahedron Letters. 2019 Oct 24;60(43):151165.
46. MuĞlu H, Yakan H, Bakir TK. Synthesis, spectroscopic studies, and antioxidant activities of novelthio/carbohydrazones and bis-isatin

derivatives from terephthalaldehyde. Turkish Journal of Chemistry. 2020;44(1):237-48.
47. Chen S, Zhang M, Feng S, Gong C, Zhou Y, Xing L, He B, Wu Y, Xue W. Design, synthesis and biological activity of chalcone derivatives containing pyridazine. Arabian Journal of Chemistry. 2023 Jul 1;16(7):104852.
48. Benouda H, Bouchal B, Challioui A, Oulmidi A, Harit T, Malek F, Riahi A, Bellaoui M, Bouammali B. Synthesis of a series of chalcones and related flavones and evaluation of their antibacterial and antifungal activities. Letters in Drug Design & Discovery. 2019 Jan 1;16(1):93-100.
49. Kumar V, Dhawan S, Girase PS, Awolade P, Shinde SR, Karpoormath R, Singh P. Recent advances in chalcone-based anticancer heterocycles: A structural and molecular target perspective. Current Medicinal Chemistry. 2021 Oct 1;28(33):6805-45.
50. Pai A, Jayashree BS. Design, synthesis and biological evaluation of novel piperidinyl chalcones. Indian Journal of Pharmaceutical Education and Research. 2019 Jul 1;53(3):S313-24.