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A Study of Synthesis, Characterization and Biological Evaluation of Pyrazolyl Diaryl Cyclohexenone Derivatives

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Abstract - Pyrazolyldiarylcyclohexenone derivatives have garnered significant attention in recent years due to their potential biological activities and diverse pharmacological properties. In this study, we focused on the synthesis and characterization of a series of novel pyrazolyldiarylcyclohexenone derivatives. The compounds were synthesized using well-established organic chemistry methods, and their structures were confirmed through various spectroscopic techniques such as NMR, IR, and mass spectrometry. The biological evaluation of these derivatives was conducted through a range of in vitro and in vivo experiments. The assessment included studies on antimicrobial activity against various pathogenic strains, antioxidant potential, anti-inflammatory effects, and cytotoxicity against cancer cell lines. Additionally, we investigated their potential as enzyme inhibitors, which could offer therapeutic possibilities for specific diseases.

Keyword - Pyrazolyl ,derivatives

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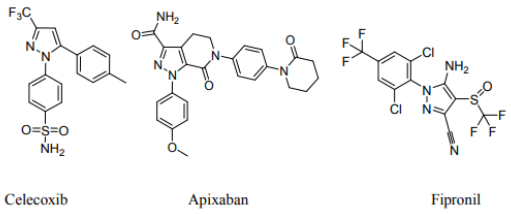
INTRODUCTION

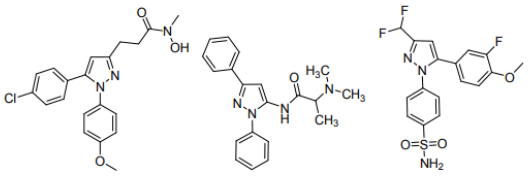
The synthesis, characterization, and biological evaluation of pyrazolicchalcone, pyrazolylpyrazoline, and pyrazolyldiarylcyclohexanone derivatives are important areas of research in medicinal chemistry. ,Pyrazolicchalcones are a class of compounds that have been shown to exhibit a range of biological activities, including anti-inflammatory, antioxidant, and antimicrobial properties. These compounds are typically synthesized by the condensation of pyrazole with a carbonyl compound, such as an aldehyde or ketone, in the presence of a base catalyst. Pyrazolylpyrazolines are a class of compounds that are formed by the cyclization of pyrazole with an enone, such as chalcone. These compounds have been shown to exhibit a variety of biological activities, including anticancer, antitumor, and antifungal properties. Pyrazolyldiarylcyclohexanones are a class of compounds that are formed by the reaction of pyrazole with diarylcyclohexanones.

Pyrazoles

The heterocyclic chemical pyrazole consists of two neighboring nitrogen atoms in a five-membered ring structure. Heterocyclic compounds linked to pyrazole showed a wide range of chemotherapeutic activity. The biological effects they exhibited ranged from anti-inflammatory to anti-microbial to anti-malarial to anti-hypertensive to anti-tubercular to anti-viral to neuroprotective to anti-depressant to anti-cancer. Some pyrazole compounds have been shown to be a valuable entity in the creation of effective chemotherapeutic medicines, while others have found usage in clinical settings.



Celecoxib Apixaban Fipronil



Tepoxalin Difenamizole Deracoxib

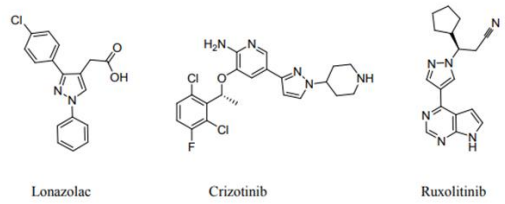
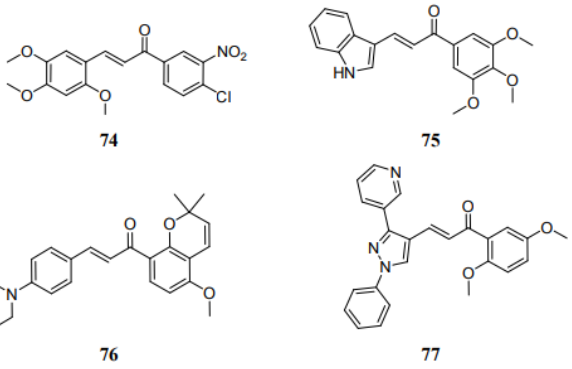


Figure 1:Pyrazole based drugs

Derivatives of PyrazolicChalcone

The promise of chalcone derivatives as chemotherapeutics has led to their study in large numbers, both naturally occurring and synthetically produced. They exhibited biological features such as those against bacteria, fungi, protozoa, malaria, leishmania, cancer, inflammation, cell division, invasion, and so on. Analogue 74 had an IC50 of 15.36 M against the MCF-7 cancer cell line. Indolylchalcone 75 was shown to be cytotoxic to the PaCa-2 cancer cell line, with an IC50 of 0.03 µM. The cytotoxicity of pyranochalcone 76 against HT-29 was determined to be 0.55 µM. The pyrazolicchalcone 77, as shown in recent research by Hawash et al., is cytotoxic to Huh7 cells with an IC50 value of 0.03 µM.



The remarkable complementarity between the anticancer activity profiles of pyrazole and chalcone inspired us to attempt the synthesis of pyrazolicchalcones. Several cell lines, including cancerous Caco-2, MIA PaCa-2, and MCF-7 cells and normal NIH-3T3 cells, were tested for cytotoxicity in vitro.

LITERATURE REVIEW

Strocchi E (2012) Herein, we detailed the use of molecular modeling in the optimization of tiny pyrazole derivatives produced by 1,3-dipolar cycloaddition of nitrile imines and functionalized acetylenes for use as kinase inhibitors. We tested the two compounds in vitro for their biological activity on cell lines developed from HCC to determine their potential as anti-HCC agents. The compounds are attractive lead compounds for future SAR studies due to their inhibitory growth effectiveness (IC(50) 50-100 M) in the SNU449 cell line, as well as their ability to halt cell cycle progression and induce apoptosis.

Wang G, (2014) Theantiproliferative effects of a newly synthesized series of pyranochalcone derivatives (3-42, 49a-49r) with an indole moiety were studied. Compound 49b, which contains a propionyloxy group at the 4-position of the left phenyl ring and an N-methyl-5-indoly group at the 5-position of the right phenyl ring, showed the highest cytotoxic activity against all tested cancer cell lines, including those with a multidrug-resistant phenotype, with IC50 values ranging from 0.22 to 1.80M. Additionally, 49b dramatically decreased tubulin polymerization and triggered cell cycle arrest in the G2/M phase. 49b's interaction with the tubulin colchicine binding site was confirmed by molecular docking. 49b showed strong anticancer efficacy against HepG2 human liver carcinoma in BALB/c nude mice in in vivo tests. These findings suggested that these chemicals are effective tubulin polymerization inhibitors, which might be used to treat cancer.

Hawash MM, (2017) Sorafenib is the only FDA-approved chemotherapeutic treatment now available for liver cancer patients, however it can only extend life by a few months at most. Liver cancer has the second-highest fatality rate of any malignancy. In this research, many compounds with structural similarities to pyrazolicchalcones were synthesized and tested for their efficacy as chemotherapeutic agents against hepatocellular carcinoma (HCC). Fourty-two alternative compounds were obtained by replacing the pyrazole moiety at C(4) with a chalcone moiety of varying substitution patterns and by modifying the pyrazole ring at C(3) with a variety of heteroaryl rings. The cytotoxicity of each of these substances was tested using the sulforhodamine B assay and with real-time cell growth monitoring. Compounds 39, 42, 49, and 52 were shown to have much higher cytotoxic effects than the standard chemotherapeutic medication 5-FU, as measured by their 50% inhibitory concentration (IC50) values, against all of the cancer cell lines examined. As a result, we decided to test these chemicals in a variety of HCC cell lines. Cell growth was inhibited and the cell cycle was arrested in HCC cells treated with chemicals 39, 42, 49, and 52, as determined by flow cytometric analysis and real-time cell growth monitoring. Consistent with these findings, western blotting of HCC cells treated with the chemicals revealed molecular alterations for cell cycle proteins. Specifically, p21 levels were found to rise independently of p53, while the levels of the major initiators of mitosis, Cyclin B1 and CDK1, were shown to decrease. In conclusion, chalcone derivatives 42 and 52 have strong bioactivities, as shown by their ability to modulate the expression of cell-cycle-related proteins and their subsequent ability to cause cell-cycle arrest in the HCC cell lines evaluated here.

Harras MF (2018) Three distinct cancer cell lines, HCT116, UO-31, and HepG2, were used to test the cytotoxic activities of a series of newly synthesized 1,3,4-trisubstituted pyrazole derivatives. The cytotoxic activities of compounds 3b, 3d, 7b, and 9 were superior to those of the reference medication Sorafenib in all of the cancer cell lines evaluated. As a result, we decided to test these chemicals in a variety of HCC cell lines. The most effective

chemicals against HCC cells here were 3b and 7b. In addition to causing a cell cycle arrest in the G2/M phase, more research into the mechanism revealed that 3b and 7b promoted apoptosis in HepG2 and Huh7 cells. Caspase-3 assays were performed, and the findings were consistent with the idea that the target chemicals' pro-apoptotic action was triggered by caspases-3. Furthermore, a CDK1 inhibition experiment was carried out, yielding IC50 values of 2.38 and 1.52 M for compounds 3b and 7b, respectively. Finally, the powerful bioactivities of the pyrazole derivatives 3b and 7b suggest that these compounds have great potential as future anticancer medicines.

Mallia A (2020) When it comes to building heterocyclic compounds with therapeutic or industrial applications, chalcones continue to be held in high esteem. Preparative procedures for this useful class of compounds have benefited greatly from the development of synthetic routes for hybrid chalcones incorporating heteroaromatic components, particularly those based on green chemistry principles. Incorporating heteroaromatic components into hybrid chalcone construction has advanced in recent decades, and this paper details those developments while also highlighting ecologically friendly procedures used in their manufacture.

Mohamed MF (2020) Lung cancer patients may benefit from a new chalcone series that has been created. The new series was validated using a variety of spectral analysis methods. The new chalcones' cytotoxic impact on the lung cancer cell line (A549) was measured using the MTT test. The two most efficient chalcones, 7b and 7c, were the subject of molecular docking research. Two chalcones, 7b and 7c, were investigated for their activity and influence on apoptosis of the A549 cell line using a variety of molecular methods.

METHODOLOGY

We utilised industrial grade reagents and solvents readily accessible to the public. Electro-thermal melting point apparatus was used to test the melting points in open capillaries, and the findings were not adjusted for temperature. Thin-layer chromatography (TLC) was used to examine the results of the reactions.Aluminium sheets that had been precoated with TLC (Silica gel 60 F254, Merck Germany) showed spots when exposed to ultraviolet light. The Agilent Cary 630 FT-IR spectrometer was used to collect the data. We will acquire 1 H and 13C NMR spectra in CDCl3 or DMSO-d6 using trimethylsilane (TMS) as an internal standard using a BrukerAvance 300/400 MHz and a BrukerAvance 75/100/125 MHz, Jeol 100 MHzspectrometer, respectively. The letters S, D, DD, T, and M indicate a single, double, triple, and multiple split, respectively. The ppm notation will be used to denote chemical shift values. The elemental analyzer ElementarVario (Vario EL-III) was used. For mass spectral recording, we utilised an Applied Biosystems AB-Sciex 2000 ESI-MS.

- **MTT Assay**

To determine cell viability, the MTT test uses the fact that certain live cells may change a yellow tetrazolium dye into a purple, water-insoluble formazan. Formazan crystal formation is strongly correlated with cell viability.Cells were cultured in a 96-well plate in a water-bathed incubator at 37 degrees Celsius (5% carbon dioxide) using DMEM medium supplemented with 10% foetal bovine serum (FBS). More and more DMSO-dissolved chemicals were deposited on the plate. Tissue culture plates with 96 wells will be dosed twice with these concentrations. After 24 hours of treatment, cell viability was determined using the MTT test. After washing away the medium, a final concentration of 10 l of MTT reagent (5 mg/ml) will be added to each well. The MTT test is what you're looking for. The reagent is taken out and the crystals are dissolved in dimethyl sulfoxide (DMSO) after a short incubation (at 37 degrees Celsius and 5% carbon dioxide). Adding DMSO to phenol red-containing tissue culture media changes the colour of the medium to yellow, but has no impact on the MTT formazan assay. Formazan dissolved in DMSO produces a uniformly blue solution. An ELISA plate reader integrated into a microplate reader (model LMR-340 M; Labexim International, Austria/Tecan, Switzerland) will be used to measure the absorbance at 570 nm.To determine the level of inhibition, apply the following formula (1):

$$\% \text{ Inhibition} = 100 - \frac{\text{Mean OD of treated cells}}{\text{Mean OD of the vehicle control cells (negative control)}} \times 100$$

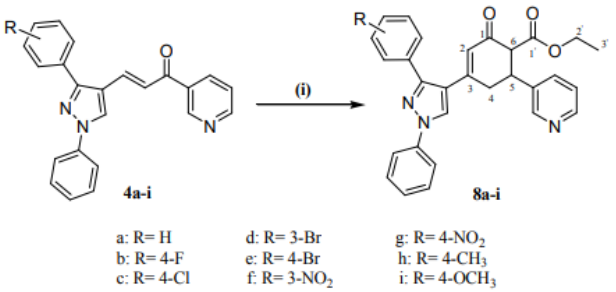
Each sample went through three different tests. The dose-response curve was used to determine the IC50 concentration, which was then published. (µM) to medication

DATA ANALYSIS

The chalcone-derived cyclic compounds known as di-aryl cyclohexenone derivatives are widely used for their antimicrobial properties. Our synthesis of pyrazolicchalcones 4a-i led us to the discovery of di-aryl cyclohexenone derivatives 8a-i, which we used in our study of chalcones' anticancer effects. The MCF-7, NCI-H460, HeLa, and HEK-293T human cancer cell lines were used as test subjects for the pharmacological activity and cytotoxicity of all synthesised compounds.

Chemistry

To get di-aryl cyclohexenone derivatives 8a-i, pyrazolicchalcones 4a-i were treated with ethylacetoacetate in ethanolic KOH. Scheme showing the synthesis of the intended compounds.



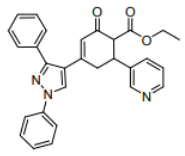
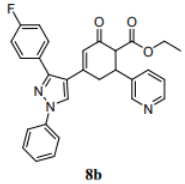
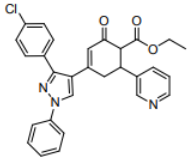
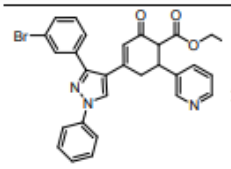
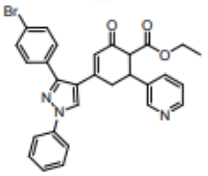
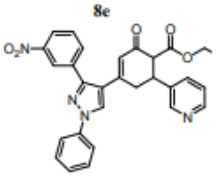
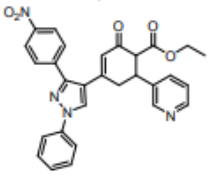
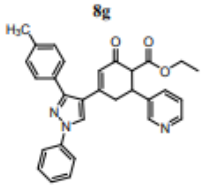
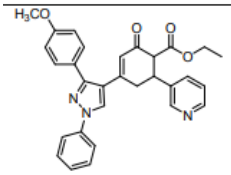
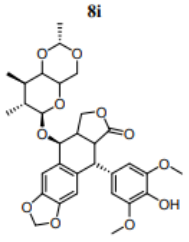
Scheme1: Synthesis of pyrazolyl di-aryl cyclohexenone derivatives: standard operating procedure (i) ethylacetoacetate, potassium hydroxide, ethyl alcohol, room temperature, and then 70 degrees Celsius.

Biology

To ascertain the cytotoxicity of all heterocyclic derivatives 8a-i, in vitro MTT experiments were carried out on a panel of three human cancer cell lines (MCF-7 (human breast), NCI-H460 (human lung), HeLa (human cervix), and HEK-293T (human embryonic kidney cell) normal cell line). The M concentrations of the substances were utilised to calculate their IC50 values. The IC50 values are shown in Table 4.1 using etoposide as the reference chemical. According to their IC50 values, the majority of these substances shown moderate to significant cytotoxicity against the human cancer cell lines NCI-H460, HeLa, and MCF-7, but only minor toxicity towards the HEK-293T cell line.

Given the medical standard of today Analogues 8d, 8e, 8f, and 8g have considerably enhanced cytotoxic effects as compared to etoposide.

Table1:When tested in vitro for cytotoxicity against four human cell lines (3 malignant and 1 normal), the IC50 values (in microM) for compounds 8a-i and etoposide are as follows:

Compounds	MCF-7	NCI-H460	Hela	Hek-293T
 8a	41.38±0.90	55.30±1.20	44.54±2.16	144.22±4.73
 8b	32.47±0.52	40.52±2.06	17.29±1.12	142.98±1.83
 8c	35.40±1.26	48.98±1.03	20.11±1.72	83.35±4.58
 8d	**14.31±0.90**	**8.55±0.35**	**7.01±0.60**	124.35±3.74
 8e	14.75±0.45	26.86±1.45	8.10±0.99	120.65±1.33
 8f	21.55±2.32	9.34±0.69	16.99±0.51	143.52±4.69
 8g	14.45±0.51	10.19±0.67	8.05±0.77	104.64±3.95
 8h	55.79±1.59	60.60±2.78	47.99±2.00	120.47±1.86
 8i	28.55±1.02	36.85±0.81	33.04±2.70	92.33±5.53
 Etoposide	32.50±0.67	16.01±2.02	11.43±0.35	12.32±0.99

Structure-activity relationship (SAR) studies were carried out to ascertain the impact of various substituents on the cytotoxic effects of the synthesised compounds. The most dangerous of the compounds tested was 8d, which has a Meta substituted Br group on the benzene ring. Nitrogen dioxide (NO2) substitutes in the benzene ring of metabolites 8f and 8g were efficient. Compounds 8d and 8f, both of which include a Meta substituted

electron-withdrawing group, are first in the cytotoxic action order for cancer cell types.

CONCLUSION

Our studies have centered on the design of new heterocyclic anticancer chemicals that are linked to pyrazoles. The pyrazolicchalcones 4a-i have been synthesized, characterized, and tested for cytotoxicity in vitro. All of the substances showed low to moderate toxicity towards normal cells while having moderate to high cytotoxic effects against the cancer cell lines evaluated. Among the synthesized compounds tested for cytotoxicity, the analogues 4f, 4g, and 4h stood out in comparison to the gold standard medication etoposide.

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