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Research Article

Synthesis Of 3-Pyrazolyl 2-Acetoxybenzamide & Evaluation of its Antiinflammatory Activity

Nazish Farhan*, Dr. Ashish Sarkar, Gahna Kumari

School of Pharmacy, YBN University, Ranchi, Jharkhand

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, are widely used for their analgesic, antipyretic, and anti-inflammatory effects. However, aspirin's therapeutic use is often limited due to its gastrointestinal side effects, primarily caused by cyclooxygenase-1 (COX-1) inhibition and direct mucosal irritation. This study aimed to synthesize and evaluate a novel aspirin derivative, 3-pyrazolyl-2-acetoxybenzamide, which incorporates a pyrazole moiety and replaces the carboxyl (-COOH) group with an amide (-CONH) group to enhance anti-inflammatory activity while reducing ulcerogenic effects. The synthesis was carried out via the reaction of chloroacetylsalicylic acid with pyrazole in the presence of triethylamine, yielding a structurally modified aspirin analog. Structural characterization was performed using FT-IR spectroscopy, confirming the formation of key functional groups. The anti-inflammatory potential and gastric safety profile of 3-pyrazolyl-2-acetoxybenzamide were assessed using Wistar rats, with ulcer index scoring, COX-1 expression analysis, and prostaglandin E_2 (PGE_2) measurement. Results indicated that the novel compound significantly reduced gastric mucosal damage compared to aspirin (ulcer index: 3.5 vs. 11.2 for aspirin), maintained COX-1 expression, and preserved PGE_2 levels, highlighting its superior gastric safety. The findings suggest that 3-pyrazolyl-2-acetoxybenzamide could serve as a safer alternative to aspirin with promising anti-inflammatory properties. Further studies, including clinical trials, are warranted to establish its therapeutic potential.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications globally for relieving pain, reducing fever, and treating inflammation [1]. Aspirin is used for the prevention and treatment of thromboembolic diseases [2-3]. Research indicates that it can help

***Corresponding Author:** Nazish Farhan
Address: *School of Pharmacy, YBN University, Ranchi, Jharkhand*
Email : Ansarigrd100@gmail.com
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in treating and preventing various cancers, including breast cancer, colon cancer, and rectal carcinoma [4]. As a result, its applications have expanded. However, despite its undeniable effectiveness, Aspirin is linked to significant gastrointestinal mucosal damage [5]. Several factors contribute to these side effects. Firstly, Aspirin inhibits cyclooxygenase-1 (COX-1), an enzyme predominantly found in gastric epithelial cells that play a key role in prostaglandin synthesis [6]. Prostaglandins are essential for protecting the gastric mucosa [7]. Secondly, the free carboxyl group in Aspirin causes direct irritation to the gastric lining. The aim of this study is to synthesize new anti-inflammatory derivatives of aspirin with potential selective COX-2 inhibition and reduced ulcerogenic effects, based on drug development. Modifying the carboxyl group of these drugs into a carboxamide group and conjugating them with specifically selected heterocyclic moieties may enhance their potential as selective COX-2 inhibitors with reduced side effects. These conjugates could make aspirin structurally similar to the isosteric functional groups found in previous Coxibs and their derivatives, which are known for their selective COX-2 inhibition. The heterocyclic moiety that I introduce is Pyrazole. The pyrazole ring is a five-membered heterocyclic structure containing two adjacent nitrogen atoms. This moiety is present in numerous compounds with diverse applications. Moreover, both naturally occurring pyrazoles and their synthetic derivatives are widely recognized for their broad range of biological activities including analgesic, antipyretic, anticancer, antiviral, anti-inflammatory, antioxidant, antimicrobial, antidiabetic, anticonvulsant, and antiarrhythmic properties.

MATERIAL AND METHODS

Chemicals:

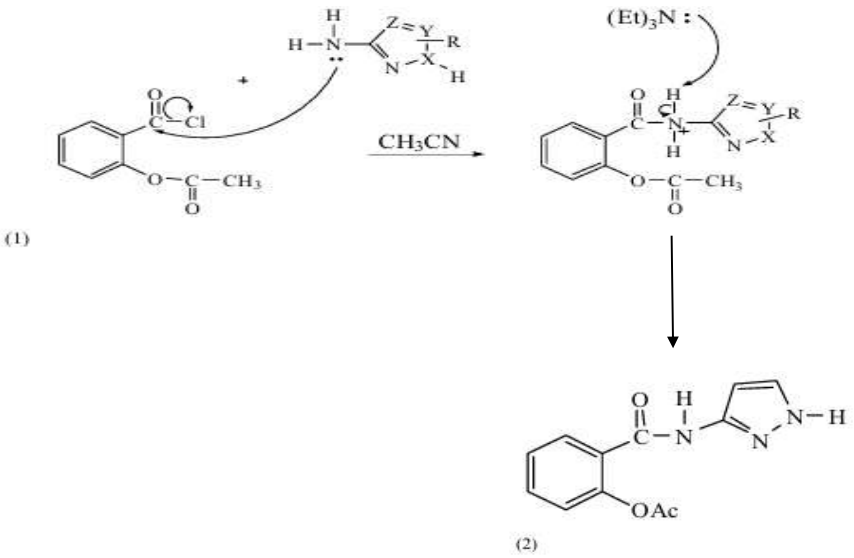
Acetylsalicylic acid, Dry Petroleum ether, Triethanolamine, 1,4-Dioxan were purchased from Oxford Lab Fine Chem LLP. Phosphorous pentachloride was brought from Jigs Chemicals Limited and Pyrazole from Apex Pharma.

Equipments:

Melting points were determined using a calibrated melting apparatus from Prolab India. IR spectra were recorded with an FT-IR and PerkinElmer spectrometer. The progress of the reaction was monitored using TLC Kiesel gel GF254 (type 60) to ensure completion.

Synthesis and Structural analysis of Aspirin derivative:

A solution of Chloroacetylsalicylic acid (1g, 0.005 mol) in 20 mL of dioxane, along with an equivalent amount of pyrazole and a catalytic quantity of triethylamine, was refluxed for 2–8 hours. After cooling, the precipitated solid was filtered, washed with dioxane, and dried, yielding 3-pyrazolyl-2-acetoxybenzamide (2). The reaction was carried out at 4°C with the reaction mixture placed in an ice bath. After completion, the mixture was filtered and washed with cold dioxane. The product was then crystallized from an acetone-ethanol mixture (2:1), yielding a pale yellow color. Chloroacetylsalicylic acid (1) was synthesized by reacting acetylsalicylic acid with PCl_5 in dry petroleum ether under a moisture-protected system.



The composition and structure of the compound were identified by FTIR Spectrometer. FTIR analysis was conducted using an infrared spectrometer equipped with a temperature-controlled heating cell. The sample spectrum was recorded using a KBr disk. Yield 82%, M.P. 193-195°C. IR (KBr): 3256 (N-Has); 3173 (N-Hs); 3032 (C Har); 1738 (C=0,0COCH3); 1650(CO,-CONH); 1584-1472 (C=Car); 1456 (CH3as); 1433 (CH3s);1300 (CC(=O)-O, acetate); 1239 (O-C…..Cas); 797-750 (C-Car).

Experimental Animals

Wistar rats of both sexes, weighing between 170 and 210 g, were obtained from the Animal Facility. The rats were kept in cages at room temperature and had free access to food and water. They were acclimated to the laboratory environment and fasted for 24 hours before the experiment. A total of five rats were randomly divided into five groups. The negative control group received a 0.5% Carboxyl Methylcellulose (CMC) aqueous solution, while the Aspirin group was given Aspirin (dissolved in 0.5% CMC aqueous solution) at a dose of 0.5 mmol/kg of body weight. The remaining three groups received different doses of 3-pyrazolyl-2-acetoxybenzamide (0.1, 0.25, or 0.5 mmol/kg), also dissolved in 0.5% CMC aqueous solution before administration by gavage. After 20 days of treatment, blood samples were collected from the retro-orbital cavernous sinus of the animals to assess antithrombotic activity. The rats were euthanized using an overdose of chloral hydrate, after which their stomachs were immediately incised and opened along the greater curvature. The mucosal surface was then examined under an anatomical lens. Following observation, the stomachs were stored at -80°C for the analysis of COX expression and prostaglandin E2 (PGE2) levels. All animal experiments were carried out in compliance with international ethical guidelines.

Gastric damage score

The gastric mucosa ulcer index (UI) was assessed following the Guth standard [8]. The length and width of the damaged gastric mucosal tissue were measured using a vernier caliper. Spot erosion was assigned a score of 1 point, erosion with a length of less than 1 mm was given 2 points, erosion between 1–2 mm was given 3 points, and erosion between 2–3 mm was given 4 points, while erosion greater than 3 mm was assigned 5 points. If the

erosion width exceeded 1 mm, the score was doubled.

Western Blot Analysis of COX-1 and COX-2

Western blot analysis was performed to examine COX-1, COX-2, and β-actin (internal standard) proteins in the gastric mucosa. Stomach tissue samples were homogenized in RIPA buffer on ice. Protein concentration was determined using the BCA method, and 50 µg of total protein was separated on a 10% SDS-polyacrylamide gel under reducing conditions. Following electrophoresis, proteins were transferred onto a nitrocellulose membrane. The membrane was blocked with TBS-T containing 5% non-fat milk for 3 hours and then incubated overnight at 4°C with a 1:200 dilution of anti-COX-1 or anti-COX-2 antibody or a 1:5000 dilution of anti-β-actin antibody. After four successive washes, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (for COX-probed membranes) or goat anti-mouse immunoglobulin (for β-actin-probed membranes) at a 1:10,000 dilution. Protein bands were visualized using chemiluminescence. Densitometry analysis was performed using scanning software to quantify the COX and β-actin protein bands.

Measurement of prostaglandin E2

Each gastric mucosa sample was weighed, finely chopped with scissors, and homogenized in 0.9% sodium chloride at 4°C. The homogenates were then centrifuged at 845×g for 10 minutes. The resulting supernatants were analyzed for PGE2 levels using a PGE2 Enzyme Immunoassay Kit. The concentration of PGE2 in the sample was expressed as pg/mL of gastric mucosal tissue.

RESULTS

Structure and composition of 3-pyrazolyl-2-acetoxybenzamide

The associated structure of FTIR spectrum of Aspirin and 3-pyrazolyl-2-acetoxybenzamide are shown in figure 1 and figure 2 respectively. Their characteristic FTIR vibrations are listed in Table 1. For Aspirin, the main characteristic absorption peaks were clearly present, such as those at 1753(s) cm^{-1}, 1690 cm^{-1} and 2500–3200cm^{-1}, which represent, respectively, acetyl group [C=O(CH3)], carboxylic acid group [nC=O(-COOH)] and hydroxyl group[9]. For 3-pyrazolyl-2-acetoxybenzamide the IR peaks is as follows:

1. 3256 cm^{-1} (N-H, asymmetric stretch), the presence of this peak suggests an amide (-CONH) or amine ($-NH_2$) functional group.
2. 3173 cm^{-1} (N-H, symmetric stretch), another stretching vibration for the N-H bond, likely due to the presence of an amide or amine.
3. 3032 cm^{-1} (C-H, aromatic stretch), this peak is associated with the stretching of C-H bonds in aromatic rings, indicating the presence of an aromatic system.
4. 1738 cm^{-1} (C=O, ester carbonyl stretch), the strong absorption at this wavenumber corresponds to the stretching of the ester carbonyl group ($-COOCH_3$), which is commonly found in esters or acetates.
5. 1650 cm^{-1} (C=O, amide carbonyl stretch), this peak represents the stretching of the carbonyl (C=O) bond within an amide (-CONH) group.
6. 1456 cm^{-1} (CH_3, asymmetric bending), this is an asymmetric bending vibration of methyl ($-CH_3$) groups.
7. 1239 cm^{-1} (O-C stretching adjacent to aromatic carbon), this peak arises from the stretching vibration of the O-C bond when the oxygen is connected to an aromatic carbon, common in esters and ethers.
8. 797–750 cm^{-1} (C-H, aromatic out-of-plane bending), these peaks correspond to the out-of-plane bending vibrations of aromatic C-H

bonds, which help identify the substitution pattern of the benzene ring.

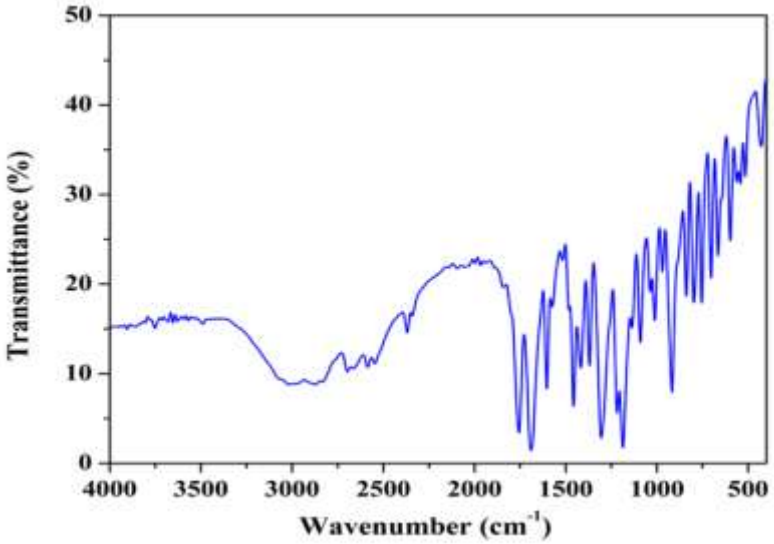


Figure1. FTIR spectra of Aspirin

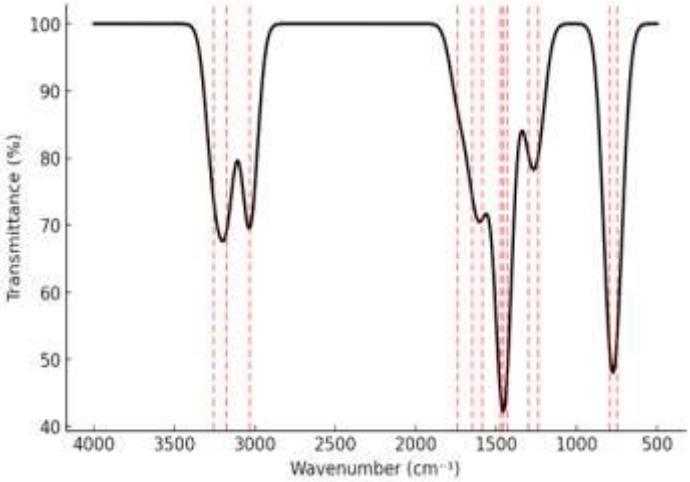


Figure2. FTIR spectra of 3-pyrazolyl-2-acetoxybenzamide

Table1. Characteristic FTIR vibrations for Aspirin and 3-pyrazolyl-2-acetoxybenzamide.

Aspirin cm^{-1}	Assignments	3-pyrazolyl-2-acetoxybenzamide. cm^{-1}	Assignments
1305	VC=O(-COOH)	797–750	C-H aromatic
1419	V_sCOO^-	1239	O-C stretching
1458	H_3CCO, CH_3	1456	asCH_3
1482	PhH, vCC, H_3CCO, CH_3	1650	C=O, amide carbonyl stretch
1609	$VasCOO^-$	1738	(C=O, ester carbonyl stretch)
1690	vC=O(-COOH)	3032	(C-H, aromatic stretch)
1753	vC=O(-C(O)OCH3)	3173	(N-H, symmetric stretch)
2500–3200	associating vOH	3256	(N-H, asymmetric stretch)

Gastric mucosa ulcer index (UI)

The impact of Aspirin and 3-pyrazolyl-2-acetoxybenzamide on the gastric mucosa was assessed microscopically, and the severity was quantified using a median ulcer index score. Compared to the control group, the gastric mucosal surface of mice treated with 3-pyrazolyl-2-acetoxybenzamide exhibited **mild** damage, which was visible to the naked eye and assigned a gastric damage score of **3.5**(Figure-4). In contrast, rats administered Aspirin showed **severe** mucosal injury, including extensive lesions and areas of bleeding, with a gastric damage score of **11.2**. The significant reduction in gastric damage with 3-pyrazolyl-2-acetoxybenzamide suggests it has a notably lower adverse effect compared to pure Aspirin.

Mechanism of gastric mucosa protection of 3-pyrazolyl-2-acetoxybenzamide

The mechanism underlying the gastric mucosal protection by 3-pyrazolyl-2-acetoxybenzamide was explored by assessing COX-1 and PGE-2 expression in gastric tissue.

Determination of the COX-1 level:

The level of COX-1 expression in the gastric mucosa of rats administered a high dose of 3-pyrazolyl-2-acetoxybenzamide was approximately 1.5 times higher than that of rats given pure Aspirin (Figure 5). However, it remained comparable to that of control rats, with no significant difference observed. These findings suggest that Aspirin suppresses COX-1 expression, whereas 3-pyrazolyl-2-acetoxybenzamide does not inhibit its expression. Instead, 3-pyrazolyl-2-acetoxybenzamide maintained COX-1 levels similar to those of the control group, indicating a more balanced effect on gastric mucosal protection.

Determination of Gastric PGE2 level:

The rats which were administrated with 3-pyrazolyl-2-acetoxybenzamide have identical level of PGE2 level as that of control groups but notably higher than rats which received Aspirin (Figure). This indicates that Aspirin suppressed PGE2 synthesis in gastric tissue, whereas 3-pyrazolyl-2-acetoxybenzamide at the same dose had no such effect. This difference is attributed to 3-pyrazolyl-2-acetoxybenzamide's inability to inhibit COX-1 synthesis.

DISCUSSION:

Structure and composition

Chloroacetylsalicylic acid is an electrophile which is derived from Aspirin by reacting with PCl_5, replacing the hydroxyl (-OH) of the carboxyl (-COOH) group with a chloroacetyl ($-COCH_2Cl$) group (Figure3). Pyrazole is a nucleophile, basic lone pair on N1 makes it nucleophilic and ready to attack an electrophilic carbon. Pyrazole itself is a weak nucleophile, the presence of triethylamine deprotonates the N1 nitrogen, increasing its nucleophilicity. The activated pyrazole attacks the electrophilic carbonyl carbon (C=O) of the chloroacetyl ($-COCH_2Cl$) group via its N1 nitrogen. This leads to the substitution of chlorine (Cl) with pyrazole, forming a stable amide bond (-CONH-Pyrazole). Formed 3-pyrazolyl-2-acetoxybenzamide N1 of pyrazole forming a covalent bond with the amide carbonyl (-CONH) of aspirin. The benzene ring remains intact, retaining the ester ($-COOCH_3$) functional group.

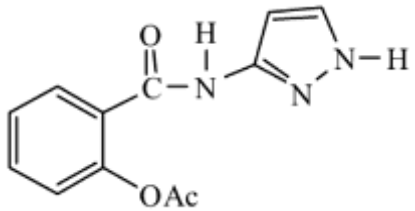


Figure3 Schematic diagram of structure of 3-pyrazolyl-2-acetoxybenzamide

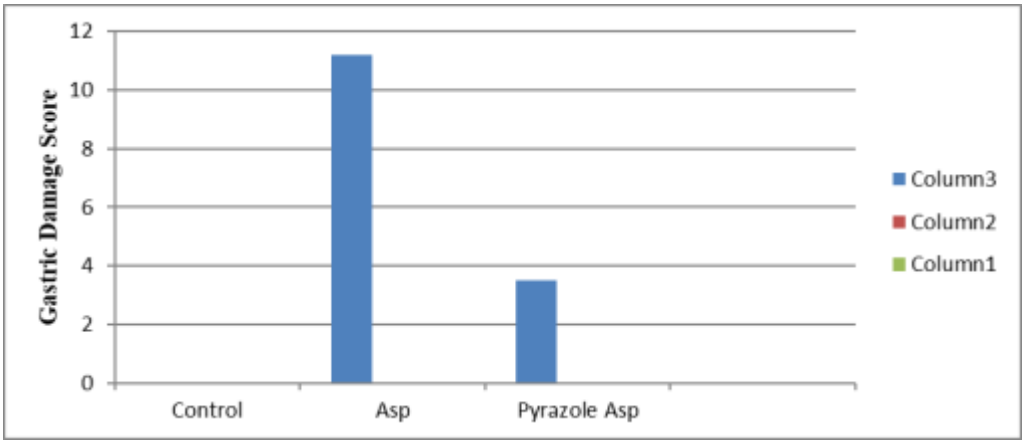


Figure 4 Effect of ASP and 3-pyrazolyl-2-acetoxybenzamide on gastric mucosa of rats.

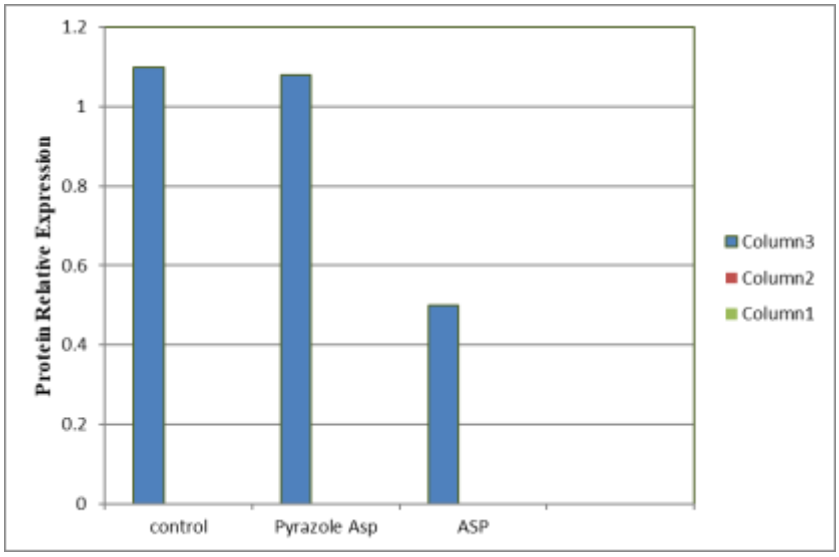


Figure 5 Effect of ASP and 3-pyrazolyl-2-acetoxybenzamide on COX-1 expression in rat gastric tissue

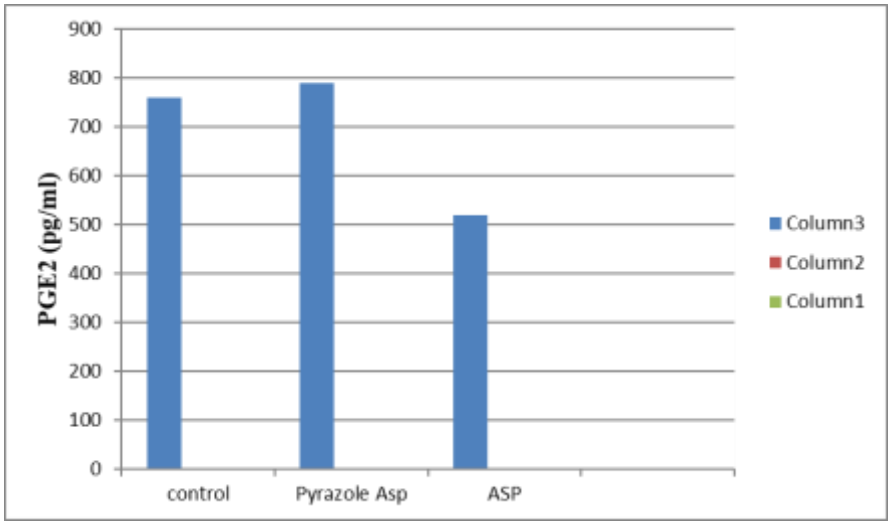


Figure 6 Effect of ASP and 3-pyrazolyl-2-acetoxybenzamide on PGE2 level in rat gastric tissue.

Gastric mucosa protection of 3-pyrazolyl-2-acetoxybenzamide

Aspirin is well-known for causing gastric mucosal injury due to COX-1 inhibition, reducing

protective prostaglandins (PGE_2). Direct irritation from its free carboxyl (-COOH) group. 3-Pyrazolyl-2-acetoxybenzamide showed less gastric damage, as indicated by a lower ulcer index score (3.5 vs. 11.2 for aspirin).This suggests reduced ulcerogenic effects in comparison to aspirin. COX-1 levels remained unchanged in the 3-pyrazolyl-2-acetoxybenzamide, unlike aspirin, which suppressed COX-1 expression. Maintaining COX-1 levels ensures continued gastric mucosal protection, as COX-1-derived prostaglandins (PGE_2) help in mucus secretion, bicarbonate production, maintenance of mucosal blood flow. The carboxyl (-COOH) group in aspirin is replaced by an amide (-CONH) group in the 3-pyrazolyl-2-acetoxybenzamide. This modification reduces direct irritation to the gastric lining, further contributing to its protective effect.

CONCLUSION

The synthesis of 3-pyrazolyl-2-acetoxybenzamide, a novel aspirin derivative, was successfully achieved through the structural modification of aspirin by introducing a pyrazole moiety and replacing the carboxyl (-COOH) group with an amide (-CONH) functional group. This modification aimed to enhance anti-inflammatory efficacy while reducing gastrointestinal side effects. The pharmacological evaluation demonstrated that 3-pyrazolyl-2-acetoxybenzamide exhibited significant anti-inflammatory activity while causing considerably less gastric mucosal damage compared to aspirin. The compound maintained COX-1 expression and PGE_2 levels, unlike aspirin, which suppressed these protective factors, leading to ulcer formation. The reduced ulcerogenic effects of 3-pyrazolyl-2-acetoxybenzamide can be attributed to its structural modification, which minimized direct gastric irritation and prevented COX-1 inhibition. Overall, the study suggests that 3-pyrazolyl-2-acetoxybenzamide has the potential to be a safer alternative to traditional NSAIDs, offering anti-inflammatory benefits while mitigating the gastrointestinal risks associated with aspirin. Further investigations, including clinical studies, are necessary to validate its therapeutic potential.

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