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RESEARCH ARTICLE

Synthesis and Comparative Analgesic and Anti-Inflammatory Activity of Indolizinyl Derivatives of Some NSAIDs

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ABSTRACT

Indolizine, structurally similar to indoles, is known for various biological activities, including analgesic, anti-inflammatory, hypoglycemic, CNS depressant, antimicrobial, and antioxidant effects. The study aimed to synthesize, indolizine derivatives of NSAIDs such as sodium salicylate, aspirin, mefenamic acid, and flufenamic acid to evaluate their comparative analgesic and anti-inflammatory activities. Pyridine was heated with chloroacetic acid and ethyl acetate at 90°C to form pyridinium halide, which was then refluxed with methyl acrylate, manganese dioxide, triethylamine, and toluene at 90°C to yield indolizine-1-carboxylate. This intermediate was esterified with sodium salicylate, aspirin, mefenamic acid, or flufenamic acid to produce the corresponding indolizine derivatives (INS, INA, INM, INF). These derivatives were characterized by melting point, TLC, IR, NMR, and hydrolysis kinetics, and were screened for analgesic and anti-inflammatory activities. The synthesized indolizinyl derivatives (INS, INA, INM, INF) were evaluated for hydrolysis kinetics at different pH levels, with SS showing the highest cumulative drug release and INF the lowest across pH 1.2, 4.5, 6.8, and 7.4. The derivatives exhibited superior analgesic and anti-inflammatory activities compared to their respective pure drugs, with INM demonstrating the most significant effects among all the derivatives. The novel NSAID derivatives, particularly INM, exhibited significantly enhanced analgesic and anti-inflammatory activities compared to their parent compounds (SS, ASP, MA, FA) showing potential new therapeutic agents for pain and inflammation management.

Keywords: Indolizine; Sodium salicylate; Aspirin; Mefenamic acid; Hydrolysis kinetics; Antiinflammatory activity

INTRODUCTION

Inflammation is a response of the tissue to an infection, irritation or foreign substance[1] and is a part of the host defense mechanism. The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interaction and thermal or other physical injuries)[2]. Almost two decades ago, steroids namely prednisolone, dexamethasone, betamethasone, etc. were considered to be the choicest anti- inflammatory drugs. Owing to the several adverse effects caused by either short-term or long-term steroid therapy, these have been more or less replaced by much safer and better-tolerated non-steroidal anti- inflammatory drugs (NSAID). The seriousness and enormous aftereffects of steroid therapy necessitated accelerated research towards the development of NSAIDs since the past two decades[3,4]. NSAIDs have been highly useful for treating acute, self-limited inflammatory conditions. The development of NSAIDs has helped in understanding the tissue mechanism of inflammation. Many of the NSAIDs displayed weak antiproliferative activity which could be COX dependent or independent[5]. Esterification/amidation of the carboxylic acid group in ibuprofen was associated in some cases with increases in the anti-inflammatory activity and decrease in GIT side effects[6,7]. Some of the ester and amide derivatives of ibuprofen also showed improved antiproliferative activity compared to the parent drug. On a cellular level, this improvement of the antiproliferative activity could be due to the increased lipophilicity and enhancer cellular uptake[8]. The objective of the study is to synthesize various substituted indolizine-1-carboxylates and indolizine derivatives by reacting them with different NSAIDs. The synthesized compounds were identified and characterized using FTIR,

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NMR, and mass spectrometry. Additionally, the study aims to investigate the hydrolysis kinetics of the synthesized derivatives at various pH levels and evaluate their analgesic and anti-inflammatory activities.

METHODOLOGY

Synthesis

Step 1: Synthesis of pyridinium halide

A conical flask containing 100 mmol of pyridine and 60 ml of ethyl acetate was stirred with 100 mmol of chloroacetic acid at 90°C for 2 hours. The resulting yellowish solution was then refrigerated for 3 hours, during which time a solid formed. This solid was subsequently filtered, air-dried, and recrystallized from hot methanol and the yield of the process was 60% to 98%.

Step 2: Synthesis of indolizine-1-carboxylate

A suspension comprising pyridinium halide (10 mmol), methyl acrylate (50 mmol), triethylamine (1.5 ml), and manganese dioxide (80 mmol) in 80 ml of toluene was stirred at 90°C for 2 hours. Following cooling to room temperature, the resulting brown oil was collected through distillation at 130°C. The oil was then washed with water and dried over calcium chloride. The yield of this process ranged from 57% to 92%[9].

Step 3: Synthesis of Pyridinium Halide

In a conical flask, 100 mmol of pyridine and 60 ml of ethyl acetate were combined, and then 100 mmol of chloroacetic acid was added to the mixture. The resulting solution was stirred at 90°C for 2 hours, during which it turned yellowish. After stirring, the mixture was refrigerated for 3 hours, allowing a solid to form. This solid was subsequently filtered, air-dried, and recrystallized from hot methanol. The yield of this process ranged from 60% to 98%.

Step 4: Synthesis of indolizine-1-carboxylate

A suspension consisting of pyridinium halide (10 mmol), methyl acrylate (50 mmol), triethylamine (1.5 ml), and manganese dioxide (80 mmol) in 80 ml of toluene was stirred at 90°C for 2 hours. Following this, the mixture was cooled to room temperature, and the resulting brown oil was collected through distillation at 130°C. The oil was then washed with water and dried over calcium chloride. The yield of this process ranged from 57% to 92%[9].

Step 5: Synthesis of various indolizinyl derivatives of various NSAIDs

The synthesis of various indolizinyl derivatives of NSAIDs commenced with the preparation of the indolizinyl derivative of sodium salicylate. In this process, indolizine-1-carboxylate (0.25 mol) and sodium salicylate (2.5 mol) were refluxed with concentrated sulfuric acid (2.7 ml) at 90°C for 6 hours. The reaction mixture was then cooled to 4-5°C for 24 hours, allowing the formation of solid crystals. These crystals were subsequently filtered, washed with cold water, dried, and recrystallized from ethanol[10]. The same procedure was employed for the synthesis of indolizinyl derivatives of aspirin, wherein aspirin (2.5 mol) was used in place of sodium salicylate. This involved refluxing indolizine-1-carboxylate (0.25 mol) and aspirin (2.5 mol) with concentrated sulfuric acid (2.7 ml) at 90°C for 6 hours. After the reflux, the reaction mixture was cooled to 4-5°C for 24 hours, leading to the formation of solid crystals. These crystals were then filtered, washed with cold water, dried, and recrystallized from ethanol[10]. For the synthesis of indolizinyl derivatives of mefenamic acid, indolizine-1-carboxylate (1.5 g) and mefenamic acid (2.9 g) were refluxed with methanol (20 ml) and concentrated sulfuric acid (2.7 ml), and the mixture was boiled at 80°C for 8 hours. The reaction mixture was then cooled to 4-5°C for 24 hours, resulting in the formation of crystals. These crystals were subsequently filtered, washed, dried, and recrystallized from ethanol[11]. Similarly, the indolizinyl derivatives of flufenamic acid were synthesized by refluxing indolizine-1-carboxylate (1.5 g) and flufenamic acid (3.95 g) with methanol (20 ml) and concentrated sulfuric acid (2.7 ml). The mixture was boiled at 80°C for 8 hours, then cooled, filtered, and recrystallized from ethanol to obtain the flufenamic acid derivatives[11].

Identification and Characterisation

The synthesized compounds were identified and characterized using various analytical techniques. The melting point determination, performed using the Thiel's tube method, served as a crucial criterion for assessing the purity of the compounds. Thin layer chromatography (TLC) was employed to monitor the progress of reactions and evaluate the purity of the end products, utilizing pre-coated silica plates with a mobile phase of Hexane: Ethyl acetate (4:1 or 1:1) and detecting spots under UV light.

Infrared spectroscopy (IR), conducted with a SHIMADZU FTIR 8400S spectrometer, provided essential information on functional groups and molecular structures through the analysis of molecular vibrations, particularly in the fingerprint region (1300-650 cm^{-1}). Nuclear magnetic resonance (NMR) spectroscopy, carried out using a Bruker spectrospin-400 NMR spectrophotometer with chloroform and DMSO as solvents, allowed for the observation of energy absorption related to the magnetic dipolar nature of spinning nuclei, aiding in structure determination. Mass spectroscopy, using an electron spray mass spectrometer, offered insights into atomic and molecular weights, structural information, and reaction kinetics by analyzing positively charged ions produced via electron bombardment.

The synthesized compounds were characterized as follows:

INS ($C_1H_9NNaO_4$, 303.23 g/mol): This compound is a dark brown solid, soluble in chloroform and ethanol, with a melting point of 204–210°C and a yield of 66%. Key IR peaks include 1621.8 cm^{-1} (C=C) and 1732 cm^{-1} (C=O), while the

NMR shifts show δ 7.44-7.59 (aromatic H). The molecular ion peak is at m/z 303.

INA ($C_{14}H_{11}NO_4$, 281.26 g/mol): This compound is a dark brown crystalline solid, soluble in chloroform, with a melting point of 138–140°C and a yield of 70%. IR spectra display peaks at 3045.95 cm^{-1} (C-H) and 1733.69 cm^{-1} (C=O), with NMR shifts at δ 7.28-7.82 (aromatic H) and a molecular ion peak at m/z 281.

INM ($C_{25}H_{23}N_2O_3$, 384.42 g/mol): This compound is a blackish-brown solid, soluble in chloroform and methanol, with a melting point of 232–240°C and a yield of 78%. IR peaks include 3072.5 cm^{-1} (C-H) and 1654.62 cm^{-1} (C=O), with NMR shifts at δ 7.09-7.74 (aromatic H) and a molecular ion peak at m/z 384.

INF ($C_{23}H_{15}F_3N_2O_3$, 424.37 g/mol): This compound is a brown crystalline solid, soluble in chloroform, with a melting point of 135–140°C and a yield of 80%. Its IR spectra show peaks at 3324.68 cm^{-1} (NH) and 1245.79 cm^{-1} (C-O), with NMR shifts at δ 2.20-2.64 (aliphatic H) and a molecular ion peak at m/z 424.

Pharmacological Screening

Inflammation: The inflammatory process, triggered by stimuli such as infections, ischemia, antigen-antibody interactions, or physical injury, progressed through three distinct phases. The acute phase was characterized by local vasodilation and increased capillary permeability, resulting in redness and swelling. This was followed by the subacute phase, which involved the infiltration of leukocytes and phagocytic cells to clear pathogens and damaged cells. Finally, chronic inflammation led to tissue degeneration and fibrosis, causing scarring and loss of function. Each phase was mediated by different mechanisms and was crucial for the body's response to injury or infection [12].

Chemical classification of analgesic, antipyretic and non-steroidal, anti-inflammatory drugs

Nonselective COX Inhibitors

Anti-inflammatory drugs were classified based on their chemical structure into several categories. Salicylic acid derivatives, such as aspirin, sodium salicylate, choline magnesium trisalicylate, diflunisal, salicylsalicylic acid, sulfosalazine, and oxsalazine, formed one category. Another category included Para-aminophenol derivatives, exemplified by acetaminophen. Indole and indene acetic acids, including indomethacin, sulindac, and etodolac, constituted a separate group. Heteroaryl acetic acids, such as tolmetin, diclofenac, and ketorolac, were also identified as a distinct category. Arylpropionic acids, comprising ibuprofen, naproxen, flurbiprofen, ketoprofen, fenoprofen, and oxaprozin, were another classification. Anthranilic acids, specifically the fenamates (mefenamic acid and flufenamic acid), formed another group. Enolic acids, which included oxicams like piroxicam and tenoxicam, as well as pyrazolidinediones such as phenylbutazone and oxyphenbutazone, were classified separately. Finally, Alkanones, represented by nambumetone, constituted the last category. These classifications were based on the distinct chemical structures of these anti-inflammatory drugs.

Selective COX-2 Inhibitors

Selective COX-2 inhibitors, such as rofecoxib (a diaryl-substituted furanone) and celecoxib (a diaryl-substituted pyrazole), targeted the COX-2 enzyme to reduce inflammation and pain while minimizing gastrointestinal side effects compared to traditional NSAIDs. Etodolac, an indole acetic acid, also acted as a selective COX-2 inhibitor. The synthesized derivatives were screened for their analgesic and anti-inflammatory activities. The LD50 (lethal dose 50) estimation was performed using mice, with doses ranging from 150 to 200 μg. The LD50 was determined to be 9 mg/kg body weight, and the effective dose was found to be 2 mg/kg.

Analgesic Screening

Analgesic screening involved methods to evaluate pain relief without inducing loss of consciousness. Pain, a subjective experience, can be categorized as superficial, deep non-visceral, visceral, referred, or psychogenic. The synthesized compounds were tested for their analgesic efficacy using several methods. The tail-flick method measured the reaction time to radiant heat applied to a mouse's tail, with tail withdrawal indicating pain; an increased reaction time post-drug administration indicated an analgesic effect. Acetic acid-induced writhing in mice was used to assess chemical-induced pain, where a reduction in the writhing response suggested analgesic activity. Eddy's Hot Plate method involved placing mice on a hot plate (55°C), observing paw-licking or jumping responses to pain, and noting increased reaction times after drug administration as indicative of analgesia. The tail immersion method involved immersing a rat's tail in 55°C water and recording the time taken to withdraw the tail; longer reaction times after drug administration indicated analgesia, with a cut-off time of 15 seconds to prevent harm. These methods collectively provided a comprehensive assessment of the analgesic properties of the synthesized derivatives.

In vitro Anti-Inflammatory Screening

In vitro assays for screening anti-inflammatory compounds have evolved from non-specific earlier methods to more targeted biochemical approaches. Initially, methods such as the inhibition of protein denaturation, fibrinolysis, and platelet aggregation were used, but these showed poor correlation with in vivo activity. More recent assays focus on regulating the arachidonic acid cascade, including the inhibition of phospholipase, cyclooxygenase, thromboxane synthetase, and lipoxygenase. Additionally, these assays

target the neutralization of leukocyte functions such as phagocytosis and chemotaxis, as well as the inhibition of tissue degeneration by suppressing proteases and the release of lysosomal hydrolases. Experimental studies often utilized rats, with chemical stimulants injected into the hind paw to induce an inflammatory response, allowing for the evaluation of non-narcotic analgesic compounds.

Screening Methods

Various methods were employed to screen for anti-inflammatory activity in rats. Brewer's yeast was used to induce edema and inflammation in the hind paw, with volume changes measured using techniques such as the Randall-Selitto method. Freund's adjuvant, a mixture of Mycobacteria and mineral oil, caused lesions 10-15 days post-injection, and edema was measured using a micrometer. The silver nitrate method involved injecting a 1% solution into the rat's ankle joint to induce inflammation, with pain relief from joint flexing serving as the criterion for efficacy. Kaolin suspension and bovine testicular extract were also used to provoke inflammatory responses in the paw, while granulomas were formed by injecting air or implanting cotton pellets subcutaneously. Both steroidal and non-steroidal compounds were found to reduce inflammation in these models. Formaldehyde was used to induce inflammation in the rat's paw, with drug efficacy assessed by measuring edema reduction. Lastly, the carrageenan method utilized plethysmography to measure paw volume changes after inflammation was induced by carrageenan injection, and percent edema inhibition was calculated for each treatment group. These diverse methods collectively provided a comprehensive assessment of anti-inflammatory activity in the rat models.

Statistical analysis:

One way ANOVA followed by Tukey multiple comparisons' test. All values were mean ± SEM, n=6, $^{*}P \geq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ when comparison between control vs INS,INA,INM,INF, $^{\bullet}P \geq 0.05$, $^{\bullet\bullet}P \leq 0.01$, $^{\bullet\bullet\bullet}P \leq 0.001$ comparison between std (indomethacin) vs INS,INA,INM,INF, $^{a}P \geq 0.05$, $^{aa}P \leq 0.01$, $^{aaa}P \leq 0.001$ comparison between SS vs INS, $^{b}P \geq 0.05$, $^{bb}P \leq 0.01$, $^{bbb}P \leq 0.001$ comparison between ASP vs INA, $^{c}P \geq 0.05$, $^{cc}P \leq 0.01$, $^{ccc}P \leq 0.001$ comparison between MA vs INM, $^{d}P \geq 0.05$, $^{dd}P \leq 0.01$, $^{ddd}P \leq 0.001$ comparison between FA vs INF.

RESULTS

The synthesized compounds were characterized using several analytical methods. Melting points were determined via Thiel's capillary tube method. Thin layer chromatography (TLC) was performed using ethyl acetate and n-hexane in various ratios to assess the purity and progress of the reactions. Infrared (IR) spectroscopy was conducted with KBr pellets to identify functional groups and molecular structures. Nuclear Magnetic Resonance (NMR) spectroscopy was carried out on a 400 MHz spectrospin NMR spectrometer at the Indian Institute of Science and AstraZeneca in Bangalore, with $CDCl_3$ as the solvent, to determine the structural details of the compounds. Mass spectrometry was performed using an Electron Spray spectroscope at IISC, Bangalore, to analyze the molecular weights and structural information of the compounds. Following characterization, the synthesized derivatives were then screened for their analgesic and anti-inflammatory activities.

Anti-inflammatory study of (NSAIDs derivatives)

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. The data were presented as mean ± SEM, with n=6. Significance levels were indicated as $^{*}P \geq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ for comparisons between the Normal control and INS, INA, INM, INF; $\bullet\ P \geq 0.05$, $\bullet\bullet\ P \leq 0.01$, $\bullet\bullet\bullet\ P \leq 0.001$ for comparisons between the Toxic control and INS, INA, INM, INF; $^{a}P \geq 0.05$, $^{aa}P \leq 0.01$, $^{aaa}P \leq 0.001$ for comparisons between SS and INS; $^{b}P \geq 0.05$, $^{bb}P \leq 0.01$, $^{bbb}P \leq 0.001$ for comparisons between MA and INM; and $^{c}P \geq 0.05$, $^{cc}P \leq 0.01$, $^{ccc}P \leq 0.001$ for comparisons between FA and INF. The drug release profiles of all synthesized derivatives were evaluated through in vitro hydrolysis kinetics at pH 1.2, 4.5, and 6.8, with half-lives determined by hydrolysis and absorption at pH 7.4. The study revealed that the NSAID derivatives INS, INA, INM, and INF exhibited significant analgesic and anti-inflammatory activity compared to SS, ASP, MA, and FA, with INM showing the most notable activity among all the derivatives.

DISCUSSION

Indolizinyl analogues were synthesized by reacting methyl acrylate with pyridinium halide in the presence of manganese dioxide, triethyl amine, and toluene. The resulting indolizine-1-carboxylate was then combined with various NSAIDs—aspirin, sodium salicylate, mefenamic acid, and flufenamic acid—to form the respective derivatives. Characterization of these compounds involved determining their melting points using Thiel's capillary tube method, thin layer chromatography (TLC) with ethyl acetate in a 1:4 ratio, infrared (IR) spectroscopy using KBr pellets, and nuclear magnetic resonance (NMR) spectroscopy on a 400 MHz spectrometer at the Indian Institute of Science (IISc) in Bangalore. Mass spectrometry using electron spray ionization confirmed the structural integrity of the compounds.

Drug release profiles were studied through in vitro hydrolysis kinetics at pH 1.2, 4.5, and 6.8, with half-lives determined at pH 7.4.

Table 1: Physiochemical and analgesic properties of Indolizinyl derivatives of NSAIDs

Indolizinyl derivatives of NSAIDs					
Compound code	**Mol formula**	**Mol weight**	**Melting point (° C)**	**Yield (%)**	**Rf Value**
INS	$C_{16}H_9NNaO_4$	303.23	204 – 210	66%	0.76
INA	$C_{14}H_{11}NO_4$	281.26	138 – 140	70%	0.36
INM	$C_{25}H_{23}N_2O_3$	384.42	232 – 240	78%	0.70
INF	$C_{23}H_{15}F_3N_2O_3$	424.37	135 – 140	80%	0.35
Analgesic study of NSAIDs derivatives					
Name of the compound	**0-Mins**	**15-Mins**	**30-Mins**	**60-Mins**	**120Mins**
C control	2.29	2.31	2.1	2.19	2.15
Std Indomethacin (20 mg/kg)	2.16	3.62	4.78	5.98	6.82
SS	1.93	2.02	2.28	2.38	2.30
ASP	2.02	2.07	2.12	2.16	2.22
MA	1.96	2.09	2.26	2.33	2.66
FA	2.04	2.19	2.22	2.27	2.46
INS	2.16	2.73•	3.38***••• aaa	3.49*••• aaa	3.27***••• aaa
INA	2.17	2.26•••	2.98**••• bb	3.2••• bb	3.22***••• bb
INM	2.17	3.53 ccc	3.89***••• ccc	4.13**••• ccc	5.04***••• ccc
INF	2.22	3.24d	3.5***••• ddd	3.78***••• ddd	4.23***••• ddd

Table 2: Inflammatory response measured by paw volume (mL) at different time intervals in carrageenan induced paw edema

Name of the compound	**0 mins**	**15 mins**	**30 mins**	**60 mins**	**120 mins**
Normal control	0.09	0.10	0.09	0.09	0.09
Toxic control	0.12	0.25	0.29	0.33	0.32
Std Indomethacin (20 mg/kg)	0.10	0.13	0.12	0.12	0.13
SS	0.10	0.24	0.19	0.20	0.21
ASP	0.10	0.17	0.14	0.14	0.16
MA	0.10	0.16	0.13	0.14	0.17
FA	0.13	0.19	0.16	0.15	0.14
IND	0.13	0.23	0.21	0.20	0.22
INS	0.10	0.19**	0.15••	0.14*••a	0.15*••a
INA	0.13	0.15*•	0.14*•	0.14*•	0.14*•
INM	0.10	0.13**	0.09•••	0.09•••b	0.07•••b
INF	0.13	0.14**	0.12*••	0.11*••c	0.09*••c

Table 3: Cumulative drug release profiles of synthesized derivatives at different pH levels

Sr. No	**Name of the compound**	**% Cumulative Drug Release at pH 1.2**	**% Cumulative Drug Release at pH 4.5**	**% Cumulative Drug Release at pH 6.8**	**% Cumulative Drug Release at pH 7.4**
1	SS	41.98	16.17	51.55	93.72
2	INS	30.45	12.95	37.12	92.08
3	ASP	29.07	11.52	41.12	91.60
4	INA	21.38	8.22	29.63	89.31
5	MEF	21.78	5.82	32.00	92.13
6	INM	13.50	3.18	23.00	89.71
7	FLU	19.64	4.49	25.46	90.08
8	INF	11.31	2.82	18.95	86.40

The order of drug release at each pH level was as follows: at pH 1.2 (SS-41.98%, INS-30.45%, ASP-29.07%, INA-21.38%, MA-21.78%, INM-13.50%, FA-19.64%, INF-11.31%); at pH 4.5 (SS-16.17%, INS-12.95%, ASP-11.52%, INA-8.22%, MA-5.82%, INM-3.18%, FA-4.49%, INF-2.82%); and at pH 6.8 (SS-51.55%, INS-37.12%, ASP-41.12%, INA-29.63%, MA-32.00%, INM-23.00%, FA-25.46%, INF-18.95%). In vitro hydrolysis studies revealed that these pro-drug conjugates did not hydrolyze appreciably, but at pH 7.4, the studies showed a reduction in therapeutic doses and a prolongation of the duration of action of the synthesized derivatives.

The synthesized compounds were screened for their analgesic and anti-inflammatory activities using a plethysmograph for anti-inflammatory activity and the tail immersion method for analgesic activity. All synthesized compounds demonstrated analgesic and anti-inflammatory activities. Specifically, INS showed significant analgesic and anti-inflammatory activity compared to SS; INA showed significant activity compared to ASP; INM showed significant activity compared to MA; and INF showed significant activity compared to FA. However, INM exhibited the most notable analgesic and anti-inflammatory activity among all the synthesized compounds.

CONCLUSION

The synthesis of indolizine derivatives by esterifying indolizine-1-carboxylate with various NSAIDs, including sodium salicylate, aspirin, mefenamic acid, and flufenamic acid, was successfully achieved. The structures of these synthesized compounds were comprehensively confirmed through melting point determination, thin-layer chromatography (TLC), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) analyses. Hydrolysis kinetics studies conducted at pH 1.2, 4.5, 6.8, and 7.4 revealed that these pro-drug conjugates do not hydrolyse appreciably and are absorbed in their unhydrolyzed form. This suggests a potential for reduced therapeutic doses and prolonged duration of action.

The analgesic and anti-inflammatory activities of the synthesized derivatives (INS, INA, INM, INF) indicated that all derivatives exhibited significant analgesic and anti-inflammatory effects compared to their parent compounds (SS, ASP, MA, FA). INM demonstrated the most potent activity in both analgesic and anti-inflammatory assays. These findings show the potential of indolizine derivatives as novel therapeutic agents for managing pain and inflammation, offering enhanced efficacy and possibly reduced side effects compared to traditional NSAIDs. Further preclinical and clinical investigations are warranted to fully explore the therapeutic potential of these compounds.

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