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DESIGN, SYNTHESIS AND CYTOTOXIC STUDY OF ARYL-METHOXY BASED AMIDE DERIVATIVES

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Pyridine, Biological Activity, Indomethacin, Anti-Inflammatory drug

Abbreviations: *NMR (Nuclear megnatic resonance), DMAP (4-(Dimethylamino) pyridine), DMSO (Dimethyl sulfoxide), NSAIDs (Nonsteroidal Anti inflammatory drugs)*

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Abstract

The paper investigates indomethacin's chemical reactions and yields. Numerous compounds' cytotoxic and anti-cancer qualities were examined. A comparison of the Hemotoxicity profiles of the generated compounds was made. Indomethacin works well to reduce pain and inflammation. There are anti-inflammatory and anti-cancer effects of indomethacin. The study assesses the cytotoxicity of synthetic derivatives. In order to improve thrombolytic efficacy, indomethacin reduces inflammation. New anti-cancer drugs are created using well-known inhibitors. A variety of derivatives have been produced using various techniques, employed for various purposes, and their thrombolytic and hemolytic activity has been examined. The synthesized derivatives of indomethacin 3a-g yields 42- 60%.

INTRODUCTION

Heterocycles based on nitrogen are essential to chemical processes in living things, including diet components and medications (Lygin & de Meijere, 2010). They form the basis of vitamins, nucleic acids, enzymes, hormones, and alkaloids (Hosseinzadeh, Ramazani, & Razzaghi-Asl, 2018). Amino acids are the most common structural element in organic substances (Arora, Arora, Lamba, Wadhwa, & Research, 2012).

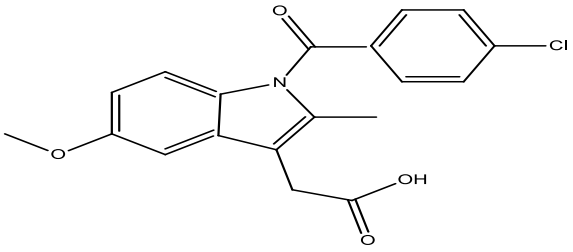

Figure 1: Structure of indomethacin

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Pyridine, a C_5H_5 heterocyclic organic molecule, is a potent anticancer, analgesic, antibacterial, antiviral, and antiulcer agent (Marinescu & Popa, 2022). It is found in various substances and is used in various applications such as dyes, anthelmintic, treatments for epilepsy, blood vessel relaxants, antimicrobials, antiparasitic drugs, antifungals, insecticides, weed control, anesthetics, and seizures (Heravi & Zadsirjan, 2020).

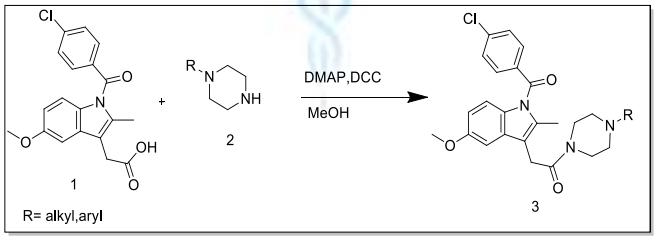
Medications that reduce inflammation are a useful treatment choice due to their anti-pyretic and analgesic properties (Lucas & Pain, 2016). However, When NSAIDs are stopped, they can cause serious problems like atherosclerosis, rheumatoid arthritis, and nasal discharge. Although NSAIDs have many positive effects, they can also cause negative ones like gastric erosion and ulceration (Davis & Robson, 2016). By preventing prostaglandin synthesis, they impair the cytoprotective function of the gastric mucosa, which causes problems for the gastrointestinal system (Huang et al., 2020).

Serious gastrointestinal problems, such as ulcers, bleeding, and inflammation, can result from long-term NSAID use (Laine & Management, 2003). The effects on organs and cell tissue are exacerbated by persistent inflammation, leading to various illnesses, including rheumatoid arthritis, inflammatory bowel diseases, metabolic disorders, cardiovascular disease, brain-related neurodegenerative diseases, and cancer (Prusakiewicz, Felts, Mackenzie, & Marnett, 2004). However, indomethacin is known to cause gastrointestinal toxicity, leading to ulcers and bleeding (Perron et al., 2013). To reduce its negative effects, researchers have developed various methods, including creating prodrugs and altering side groups (Ramos-Inza et al., 2022).

As a non-selective COX inhibitor, indomethacin helps stop the proliferation of cancer cells and suppresses the formation of gastrointestinal tumors (Munjal & Allam, 2024). Its detrimental effects on the gastrointestinal system have been eliminated by the synthesis of indomethacin derivatives that show selectivity for COX-2 inhibition (Rotella, 2021).

Experiments

General scheme for the production of indomethacin derivative is as follows:



General information

The glassware was dried at 1000C, and reactions were conducted using liquid chemicals and reagents. 1H-NMR-Spectra and 13C-NMR-Spectra were evaluated using a 500 MHz and 100 MHz NMR spectrometer. Spots were identified using UV lights and thin-layer chromatography (TLC). Analytical-grade solvents were employed, with easily accessible industrial solvents purified through evaporation.

General Procedure

A compound made from acetic acid and a chlorobenzene group was treated with methanol to create nitrogen-containing amide derivatives. This resulted in a methoxy-substituted indole derivative, including one connected to a methyl piperazine group. A substituted piperazine (a carbodiimide compound) and an organic catalyst (DMAP) were used as reaction aids.

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Cytotoxic assay

very freshly created derivative has its hemolytic activity evaluated and is provided with the hemolysis % explained. The toxicity of each derivative to red blood cells is shown in this table. Some substances are highly poisonous, whereas others are not. The indomethacin derivative 2a-g were investigated against all type of arthritis disease. The corresponding infected cell were matured at 37℃ in DMEM (Dulbecco's Modified Eagle's Medium) added with FBS (fetal bovine serum). Cell viability of the synthesized compounds has been measured by MTT assay (Zahoor et al., 2023).

Prepared compounds has been added into DMSO. For infected cells, 0.05% DMSO was added for 48 hours and employed as control in these experimental procedures. MTT reagent (500µg/ml) was incubated at 37℃ for 04 hours. To examine the absorbance of derivative solution in DMSO was employed. Absorbance was recorded at 575nm on thermos scientific microplate reader the cell viability was calculated in %age (Sadiq Ahmad et al., 2016) (Sajjad Ahmad et al., 2016)

Characterization

FTIR spectroscopy can be used to assess the chemical composition of various indomethacin derivatives and analyze how it is produced from various components. NMR investigations were conducted using DMSO-d6 ultra-precision NMR tubes and a Varian 600 MHz analyzer tuned to 300K. Compounds that had been immersed in DMSO-d6 up to a final dosage of 10 Mm were subjected to several tests.

Statistical analysis

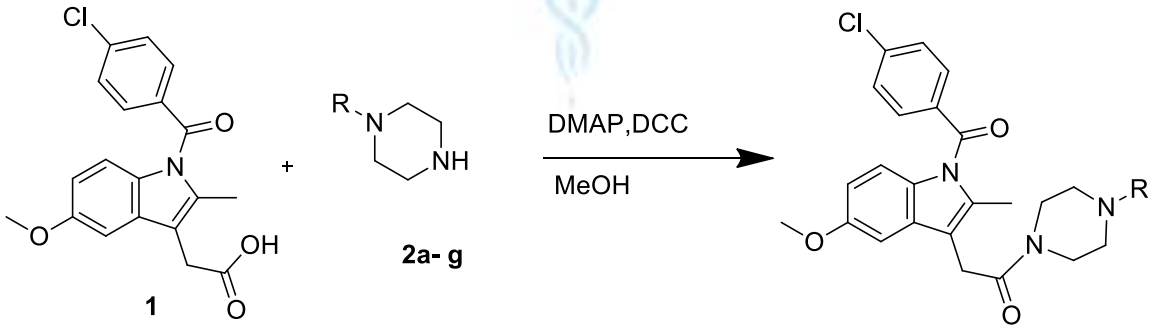
A statistical analysis was performed using MS Word 2010 and above. All the results are taken in triplicates.

Results

This study aims to prepare nitrogen-based derivative equivalents using novel techniques and evaluate their biological impact. Nitrogen-based derivatives are effective pharmacological compounds with clean work-up, reduced reaction time, and effective yield.

General Outline: Synthesis of Compound

General procedure to produce indomethacin derivatives is given below.



R=

2a= methyl piprazine
2b= 2,4,6- trichloroaniline
2c=2,4,6- tribromoaniline
2d=2- nitroaniline
2e=2,4- dinitroanilile
2f= ethylpiprazine
2g= 1- isopropylpiprazine

Scheme 1: General outline scheme for the synthesis of derivatives of indomethacin.

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Table 1: Spectral characterization of compounds

Compound	M.P. (°C)	YEILD (%)	FT-IR (cm^{-1})v_{max} / ^{1}HNMR , C^{13}NMR (500 MHz, $CDCl_3$)/ MS (EI) (m/z)
3a	496.5	60	1750 (C=O), 1323(C-N), 1250 (C-O)/ 2.03, (s, 3H, CH_3), 2.15 (t, 7H, 3H-CH_3, 4H-$(CH2)_2$, 3.20 (t, 4H,$(CH_2)_2$), 3.33 (s, 2H, CH2), 3.51 (s, 3H, CH3), 6.30 (d, 1H, H-Ar), 7.1 (d, 1H, H-Ar), 7.20 (d, 1H, H-Ar), 7.38 (d, 2H, H-Ar), 7.49 (d, 2H, H-Ar)/ 12.0, 29.1, 46.6, 49.6, 51.5, 55.8, 100.6, 108.2, 112. 2, 113.4, 128.7, 129.3, 131.1, 131.2, 131.3, 135.1, 137.4, 140. 1, 154, 160, 167.7/ 139.1
3b	673.7	42	1700 (C=O), 1300(C-N), 1250 (C-O)/ 2.10 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 4.02 (s, 2H, OCH_2), 6.42 (d, 1H, H-N), 6.76 (d, 1H, H-Ar), 7.11 (s, 1H, H-Ar), 7.00 (d, 1H, H-Ar), 7.28 (d, 2H H-Ar), 7.59 (d, 2H, H- Ar), 7.91 (s, 1H, H-Ar)/ 13.2 35.1, 55.2, 124.6, 128.7, 129.3, 131.3, 132.6, 135.1, 137, 140.1, 146.7, 154, 167.7/ 536
3c	762.8	50	1720 (C=O), 1200(C-N), 1250 (C-O)/ 2.26 (s, 3H, CH_3), 3.20 (s, 3H, CH_3), 4.02 (s, 2H, OCH_2), 5.71 (d, 1H, H-N), 6.20 (d, 1H, H-Ar), 7.11 (s, 1H, H-Ar), 7.40 (d, 1H, H-Ar), 7.68 (d, 2H H-Ar), 7.79 (d, 2H, H- Ar), 7.81 (s, 1H, H-Ar)/ 13.2, 134.2, 55.8, 100.5, 106.3, 108.5, 112.2, 114.3, 126.1, 128.7, 129.3, 134.4, 137.6, 137.6, 140.1, 142.8, 152.2, 154, 167.7, 201.7/ 667.85
3d	840.5	66	1770 (C=O), 1200(C-N), 1250 (C-O), 1383 (N-O)/ 2.0 (s, 3H, CH_3), 3.31 (s, 3H, CH_3), 4.02 (s, 2H, OCH_2), 6.70 (d, 1H, H-Ar), 6.95 (d, 1H, H-Ar), 7.11 (s, 1H, H-Ar), 7.21 (t, 1H, H-Ar), 7.32 (t, 1H, H-N), 7.62-7.68 (d, 4H, H-Ar), 7.79 (d, 2H, H-Ar)/ 13.2, 34.6, 55, 100.5, 106.3, 112.2, 113.4, 116.3, 121.6, 128.7, 131.1, 131.3, 136.1, 137.6, 139, 140.1, 144.8, 154, 167.7, 201.7/477.11
3e	800	57	1760 (C=O), 1230(C-N), 1250 (C-O), 1383 (N-O)/ 2.06 (s, 3H, CH_3), 2.81 (s, 3H, CH_3), 4.02 (s, 2H, OCH_2), 6.21 (d, 1H, H-Ar), 7.00 (s, 1H, H-Ar), 7.20 (d, 1H, H-Ar), 7.42 (d, 1H, H-N), 7.51 (d, 1H H-Ar), 7.78 (d, 2H, H-Ar), 7.79 (d, 2H, H-Ar), 8.52 (d, 1H, H-Ar)/ 13.2, 33.6, 35, 100.5, 106.3, 112.2, 113.4, , 122.5, 128.7, 129.3, 130.7, 131.1, 131.3, 135.1, 136, 137.6, 138, 140.1, 150.4, 154, 167.7, 201.7/ 522.09
3f	507. 8	60	1760 (C=O), 1230(C-N), 1250 (C-O), 1383 (N-O)/ 1.03 (t, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.38 (q, 2H, OCH_2), 2.48 (d, 2H CH_2),, 3.21 (t, 4H,$(CH_2)_2$), 3.73 (s, 2H, CH_2), 2.81 (s, 2H, CH_2), 6.70 (d, 1H, H-Ar), 7.11 (d, 1H, H-Ar), 7.40 (d, 1H, H-Ar), 7.68 (d, 2H, H-Ar), 8.0 (d, 2H, H-Ar)/ 12.8, 24.5, 49.6, 49.9, 55.8, 100.6, 106.3, 112.2, 113.4, 128.9, 131.1, 131.3, 131.4, 140.1, 154, 160.4, 167.7/ 453.18
3g	499	50	1760 (C=O), 1230(C-N), 1250 (C-O), 1383 (N-O)/ 1.00 (d, 6H, $(CH_3)_2$), 2.26 (s, 3H, CH_3), 2.02 (t, 4H $(CH_2)_2$ peprazine), 2.29 (d, 1H, peprazine), 3.40 (t, 4H,$(CH_2)_2$), 3.73 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 6.70 (d, 1H, H-Ar), 7.11 (d, 1H, H-Ar), 7.40 (d, 1H, H-Ar), 8.0 (d, 2H, H-Ar), 8.09 (d, 2H, H-Ar)/ 12.8, 18.1, 29.5, 46.7, 50.2, 55.8, 63.2, 100.6, 106.3, 113.4, 129.3, 131.1, 131.2, 131.3, 135.1, 140.1154, 160.4, 160.7/ 467.20

Discussion

The synthesized compounds (3a-3g) were evaluated for their physicochemical properties, yield, and biological activities, focusing on hemolysis and thrombolysis percentages (Hafeez, Zahoor, Ahmad, Ahmad, & Faiz, 2019). Analytical data from melting point determination. Compound 3d exhibited the highest yield (66%), possibly due to intermediate stability or favorable reaction conditions. The compounds showed notable trends in hemolytic and

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thrombolytic activities, with compounds 3a, 3d, and 3f exhibiting higher thrombolysis percentages, indicating their potential as thrombolytic agents. Compound 3e showed the least hemolysis (0.7%), suggesting minimal cytotoxicity, which is desirable for therapeutic applications. The structure-activity relationship suggests that compounds with higher substitution and electron-withdrawing groups generally exhibit enhanced thrombolytic activity. The compounds demonstrate promising thrombolytic potential and a balanced cytotoxicity profile, essential for therapeutic safety (Hafeez et al., 2022).

Derivatives	%ageof hemolysis	%age of thrombolysis
3a	45.7 ± 0.132	30 ± 0.128
3b	50.8 ± 0.012	70 ± 0.018
3c	57.7 ± 0.092	66 ± 0.08
3d	0.7 ± 0.012	40 ± 0.018
3e	53.8 ± 0.110	62 ± 0.121
3f	57.9 ± 0.10	65 ± 0.118
3g	37.7 ± 0.10	75 ± 0.118

Conclusion:

The synthesized series of compounds (3a-3g) showed significant potential in terms of structural diversity, yield, and biological activity. Compound 3d had the highest yield (66%), indicating specific structural features. Compounds 3a, 3d, and 3f showed notable thrombolytic activities, while compound 3e had minimal cytotoxicity. Electron-withdrawing groups and structural alterations had a considerable impact on thrombolytic efficacy, according to the structure-activity relationship analytical methodology. These findings suggest further studies on these compounds' therapeutic applications, including mechanistic studies and optimization for enhanced bioactivity.

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