Research Article

Murad Abualhasan*, Nidal Jaradat, Raghad Maslamani, Dana Nofal and Linda Omar

Synthesis of enhanced lipid solubility of indomethacin derivatives for topical formulations

https://doi.org/10.1515/hc-2022-0013
received March 14, 2022; accepted June 10, 2022

Abstract: Indomethacin is a nonselective nonsteroidal anti-inflammatory drug with serious side effects such as depression, hallucination, and gastrointestinal irritation. This study aims to enhance indomethacin lipid solubility of indomethacin derivative to use it for the topical formulation since topical formulation may lower the unwanted side effects. The lipid solubility was achieved by adding various alkyl groups (methyl, ethyl, propyl, and isopropyl) to the drug via an ester linkage. The measured log *p* of these compounds was higher compared to the underivatized indomethacin. Furthermore, an ointment of each ester was formulated and was tested on mice skin using Franz diffusion. The best absorption was observed for methyl indomethacin with threefold increase in permeability compared to indomethacin. This study approves using derivatized indomethacin as a topical formulation with improved efficacy compared to the present gel formulation in the market.

Keywords: indomethacin, franz, diffusion, lipid, transdermal

1 Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as antipyretic, anti-inflammatory, and analgesic agents approved by the Food and drug Administration. These effects are useful for the treatment of muscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, and migraines and are used as opioid-sparing agents in certain acute trauma cases [1–3]. Moreover, they are responsible for approximately 5–10% of all medications prescribed in 1 year. Ninety-six percent of the prevalence of NSAIDs was used in 65-years-old patients in a general practice setting, and at least one NSAID prescription was filled by 7.3% of elderly patients [4–6].

COX-1 is essentially expressed in the body. It is crucial in maintaining gastrointestinal mucosa lining, kidney function, and platelet aggregation. COX-2 is not essentially expressed in the body and is expressed during inflammatory response [7,8]. Since COX-1 is essentially found in the body and has multiple functions, it maintains gastrointestinal mucosa lining by the production of prostacyclin, and its inhibition may cause irritation of the stomach lining and other side effects, while COX-2 works only when inflammatory stimuli is found, which means when the unwanted side effects caused by the inhibition of COX-1. Therefore, the drugs that are more selective toward COX-2 have fewer side effects [9]. As a result, the determination of selective inhibitors of COX-2 will lead to advances in therapy [10].

Indomethacin (Figure 1) is a potent nonselective NSAID. Its importance lies in being an antipyretic, anti-inflammatory, and analgesic agent. Clinicians often use indomethacin in conjunction with disease-modifying antirheumatic drugs in the treatment of rheumatoid arthritis [11].

Several side effects of indomethacin were reported. These side effects include central nervous system effects such as depression, hallucination, gastrointestinal irritation and bleeding, rashes, and some abnormal liver function [12]. Indomethacin has poor skin absorption, and hence, multiple types of researches were performed to enhance its lipid solubility, either through chemical derivatization or through formulation [13,14].

There are some factors responsible for reduced drug efficacy, such as poor absorption, short half-life, and others. Making lipophilic pro-drugs can overcome these problems [15]. Many drug research showed that increasing lipophilicity increases the passage of molecules across cellular barriers, and the highest lipophilicity has the highest intestinal absorption [16,17].

Adding a chemical derivative of the hydrophilic agent with lipophilic pro-moieties can significantly elevate drug diffusion across absorptive membranes [15].

* **Corresponding author: Murad Abualhasan,** Department of Pharmacy, Faculty of Medicine & Health Sciences, An-Najah National University, Nablus, 12345, Palestine,
e-mail: m_abualhasan@najah.edu
Nidal Jaradat, Raghad Maslamani, Dana Nofal, Linda Omar: Department of Pharmacy, Faculty of Medicine & Health Sciences, An-Najah National University, Nablus, 12345, Palestine

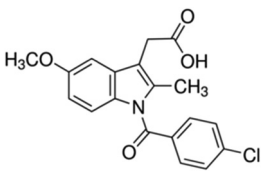

Figure 1: Indomethacin chemical structure.

Transdermal delivery of NSAIDs may be a good strategy for delivering these drugs to the wanted site, but it might be ineffective due to low skin permeability [18].

Several studies have been done to see the efficacy of transdermal delivery against oral delivery, and one of these studies was about vitamin D, where the results showed a promise that transdermal delivery might be an effective administration route for vitamin D3 when ethanol and dodecylamine are used as penetration enhancers [19].

Systemically NSAIDs may cause unwanted adverse effects, but a local application of NSAIDs can support analgesic and anti-inflammatory effects with little risk of causing adverse effects. NSAIDs are usually used topically for 1–2 weeks to help relieve pain caused by musculoskeletal, mainly after injuries, soft tissue pain, and rheumatic diseases [20].

The objective of this study was to synthesize a small library of pro-drug esters of indomethacin for enhanced lipid solubility. The compounds were characterized using proton and carbon nuclear magnetic resonance (NMR). The synthesized compounds were formulated in an ointment topical dosage form. The drug transmission through the skin was tested using the Franz diffusion test.

2 Methodology

2.1 Materials and chemical reagents

Indomethacin powder was given as a gift by Sama Pharmaceutical Company, Palestine. The other reagents that were used in the synthesis and the formulation were purchased from reliable sources: methanol and ethanol were obtained from Sun Pharm Ltd., Palestine; propanol was purchased from Avocado Ltd, Lancs; and isopropanol from Omega Raw Materials Drugstore, Palestine. Hexanol was obtained from Riedel DeHaen, Germany. Benzyl alcohol, sulfuric acid, hexane, octanol, and ethyl acetate were obtained from CS Ltd, and silica, anhydrous sodium sulfate, and diethyl ether was obtained from SDFCL, Mumbai.

2.2 Instruments

The following instruments were used throughout the research:

Infrared (IR) spectrophotometer (Nicolet iS5, Thermo-Scientific) was used to scan all the synthesized indomethacin pro-drug esters using attenuated total reflectance-fourier transform infrared at the range of 500–4,000 nm. Centrifuge (REF 1401, Hettich, Germany) was used to separate compounds. Rota evaporate (modal STONE, ST15 OSA, UK) was used to evaporate extracting solvent. UV/Vis spectrophotometer (Model 7315, Jenway, UK) was used to measure the absorbance. Franz diffusion cell using pump (modal PP-X-575) was used to measure transdermal penetration through mice skin of the synthesized drugs. Other instruments such as magnetic bar stirrer (modal LMS-1003) and Shakers (Model LSB-015S, Korea) were used.

2.3 Synthesis

Indomethacin esters were synthesized by esterification reactions with methanol, ethanol, propanol, and isopropanol reagents. The reaction progress was checked using thin-layer chromatography (TLC; Mineralight® light, Model UVGL-58, USA). The TLC mobile phase used hexane and ethyl acetate at the ratio of 7:3.

The reaction started by weighing (500 mg) indomethacin in a round bottom flask. Subsequently, it was dissolved in 20 mL of reaction solvent (methanol, ethanol, propanol, and isopropanol). Then, four drops of concentrated sulfuric acid (H_2SO_4) were added to the reaction mixture. The reaction was refluxed for 48 h and was checked by TLC. The reaction was neutralized by adding 10 mL sodium bicarbonate. The ester was extracted using 30 mL dichloromethane by the separatory funnel. The organic layer was dried using sodium sulfate. The organic layer was evaporated using a rota evaporator. The dried residue was purified by silica gel column chromatography at the hexane:ethyl atetate (7:3) mobile phase. The synthesis reaction is shown in Figure 2.

Methyl-indomethacin (Comp 1): a pure white semi-solid was obtained (280 mg, 54% yield). IR: ATR, υ_{max} (cm^{-1}): 3375.2 (stretch for carboxylic acid).

^{1}H NMR (dimethyl sulfoxide (DMSO)-d_6, 500 MHz) δ ppm: 2.31 (3H, s, CH_3), 3.35 (2H, s, $-CH_2CO-$), 3.59 (3H, s, $-O-CH_3$), 4.19 (3H, s, $CO-O-CH_3$), 6.59–7.13 (7H, m, Ar–H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 172.5, 153.5, 134.3, 133.1, 132.3, 130.5, 129.1, 112.3, 111.1, 111.5, 110.5, 100.4, 55.7, 39.9, 29.9, 11.8.

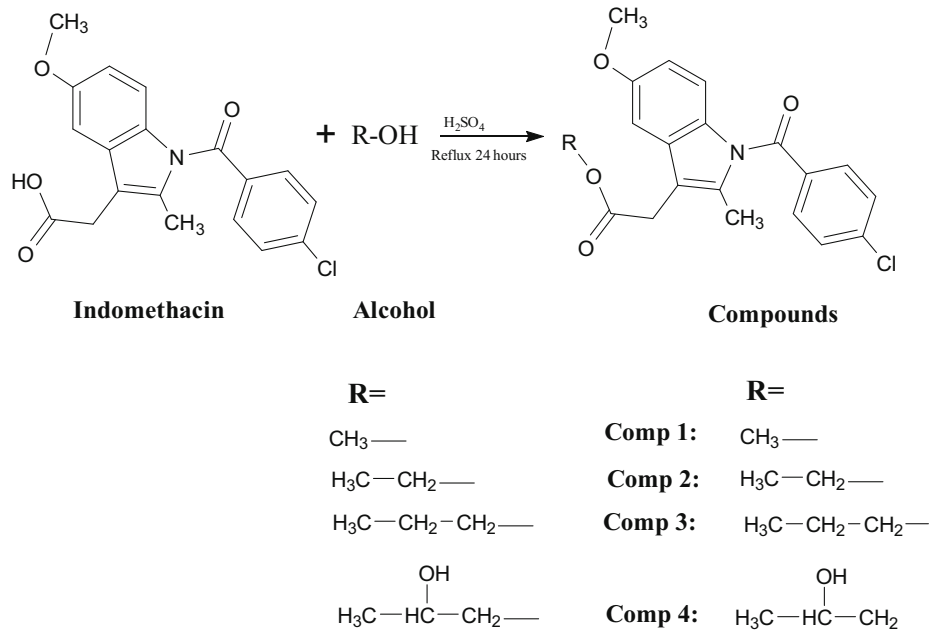

Figure 2: Esterification reaction of Indomethacin.

Ethyl-indomethacin (Comp 2): a pure yellow powder was obtained (310 mg, 57.4% yield). IR: ATR, υ_{max} (cm^{-1}): 3380.2 (stretch for carboxylic acid).

^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.18 (3H, t, CH_3), 2.28 (3H, s, CH_3), 3.39 (2H, s, $-CH_2CO-$), 3.57 (3H, s, $-O-CH_3$), 4.17 (2H, q, $CO-O-CH_2-$), 6.59–7.13 (7H, m, Ar–H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 169.2, 167.7, 154.0, 140.1, 137.6, 135.1, 131.3, 131.1, 129.3, 128.7, 114.5, 113.4, 112.2, 100.6, 61.3, 55.8, 31.9, 14.1, 13.2.

Propyl-indomethacin (Comp 3): a pure yellow powder was obtained (350 mg, 62.5% yield). IR: ATR, υ_{max} (cm^{-1}): 3390.2 (stretch for carboxylic acid).

^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.18 (3H, t, CH_3), 1.73 (2H, p, CH_3CH_2-), 2.28 (3H, s, CH_3), 3.39 (2H, s, $-CH_2CO-$), 3.57 (3H, s, $-O-CH_3$), 4.17 (2H, q, $CO-O-CH_2-$), 6.59–7.13 (7H, m, Ar–H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 170.2, 166.7, 153.0, 141.1, 136.6, 134.1, 131.3, 131.1, 129.1, 126.6, 114.2, 113.1, 111.1, 100.2, 65.2, 54.8, 31.7, 21.7, 12.1, 10.2.

Isopropyl-indomethacin (Comp 4): A pure yellow powder was obtained (290 mg, 51.8% yield). IR: ATR, υ_{max} (cm^{-1}): 3360.2 (stretch for carboxylic acid).

^{1}H NMR (DMSO-d_6, 125 MHz) δ ppm: 1.19 (6H, d, $-(CH_3)_2$), 2.28 (3H, s, CH_3), 3.39 (2H, s, $-CH_2CO-$), 3.57 (3H, s, $-O-CH_3$), 4.97 (1H, hept, $CO-O-CH-$), 6.59–7.13 (7H, m, Ar–H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 168.2, 166.7, 153.0, 136.6, 134.1, 131.3, 131.1, 129.4, 129.1, 128.6, 113.5, 112.4, 111.2, 68.4, 53.8, 31.2, 20.6, 12.2.

2.4 Log *P* determination

Log *P* determination was performed for the synthesized compounds to evaluate the lipophilicity of the synthesized pro-drugs and to judge its suitability for transdermal formulation. The test was done by measuring the concentration of the examined drug between two solvents, namely, octanol and water [21]. The concentrations of indomethacin esters were calculated using the absorbance at the predetermined λ max for each pro-drug determined by a spectrophotometer (Model 7315, Jenway, UK).

A serial standard solutions were prepared from stock solution (1 mg/mL) and were dissolved in octanol (0.1, 0.01, 0.02 mg/mL, and 0.04 mg/mL). The regression line equation from the calibration curve was used to demine the concentration of the pro-drug in octanol and consequently in the water.

To determine the log *P* of the compounds, 10 mg of indomethacin esters were added to 10 mL octanol and 10 mL distilled water in a test tube. The tube was placed on a shaker (Model LSB-015S, Korea) for 30 min at room temperature. Then, it was moved to a centrifuge (REF 1401, Hettich, Germany) for 10 min at 4,000 rounds per min. From the absorbance value of indomethacin esters, the concentration of the indomethacin derivative in both layers was determined, and consequently, the log *P* values were determined.

2.5 Dosage formulation

Ointment formulations were prepared for methyl indomethacin, ethyl indomethacin, propyl indomethacin, and isopropyl indomethacin (0.5% w/w). The ointment formulations were prepared by weighing 50 mg of the active ingredient and were dissolved in benzyl alcohol (0.5 g), which is used as a penetration enhancer. Vaseline (8.2 g) and paraffin (1.25 g) were dissolved at 75°C, and the two mixtures were added to each other and stirred until they cooled.

2.6 Transdermal testing using Franz cell diffusion

The dead animal abdomen skin used in the experimental part was provided by An-Najah National University animal house. The animal house provided the items after taking the required approval from the university ethical committee. All the animal experiments and methods were carried out in accordance with ARRIVE guidelines and regulations.

Franz cell diffusion test was carried out to measure the ability of Indomethacin and its esters to penetrate the skin and reach the blood circulation. The test was carried out by placing an ointment of 50 mg on rat skin. Diffusion cell apparatus was filled with absolute ethanol at 37°C, and the skin was placed above the solvent. Samples aliquots were taken every 30 min over 24 h, and the absorbance was measured by spectrophotometer. The drug concentration was calculated using the regression line equation of the previously generated calibration curve.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

3 Results and discussion

3.1 Synthesis

The proton and carbon NMR results show that all the targeted libraries of indomethacin derivatives were successfully synthesized. Moreover, the IR spectra of the compounds confirm the success of the synthesis of the targeted compound. The IR spectra show a strong stretch at around 1,700 cm^{-1} and the absence of broad starch at 3,500 cm^{-1}, which was present in the nonesterified indomethacin. One of the synthesized compounds, the isopropyl derivative (**comp 4**), was tested for its mass spectrometry. The expected mass was found to be 400.88, and the result was 401.

3.2 Log *P*

Log *P* for the indomethacin esters was calculated using the calibration curve and the absorbance from the experiments. The results showed an increase in the log *P* with an increase in the alkyl chain. The highest log *P* was propyl indomethacin derivative compared (4.63) and was more than isopropyl (4.63). The detailed results are presented in Table 1.

Many researches showed that a good penetration occurs with higher log *P*. The log *P*, along with other factors, will affect the penetration, such as the molecular

Table 1: Log *P* of indomethacin esters

Synthesized compound	Log *P*
Methyl indomethacin	3.57
Ethyl indomethacin	4.10
Propyl indomethacin	4.63
Isopropyl indomethacin	4.44

Table 2: The concentration of the synthesized compounds in the France diffusion cell

Time (hours)	Indomethacin Conc. (mg/10 mL)	Methyl-indomethacin Conc. (mg/10 mL)	Ethyl-indomethacin Conc. (mg/10 mL)	Propyl-indomethacin Conc. (mg/10 mL)	Isopropyl-indomethacin Conc. (mg/10 mL)
0.5	0.0037	0.01078	0.00529	0.00376	0.00457
1	0.00745	0.01326	0.00682	0.00534	0.006
1.5	0.0075	0.01360	0.00777	0.00688	0.00976
2	0.0076	0.01515	0.00838	0.00849	0.012
2.5	0.00864	0.01905	0.00954	0.00905	0.011
3	0.00941	0.02073	0.01045	0.01062	0.0101

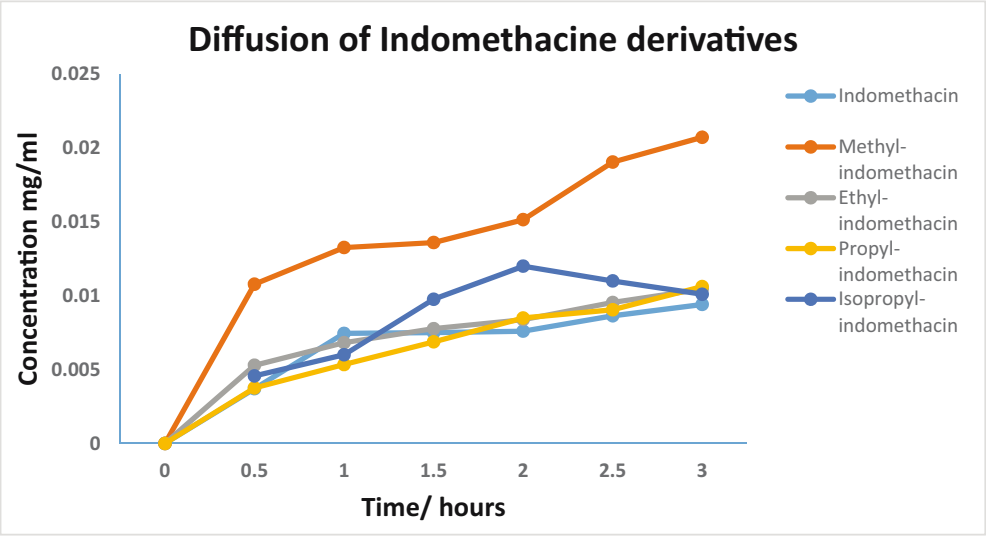

Figure 3: The concentration of indomethacin and its derivatives in the Franz diffusion cell after 3 h.

weight of the synthesized compound [22,23]. The results also show an increase in the log *P* value when compared to underivatized indomethacin, which has a value of 1.72×10^{-1} [24].

3.3 Franz diffusion

All concentrations have been calculated from the absorption value of ethanol in the Franz cell released from the tested ointments. The results show the calculated concentration of each ointment formulation of the derivatized indomethacin using the predetermined regression line equation of the generated calibration curve for the synthesized compound. The result demonstrates the highest release of the drug after 3 hours (0.02073), which is almost equal to 10% of the total drug present in the ointment. However, underivatized indomethacin showed the lowest release (0.00941), which constitutes 3.76 release from the ointment. The detailed results are presented in Table 2. In the market, indomethacin topical preparation is used in a gel dosage. The absorption of this dosage from the gel in the lipophilic dermal is limited. Our novel derivatization approves the improvement of the skin penetration in an ointment formulations.

The concentrations were represented on a graph, which shows the differences between the concentrations as shown in Figure 3.

4 Conclusion

In this study, we successfully synthesized a small library of synthetically modified indomethacin. The synthesized compounds had higher log *P* compared to the parent drug. All the synthesized indomethacin derivatives showed better penetration through the mice's skin. When formulated in an ointment dosage form, methyl-indomethacin showed the best diffusion through the mice's skin. This research concept can be applied to many other drugs with low skin penetration.

Acknowledgment: The authors would like to acknowledge the department of pharmacy An-Najah National University for facilitating the accomplishment of the current work.

Funding information: This work was supported by the deanship of Scientific Research at an Najah National University under fund identification number (ANNU-2021-Sc0011).

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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