

Aspirin Derivatives Exploration: A Review on Comparison Study with Parent Drug

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Abstract

In recent decades, drug modification is no longer unusual in the pharmaceutical world as living things are evolving in response to environmental changes. Aspirin as one of the non-steroidal anti-inflammatory drugs (NSAID) is a common over-the-counter drug due to its analgesic, antipyretic and anti-inflammatory activity. This review article highlights on the recent derivatives of aspirin, which were developed either to reduce the risk of side effects or to obtain better physicochemical properties. Aspirin derivatives can be synthesized in various pathways and have been reported to give better biological activities compared to the parent drug. Nitric oxide (NO)-aspirin gives a potent anticancer drug as it able to inhibit lung and prostate cancer cells. Meanwhile NOSH-aspirin that release hydrogen sulphide (H₂S) and NO moiety is a potent anti-inflammatory agent that stimulate the gastric and colonic secretion, prevent the penetration of acid in gastrointestinal. It also has anticancer action that is effective in hindering the proliferation of pancreatic and colon cancer cells. Aspirin-thiourea has been studied its antimicrobial activity. Still, it resulted in poor inhibition due to steric hindrance of the compounds and influence its penetration into the enzyme's active site. However, aspirin-amide has managed to inhibit the bacterial and fungal, and compound with halogen substituents is reported with the highest inhibition. Aspirin derivative linked with chalcone has poor antibacterial and antioxidant due bulky structure of the compounds, but it has a superior anticancer that induce cancer cells apoptosis by reactive oxygen species (ROS) treatment. The modification of azo-aspirin has more potential in antibacterial activity compared to ampicillin especially when the presence of halogens substituents is involved. Overall, these aspirin derivatives are safe to be considered as potential pharmaceutical agents.

Keywords: Aspirin, Aspirin derivatives, Biological activities, Chemical modification, NSAIDs

Introduction

Drug modification is vital in drug discovery and development process as it is usually done by altering the molecular structure of the formerly characterized lead compound to improve drug potential for treatment of diseases. Some of the chemical alterations are done either by the specification of a particular body target site, modification of time course in the body, or by increasing the rate and degree of absorption. It is also able to improve the potency of drugs, provide the desired feature by decreasing the toxicity or changing the physical as well as chemical properties.

Aspirin is one of the most common over-the-counter drugs, which has been widely known as a fever reducer and anti-inflammatory drug for years. At low dose (75-100 mg), aspirin is selective to inhibit COX-1 activity. As a result of that interaction, aspirin can promote antithrombotic purpose and suppressing platelet aggregation without damaging vascular endothelial cell function which express cyclooxygenase enzyme⁽¹⁾. Thus, it can be used prophylactically in patient with heart attack, high cardiovascular risk, and stroke by taking it in daily low dosage⁽²⁻⁴⁾. However, prolonged use of the aspirin may result in vomiting, major gastrointestinal (GI) bleeding⁽⁵⁾, and other side

effects such as hypertension, renal or GI toxicity due to dosage-related⁽³⁾.

Therefore, aspirin derivatives are being explored in order to get better biological activity. The presence of significant moieties such as nitric oxide (NO), NOSH, thiourea, azo, amide, and chalcone on the modified aspirin plays an important role in achieving desired biological activities. The addition of the halogen in the modification has also become a preference among researchers as it also affects the actions due to its ability to hinder bacterial activity^(6,7).

Aspirin

Aspirin or acetylsalicylic acid (Fig. 1) is an NSAIDS approved by the FDA to be used as antipyretic, antiplatelet, and analgesic agents^(8,9). Aspirin can inhibit the synthesis of prostaglandin by blocking the cyclooxygenase (COX) which contributes to its properties such as anti-inflammatory, antipyretic, antiplatelet, etc. Aspirin is also being considered as a chemopreventive agent because of its antithrombotic effects through the COX's inhibition,^(10,11) and its antioxidant action that inhibit the cancer cells growth by donating their electron to the free radicals that cause proliferation, induce apoptosis or necrosis to the cells^(12,13). However, the prolonged use of aspirin can cause heartburn, ulceration, and gastro-toxicity in children and adults.

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It contains an aromatic ring with carboxyl functional groups. Carboxyl group plays many important roles in pharmaceuticals like acting as solubilizer or cell permeation for antibiotic or antihistaminic drug class, prodrug and/or bio-precursor that activated at specific conditions to act as an antihypertensive, antithrombotic or antiviral⁽¹⁴⁾.

Aspirin is prepared by reacting acetic anhydride and salicylic acid in the presence of acid catalyst (H_2SO_4/H_3PO_4) (Scheme 1). The hydroxyl

group of salicylic acid is converted to an ester, with acetic acid as a byproduct⁽¹⁵⁾.

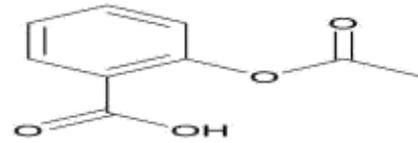
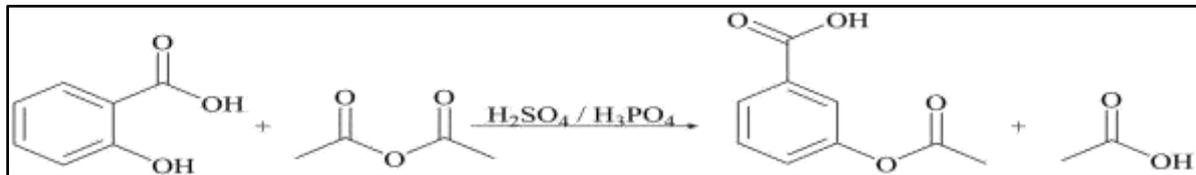


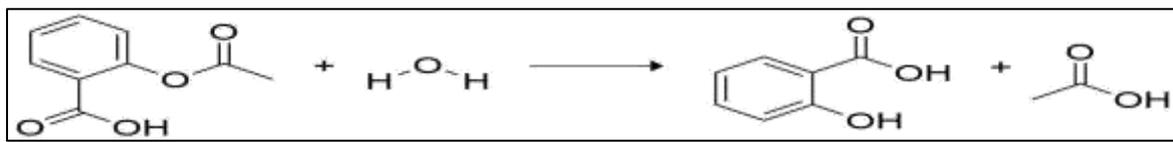
Figure1. Structure of Aspirin



Scheme 1. Synthesis of aspirin

In the body, aspirin mainly absorbed in the stomach and upper part of small intestine after oral administration. It reacts with water in the plasma, liver and within the cells, by esterases to give salicylic acid and acetic acid (Scheme 2)^(16,17). The plasma half-life of salicylic acid is 15-20 min. In the

liver, most of salicylic acid is metabolized into salicyluric acid, salicylic acid and phenolic glucuronides, and a small part of it is metabolized into genistic acid. These metabolites are mainly discharge by the kidneys to the urine⁽¹⁸⁾.



Scheme 2. Hydrolysis of aspirin

However, aspirin lead to GI side effects by reducing mucosal prostaglandin synthesis that affects leukocyte adherence and decrease in bicarbonate, mucus secretion, and blood flow⁽¹⁹⁾. Mucus is mainly secreted from the surface epithelial cell and foveolar cells. The mucus bicarbonate is being used to regulate the pH gradient in the GI

tract⁽²⁰⁾. It protects the stomach from a highly acidic environment. Aspirin will inhibit the synthesis of the prostaglandins and decrease gastric mucus secretion. Thus, mucosal blood flow not maintained effectively, stomach epithelium can be damaged as mucus layer is disrupted⁽²¹⁾.

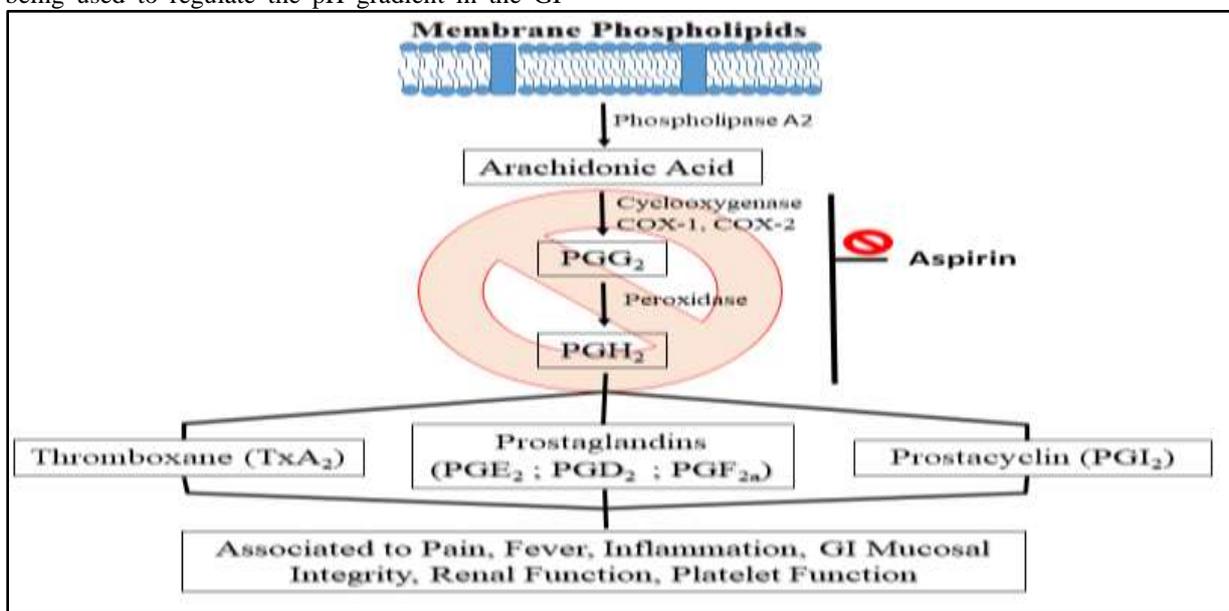
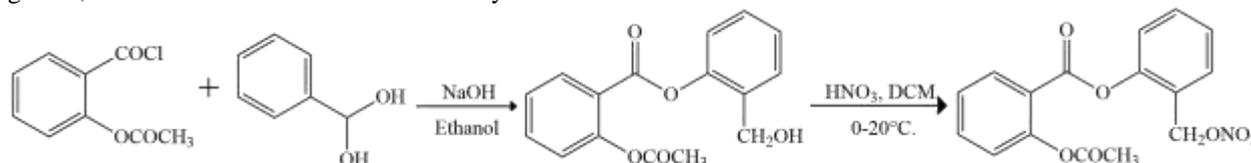


Figure. 2. Mechanism of action of aspirin

Aspirin is an irreversible-COX inhibitor that causes the inhibition of prostaglandin synthesis⁽²²⁾. Aspirin can inhibit both COX-1 and COX-2 (Figure. 2). As it is being administered, the aspirin transfers its acetyl group to a serine residue in the cyclooxygenase (COX) active site, making it unable for arachidonic acid to becoming prostaglandin H₂, resulting in cyclic prostanoid (beckoning the molecules to mediate inflammation and other immune response) not to be synthesized⁽²³⁾. The pharmacological activity of aspirin is proven to be antiplatelet by inhibiting thromboxane A₂ and anti-inflammatory by preventing prostaglandin I₂, E₂, D₂, and F_{2a}. Turning off the COX-1 enzyme can upset the stomach and cause ulcers or GI bleeding.

Aspirin bearing Nitric Oxide Moiety (NO)

NO-aspirin (Fig. 3) is one of gaseous mediator prodrug that is synthesized to improve the efficacy of parent aspirin, and to decrease the side effect that is associated with GI bleeding or ulcer⁽¹⁰⁾. NO is needed to regulate the physiological pathways, particularly regarding the homeostasis of the GI tract. It is usually formed in esophageal, gastric, and intestinal mucosa via the enzymatic



Scheme 3. Synthesis of NO-aspirin

NO-Aspirin hybrids as a promising anticancer activity

One of the research reviews has indicated that the biological analysis by using NO-aspirin derivative (Figure. 3) was associated with reduced GI risk and could be consider as a potentially an anticancer agent⁽²⁸⁾.

NO-aspirin exhibited lower IC₅₀ value (1 μM) in comparison to aspirin alone (>1000 μM). It was reported that proliferating cell nuclear antigen expression was reduced to 54.5%; meanwhile, at G₀/G₁ phases, over 83.9% of tumor cells were blocked after being treated with NO-aspirin⁽²⁹⁾. NO has dual role in anticancer activity depending on the type of cancer, the tumor microenvironment, the type of NO synthase and the concentration of NO itself. A low-rate NO donor will end up with tumorigenesis, whereas a high-rate NO can cause death to cancer cells⁽²⁹⁾. As an anticancer agent, NO has ability to sequester iron into iron-nitrosyl complexes, resulting in a loss of intracellular iron and the inhibition of mitochondrial respiration and DNA synthesis in the tumor cells. Meanwhile, aspirin is also known for its ability to bring cell cycle arrest, apoptosis, and lead to cell proliferation suppression^(30,31).

activity of NO synthases; neuronal (nNOS), endothelial (eNOS), and inducible (iNOS)⁽²⁴⁾.

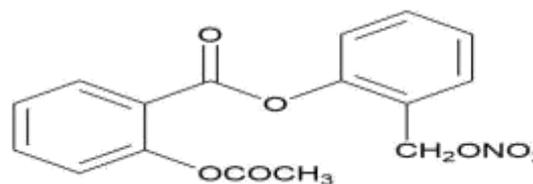


Figure 3. Nitric oxide aspirin (NO-Aspirin)

In various clinical conditions, NO-aspirin is a potential therapeutic agent and typically synthesized by esterification of a NO-releasing moiety to the NSAIDs⁽²⁵⁾. In addition, it has related parts in cancer biology, such as anti-inflammatory and anti-tumor properties, mainly exerted by NO-activated apoptotic pathways⁽²⁶⁾.

The summary of the synthesis of NO-aspirin can be seen in Scheme 3. The halide from salicylic acid derivative react with hydroxybenzylalcohol in the presence of base, giving 2-(hydroxymethyl) phenyl 2-acetoxybenzoate continue reaction with nitric acid in organic acid. It is recrystallized using selected solvent to form final product, NO-aspirin⁽²⁷⁾.

It can be concluded that the hybrid of aspirin and nitrate ester-based on NO donor is significantly potent anti-proliferate and apoptosis induction against the colon tumor cells compared to the aspirin itself^(28,32).

NO- Aspirin as a potential anti-lung cancer

NO-aspirin has been studied as a highly potent in preventing lung cancer within high-risk populaces⁽¹⁰⁾. When NO-aspirin (Fig. 3) was administered, the antiproliferative and apoptotic effect of erlotinib (an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor) considerably increased. The study also indicated that NO-aspirin managed to inhibit inflammation-induced lung tumorigenesis in mice⁽¹⁰⁾.

The drug was used to inhibit the proliferation of tumorigenic bronchial cell line (1170), non-small cell lung cancer (NSCLC), and colony formation by NSCLC cells. Effect of NO-aspirin on the 1170 and NSCLC cells was deduced by MTT (3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide) assay, annexin v/propidium iodide apoptosis assay, colony formation assay, and tumor bioassay using mice⁽¹⁰⁾.

Cell viability assay was used to determine the antiproliferative outcome of NO-aspirin against 1170 and NSCLC cells. The result showed that the proliferation of cells had been reduced in dose-dependent by 30%, 56%, and 71% after being compared to treatment-free cells. Furthermore, the apoptosis effect of cells increased when exposed to NO-aspirin in dose-dependent manner as 8%, 18%, and 24% using flow cytometry-based analysis of Annexin V and PI stained cells⁽¹⁰⁾.

Aspirin inhibited COX and platelets activation, which caused the anticancer effect. Activated platelets were not only able to activate the expression of COX-2 in epithelial cells but also capable of repressing T-cell immunity on cancer⁽³³⁾. It has been suggested another potential mechanism of anticancer since NO-aspirin is unable to balance the COX-2 level in mouse lung tumor tissue. Though, it was clear that phosphorylation of EGFR and the downstream effectors Akt, ERK, and STAT3 in 1170 and NSCLC cells had been restricted by the presence of NO-aspirin⁽¹⁰⁾. There is also a study that found NO moiety caused cell growth, apoptosis, and cancer invasion mostly over phosphorylation transition proteins, PI3K/Akt pathway, and downstream proteins⁽³⁴⁾.

NO-Aspirin as a potential anticancer agent for metastatic prostate cancer

One of the most prevalent malignant tumors identified in men is prostate cancer^(35,36). Most cancer-related death is caused by metastatic castrate-resistant prostate cancer (CRPC)⁽³⁷⁾. Based on the phase of prostate cancer, either surgery, androgen deprivation or chemotherapy can be alternatives for the treatment⁽³⁸⁾. The influence of NO-aspirin inducing apoptosis in metastatic castration-resistant prostate cancer (CRPC) (PC3) cell via hydrogen peroxide (H₂O₂)-mediated oxidative stress has been reported⁽³⁸⁾.

The reactive oxygen species (ROS) or oxygen radical is comprised of both radical and non-radical depend on its reactivity⁽³⁹⁾. Radicals are the species which contain at least one unpaired electron in the shells around the atomic nucleus and are capable of independent existence, such as superoxide radical (O₂^{•-}), hydroxyl (OH), nitrogen monoxide (NO), nitrogen dioxide (NO₂⁻), and etc. While non radical species are not free radicals but can easily lead to free radical reactions in living organisms, for examples hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl), ozone (O₃), and etc.^(38,40). In order to regulate normal physiological functions that are required in development, ROS is crucial. However, excessive levels of ROS harms proteins and membranes, which results in apoptosis or cell death⁽⁴¹⁾. Compared to normal cells, cancer cells are more high-level in ROS, thus causing oxidative stress. Oxidative stress is an inconsistency between the output of ROS in the body that interrupts its ability

to purify reactive immediate or restore the damage to the organ and cellular systems initiated by ROS⁽⁴²⁾. The free radical-induced oxidative stress can damage cellular, tissue, and organ systems, leading to several diseased conditions such as cardiovascular, asthma, and various cancers (colorectal, lung, prostate)^(43,44). The majority of chemotherapeutic drugs display anticancer mechanisms by bringing free radicals into cancer cells^(38,40). For example, nitric oxide as a free radical conjugated with aspirin in order to have anticancer properties to reduce the chance of proliferation of prostate cell cancer.

The PC3 cells viability had been tested for antiproliferative effect by using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, by treating it with numerous concentrations of NO-aspirin and parent compound aspirin. Untreated cells were used as control and incubated according to condition, read under spectrometer, and percent cell viability was recorded⁽³⁸⁾. Anticancer activity was investigated using three methods; colony formation assay, Annexin V-FITC/Propidium Iodide assay, and cell cycle analysis by flow cytometry⁽³⁸⁾.

MTT assay showed that NO-aspirin almost completely inhibited PC3 cells at 100μM, compared to aspirin at 100mM. As anticipated, NO-aspirin is more practical in hindering PC3 cell viability compared to aspirin as an anticancer agent⁽³⁸⁾.

The phosphatidylserine in the Annexin FITC/PI staining gives eat-me signals, making the identified and phagocytosis of dying cancer cells. Thus, apoptotic cells can be elucidated⁽⁴⁵⁾. Histogram of cell cycle analysis indicated that NO-aspirin induced G₀ phase arrest at almost 90% concentration compared to untreated cells. The presence of high concentration of H₂O₂ also leads to cancer cell apoptosis. NO-aspirin has induced oxidative stress via NO group, which turned into H₂O₂, resulting in cell cancer PC3 apoptosis⁽³⁸⁾.

This concluded that NO-aspirin has an anticancer effect on colon, lung, and prostate cell cancer.

NOSH-Aspirin as Anti-Inflammatory and Anticancer Agent

NOSH-aspirin is developed as a substitute aspirin with broader application ranges to decrease the risk of hemorrhage stroke in aspirin users⁽⁴⁶⁾. It is a novel hybrid between hydrogen sulphide (H₂S) and NO moiety covalently bonded at 1, 2 positions of aspirin (ortho-NOSH-aspirin)^(47,48) which is also known as NBS-1120 (Fig. 4)⁽¹⁹⁾. The synthesis summary of the ortho NOSH-aspirin can be seen as scheme 4 below⁽⁴⁸⁻⁵⁰⁾.

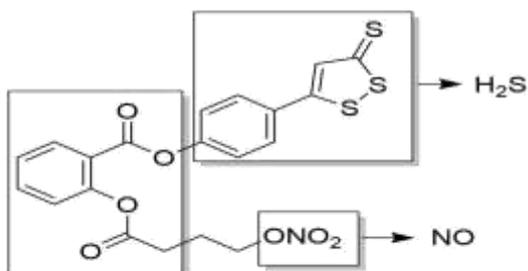


Fig. 4 Structure of NOSH-Aspirin releasing H₂S and NO

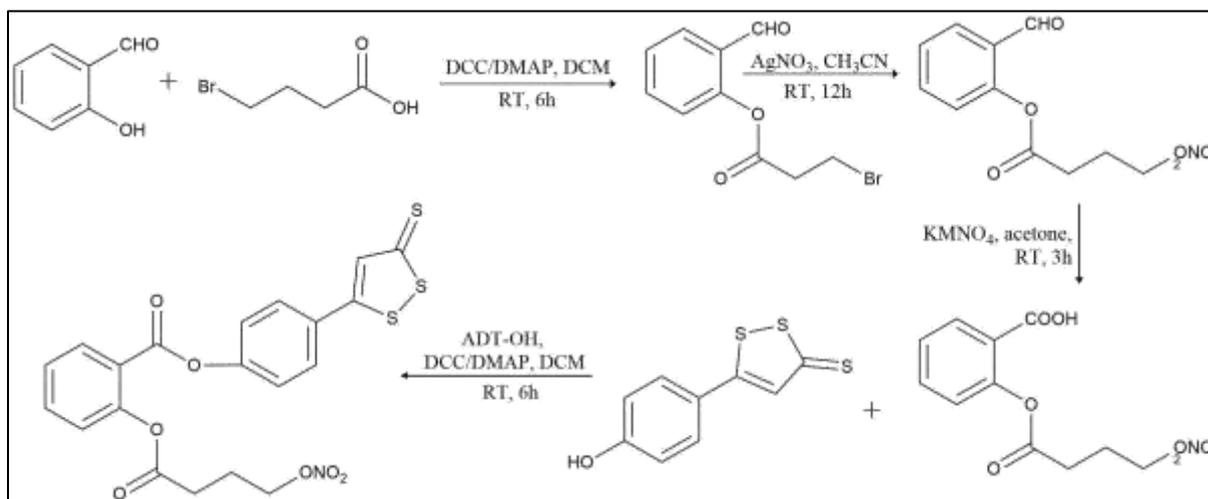
Aspirin and NOSH-aspirin had been evaluated the effect on rats' stomachs when the drugs were being administered orally. After being treated with aspirin, the rats' stomach showed ulceration and bleeding while NOSH-aspirin-treated was free from ulceration⁽⁴⁷⁾. While aspirin regulates prostaglandin, the NO and H₂S donors have the same properties as prostaglandins that protect gastric mucosa^(51,52). The gastric mucosa defense mechanism requires mucus to block the penetration of acid and pepsin by creating a viscous gel layer that assists a pH gradient in the epithelial surface of the stomach, thus blocking the penetration of acid and pepsin. NO, and H₂S donors improve barrier function by stimulating

the gastric and colonic secretion, which leads to reduce GI toxicity^(51,53). Both donors also play a protective role in reducing oxidative stress, which is good in preventing cancer⁽⁵⁴⁾.

NOSH-aspirin is a potent anti-inflammatory agent compared to aspirin parent by using Carrageenan-induced inflammation on rat paw.

Inflammation is usually linked with cancer⁽⁵⁵⁾. As anti-inflammatory agents are capable to hinder with tumor development, they are significant in the prevention and treatment of cancer⁽⁵⁶⁾. Accordingly, there was a study mentioned that NOSH-aspirin showed 5 times more potency in targeting mouse model of colon cancer, which it lessens the cell proliferation and cell cycle arrest leading it to apoptosis⁽⁴⁷⁾.

The latest study on NOSH-aspirin also stated that it was highly potent in inhibition of tumor growth in pancreatic cells. This was due to the ability of the drug to arrest cells in the G₀/G₁ phase transition and caused apoptosis in vitro⁽⁵⁷⁾.



Scheme 4. The Synthesis of the ortho NOSH-Aspirin (NBS-1120)

Aspirin-Thiourea Bearing Alkylated Amine Derivatives as Antimicrobial Agents

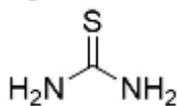


Fig. 5. Thiourea Structure

Thiourea (Fig. 5) is an organosulfur compound with the formula of S=C(NH₂)₂. This compound and its derivatives, in particular, have showed various pharmacological activities such as anti-fungal^(58,59), antiviral^(58,60), anticancer⁽⁶¹⁾, anti-tuberculosis⁽⁶²⁾, antimicrobial⁽⁶³⁾ and anti-

inflammatory⁽⁶⁴⁻⁶⁶⁾. Thiourea has gained significant values since being studied for their application in commercial and industrial applications; plastics, textiles dyes, elastomer, herbicides, pesticides, rodenticides and catalytic^(65,67,68).

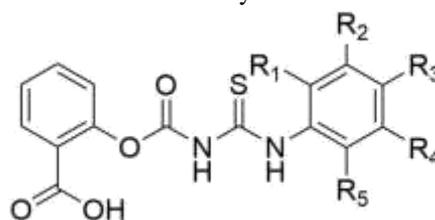
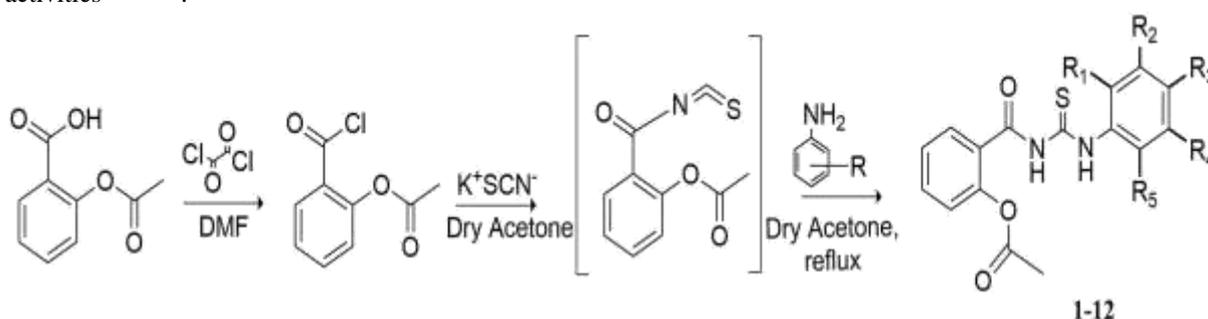


Fig. 6. Structure of Aspirin-Thiourea bearing Alkylated Amine

A study showed that compounds with two or more thiourea moiety hold better antimicrobial activity⁽⁶⁷⁾. Moreover, it gained much attention from researchers as it contains carbon, nitrogen, hydrogen, and sulfur elements⁽⁶⁹⁾. At acidic conditions, C=S and N-H functional groups can be protonated, which gives the thiourea ideal potential site for electrostatic binding on the bacterial surface, which consist of carboxyl and phosphate group (anionic), thus complementing its biological activities^(67,70,71).

Aspirin-thiourea (Fig. 6) with alkylated amine derivative as potential antimicrobial agents has been reported. The synthesis of aspirin-thiourea by reacting acetoxybenzoyl isothiocyanate with series of methyl, methoxy anilines, and alkylated anilines had been prepared through Williamson esterification (Scheme 5)



Compound 1-12:

1,	R ₁ = -CH ₃ ,	R ₂ = -CH ₃ ,	R ₃ = -H,	R ₄ = -H,	R ₅ = -H
2,	R ₁ = -CH ₃ ,	R ₂ = -H,	R ₃ = -CH ₃ ,	R ₄ = -H,	R ₅ = -H
3,	R ₁ = -CH ₃ ,	R ₂ = -H,	R ₃ = -H,	R ₄ = -CH ₃ ,	R ₅ = -H
4,	R ₁ = -CH ₃ ,	R ₂ = -H,	R ₃ = -H,	R ₄ = -H,	R ₅ = -CH ₃
5,	R ₁ = -H,	R ₂ = -CH ₃ ,	R ₃ = -H,	R ₄ = -H,	R ₅ = -H
6,	R ₁ = -OCH ₃ ,	R ₂ = -H,	R ₃ = -H,	R ₄ = -OCH ₃ ,	R ₅ = -H
7,	R ₁ = -H,	R ₂ = -OCH ₃ ,	R ₃ = -OCH ₃ ,	R ₄ = -H,	R ₅ = -H
8,	R ₁ = -H,	R ₂ = -OCH ₃ ,	R ₃ = -H,	R ₄ = -OCH ₃ ,	R ₅ = -H
9,	R ₁ = -H,	R ₂ = -H,	R ₃ = -OC ₆ H ₁₃ ,	R ₄ = -H,	R ₅ = -H
10,	R ₁ = -H,	R ₂ = -H,	R ₃ = -OC ₁₀ H ₂₁ ,	R ₄ = -OCH ₃ ,	R ₅ = -H
11,	R ₁ = -H,	R ₂ = -H,	R ₃ = -OC ₁₂ H ₂₅ ,	R ₄ = -OCH ₃ ,	R ₅ = -H
12,	R ₁ = -H,	R ₂ = -H,	R ₃ = -OC ₁₄ H ₂₉ ,	R ₄ = -OCH ₃ ,	R ₅ = -H

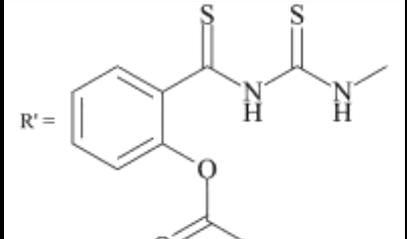
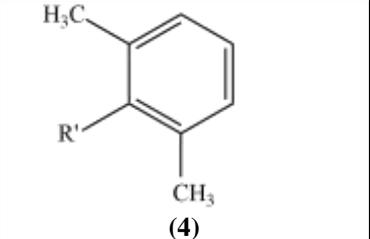
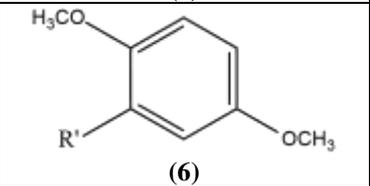
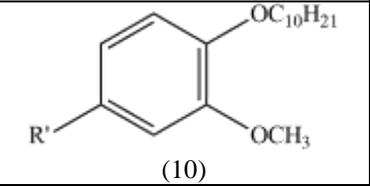
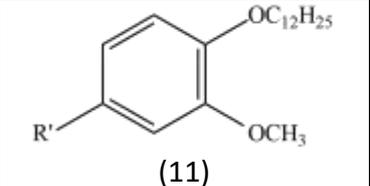
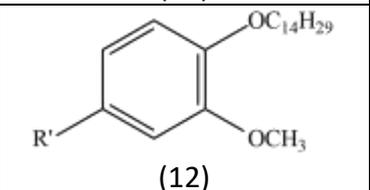
Scheme 5. Synthesis of Aspirin-thiourea Derivatives

The modification of alkylated amine on aspirin-thiourea gave various results on antibacterial activity against *E. coli* and *S. aureus*. The presence of C=O, C=S, and NH group, had increased the activities of antibacterial activities through the

interaction on bacterial surface that contained carboxyl and phosphate group⁽⁷²⁾.

The synthesized compounds 1-12 were studied on their antibacterial activity. However, it was found that compounds 4, 6, 10, 11, and 12 did not give any inhibition towards *E. coli* and *S. aureus*.

Table 1. Results on the alkylated amine on aspirin-thiourea based on their substituents.

R'	Compounds	Result
	 <p>(4)</p>	The presence of methyl (4) and methoxy (6) groups in the structure have reduced the biological activity due to steric hindrance (75)..
	 <p>(6)</p>	
	 <p>(10)</p>	As the alkyl chain increased from the compound (10), (11) and (12), a parabolic effect had been displayed (71)
	 <p>(11)</p>	
	 <p>(12)</p>	
Longer the alkyl chain (>10), gave higher chance to hinder the cell membrane penetration, which prevents inhibition on bacterial growth ⁽⁶³⁾ .		

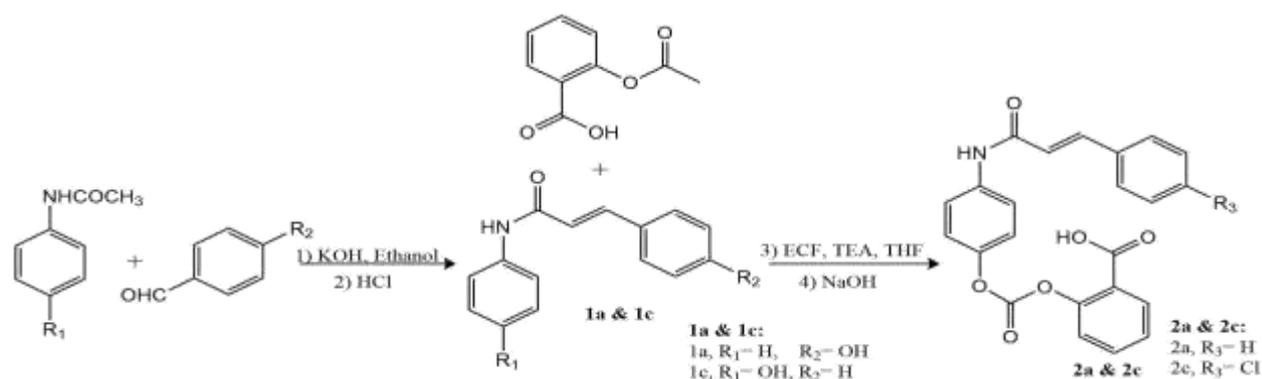
By comparing the result of the biological testing of the prepared derivatives on *E. coli* and *S. aureus*, it was found that *E. coli* was easier to be inhibited⁽⁶³⁾. This is due to the characteristic of *S. aureus* that is hard to penetrate because of its thick peptidoglycan layer that increases cell wall rigidity⁽⁷³⁾.

N- Phenylcinnamide- Aspirin for Antimicrobial and Antifungal Activity

Amide functional group contains (R-N-C=O) has been chemists' choice since it has a lot of potentials. There are amide derivatives reported to be potent anticancer⁽⁷⁴⁾, anti-inflammatory⁽⁷⁵⁾, antioxidant⁽⁷⁶⁾, antibacterial, antifungal, antimalarial^(77,78) and etc. As aspirin also has a lot potential, the modification of aspirin with amide is

being studied, especially the antibacterial and antifungal activity.

The synthesis of N-phenylcinnamide derivatives that linked with aspirin (Scheme 6), started with dissolving the aryl aldehyde, giving substituted acetanilide chalcones compounds. The compounds were linked with aspirin by using mixed anhydride method, producing N-phenylcinnamide-aspirin and continued with antimicrobial screening and antifungal assay⁽⁷⁹⁾. The final product of aspirin-N-phenylcinnamide had three phenyl rings but was able to inhibit *S. aureus* and *E. coli*. Antimicrobial screening found that the compound 2c gave the highest inhibition against *E. coli*, 19 mm but 16 mm for the *S. aureus*, meanwhile 2a gave the highest inhibition of *S. aureus*, 18 mm, but against *E. coli*, 16 mm. As for the antifungal assay, 2c gave the largest inhibition of *C. albicans*, 18 mm, while 2a only gave 10 mm⁽⁷⁹⁾.



Scheme 6. Synthesis of N-phenylcinnamamide-Aspirin of 2a and 2c

Table (2) Results on the antibacterial and antifungal of N-phenylcinnamamide-Aspirin (2a & 2c).

Compounds	Antibacterial		Anti-fungal
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
2a	16 mm	18 mm	10 mm
2c	19 mm	16 mm	18 mm

Although the aspirin with substituted amide (2a) has good antibacterial activity, the presence of -Cl substituent in the compound (2c) is slightly higher. It was determined that its high lipophilicity which penetrated the bacterial cell wall has contributed to the higher success rate of inhibition⁽⁷⁹⁾. This may be caused by the lipophilic characteristic of N-phenylcinnamide due to interaction of its active site to the bacterial cell membrane and gain access to its target and restrained the bacteria^(80,81).

A study reported that halogenation also affects *C. albicans* virulence activity due to their steric effect that provides the best fitting of small molecule to conquer the target's binding site. The -Cl substituent also found to be the most stable halogen that tolerates a steady docking on *C. albicans*. As the result on these studies, -Cl substituent on the compound was found to give the most stable derivatives⁽⁸²⁾.

Aspirin-Chalcone Derivatives

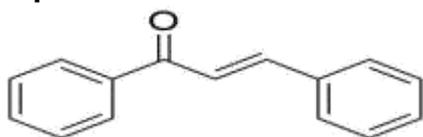


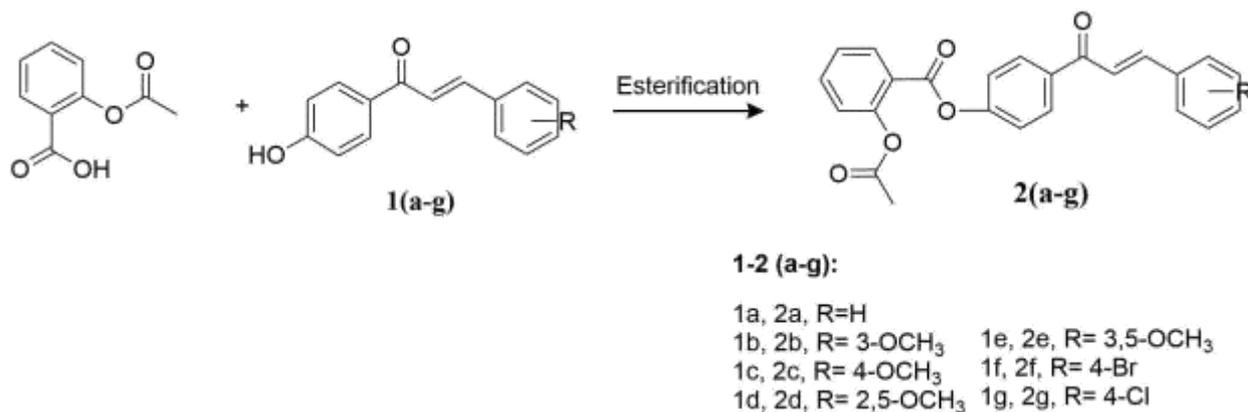
Fig. 7. Chalcone structure

Chalcone (Fig. 7) comprises of two aromatic rings that are highly interconnected by three-carbon α,β -unsaturated ketones that contribute to the pharmacological activity^(83,84). In medicinal chemistry, chalcone is a simple scaffold that originates in countless naturally occurring compounds and is being used widely as an efficacious model for drug discovery⁽⁸⁵⁾. It is stated that chalcones have many benefits, for instance, low interaction with DNA and low-risk of mutagenicity

⁽⁸⁶⁾. Chalcone is known as the precursor for the synthesis of flavonoids, which is practical as antiplatelet⁽⁸³⁾, anticancer⁽⁸⁴⁾, anti-inflammatory, antioxidant, anti-diabetic, and antimicrobial⁽⁸⁷⁻⁹⁰⁾.

Researches on chalcone derivatives reported that chalcones have high antioxidant activity^(91,92). Since antioxidants have the ability to donate electrons, they can neutralize free radicals and prevent any damage to biological compounds in the body⁽⁹³⁾. As chalcones are known as minor flavonoids⁽⁹⁴⁾, they can scavenge free radicals⁽⁹²⁾. Excess of free radicals and ROS (reactive oxygen species) in the human body may cause diseases like cancer, cardiovascular, and cerebrovascular due to damage to lipids, proteins, and nucleic acids⁽⁹⁵⁾. That is the reason why the proper physiological function depends on the balance between free radicals and antioxidants⁽⁹⁶⁾. The accumulation of ROS in the body can be influenced by several factors including endogenous factors such as by-products of mitochondrial activity, exogenous factors including ultraviolet radiation, and even the lack of antioxidant agents in the body such as glutathione, vitamins A, C, and E⁽³⁹⁾. The accumulation of oxidative damage can be prevented by avoiding the excessive ROS formation through optimal functioning of oxygen metabolism and avoidance of environmental pollutants, as well as increasing the neutralization of ROS by having appropriate antioxidant intake⁽⁹⁶⁾. Therefore, a study regarding aspirin-chalcone with antibacterial effect was done. **Aspirin-Chalcone with antibacterial and antioxidant activities**

The synthesis of aspirin-chalcone was done by the reaction of aspirin and chalcone derivatives by esterification, giving aspirin-chalcone (Scheme 7)⁽⁹⁷⁾.



Scheme 7. Synthesis of aspirin chalcone a-g

The antibacterial evaluation of the aspirin-chalcone derivatives was analyzed against *E. coli* and *S. aureus*. However, the result indicated that most derivatives gave no inhibition against *E. coli* and *S. aureus* when compared to ampicillin⁽⁹⁷⁾. The similar result had been also conducted by Ngaini et al. on the previous research., in which aspirin chalcone derivatives gave no inhibition against *E. coli* for antibacterial assay⁽⁹⁸⁾. There is a high possibility it occurs due to *E. coli* is easier to accumulate resistance genes, making it more resistance toward older antibiotics like phenicols, sulfonamides, and trimethoprim⁽⁹⁹⁾. The asymmetric lipopolysaccharide (LPS)-phospholipid bilayer of the outer membrane of *E. coli* causes a weaker permeable barrier for both hydrophobic and hydrophilic compounds⁽¹⁰⁰⁾.

Nevertheless, it was discovered that the aspirin-chalcone displayed poor antioxidant properties on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in comparison to ascorbic acid was due to low phenolic pharmacophore and steric hindrance which also cause bulky structures⁽⁹⁷⁾. The phenolic group was necessary for getting high antioxidant activity⁽¹⁰¹⁾.

The presence of phenyl ring in the compound may also contribute to the bulkiness and cause steric hindrance, making it harder for penetration into phospholipid bilayer of *S. aureus* and *E. coli*^(102,103).

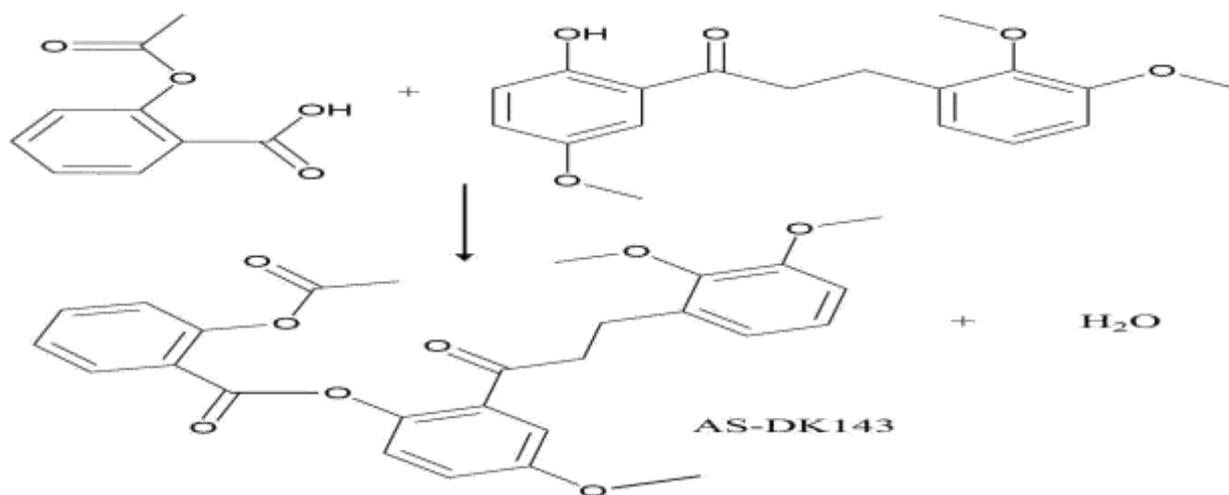
Aspirin-chalcone with anticancer activity

The studies of the aspirin-based drug for anticancer effect have been discovered and encouraged since a while back⁽¹⁰⁴⁾, including breast cancer that is highly prevalent among women worldwide⁽¹⁰⁵⁾. Anticancer drugs usually kill these cancer cells by inducing ROS generation since the high level of ROS causes cell damage as well as apoptosis, autophagic and necrotic cell death^(106,107). Cancer cells have abnormal metabolism; thus, they have higher ROS compared to normal cells, making them more susceptible to ROS-induction treatment⁽¹⁰⁷⁾.

Chalcone derivatives have received significant attention as they exhibited potent anticancer activity against some cancer cell lines, such as naphthalene-chalcone derivatives that displayed potent antiproliferative activity against breast cancer cells (MCF-7)⁽¹⁰⁸⁾. Chalcone inhibited proliferation in MCF-7 by inducing apoptosis and hindering cell cycle development⁽¹⁰⁹⁾ by increasing ROS⁽¹¹⁰⁾. Aspirin also prevents breast tumor cell growth through induction apoptosis⁽¹¹¹⁾. Thus aspirin-conjugated chalcone polymeric micelles for anti-breast cancer activity is an interest⁽¹¹²⁾. Polymeric micelle unsurprisingly is a decent delivery system for anticancer drugs with lower water solubility. At the size of 10-100 nm, it is able to elongate circulation time of the drugs in the blood⁽¹¹²⁾. Aspirin also compromised the condition of chalcone which is a hydrophobic polyphenol with poor aqueous solubility⁽¹¹⁰⁾.

It has been studied that aspirin-conjugated chalcone derivative-loaded nanoparticles (AS-DK143-loaded NP) as potential chemotherapy agents with anticancer efficacy. Synthesis of AS-DK143, which is also known as (E)-2-(2,3-dimethoxyphenyl)acryloyl-4-methoxyphenyl-2-acetoxybenzoate, started with preparation on one of the chalcone derivatives from the previously reported study by the same author, which is known as (E)-3-(2,3-dimethoxyphenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1 or DK143⁽¹¹³⁾.

The method of the synthesis can be seen in Scheme 8. Then the process continued with the preparation of AS-DK143 polymeric micelles by thin-film hydration method. The AS-DK143 undergoes cell viability and animal studies for testing the anticancer effect towards nude mice⁽¹¹⁰⁾.



Scheme 8. Synthesis of AS-DK143-loaded-NP

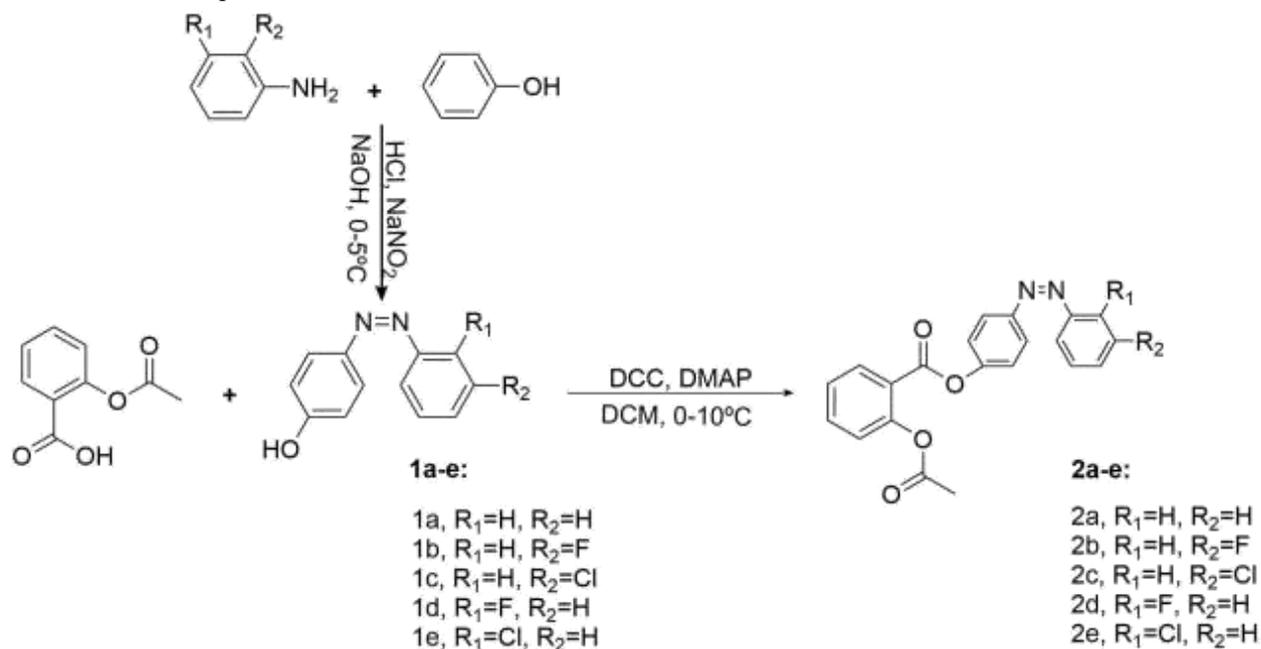
AS-DK143 was synthesized and characterized using ^1H NMR and IR spectroscopy, and the nanoparticles were tested in 4T1 cell viability. The chalcone-based compound can be used as a potent anticancer agent as it induces cancer cell apoptosis by increasing ROS production^(106,110). However, due to its non-polar properties, they increased the bioavailability by interlinking $-\text{OH}$ in DK143 with the polar group of aspirin, $-\text{COOH}$ in the form of nanoparticles⁽¹¹⁰⁾. AS-DK143 showed significant reduction of 4T1 cell viability to 25.97%, $\pm 5.69\%$ and $11.02\% \pm 0.01\%$. It was also found that the IC_{50} of aspirin and AS-D143 gave $4955\mu\text{M}$ and $39.61\mu\text{M}$. Thus proving that the modification of aspirin-chalcone derivative (AS-DK143) against 4T1 cell gave greater anticancer effect at the lowest concentration compared to chalcone derivative

(DK143) itself.

Azo-aspirin with Antibacterial Properties

In order to increase the antibacterial activity, it is recommended to introduce azo moiety into the structure as $-\text{N}=\text{N}-$ is important in bactericidal activities⁽¹¹⁴⁾

The study for antibacterial properties of aspirin derivatives continued on pursuing on azo-aspirin as it gives good biological activity. The synthesis started with a phenol and aniline derivatives and produced azo derivatives. The product reacted with aspirin, becoming azo-aspirin. Further explanation on synthesis can be seen in Scheme 9⁽¹⁰²⁾.

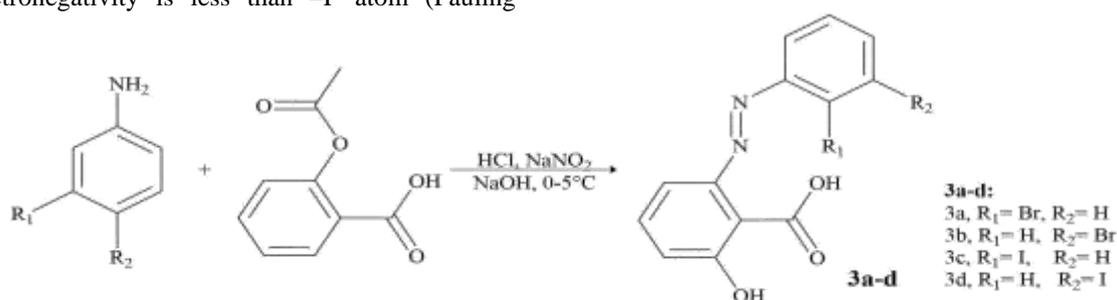


Scheme 9. Synthesis of Azo and Azo-Aspirin Derivatives

E. coli and *S. aureus* had been chosen for the antibacterial activity of azo-aspirin. Presumably, halogen being chosen as substituent, it is due to its high reactivity, which can be deadly to bacteria in a sufficient amount.

The ortho-fluorine aspirin-azo derivatives (compound 2d) gave better antibacterial activity against *E. coli* (156.3 ppm), meanwhile meta-fluorine aspirin-azo (compound 2b) gave better results against *S. aureus* (194.1 ppm) in comparison to parent aspirin (>220 ppm). The result indicated that -F substituted compounds showed superior antibacterial activity compared to -Cl substituted compounds⁽¹⁰²⁾. This is because of larger atomic radius of -Cl atom, which creates a larger steric hindrance than -F. In other hand, although its electronegativity is less than -F atom (Pauling

electronegativity of 4.0), -Cl (Pauling electronegativity of 3.2) can form very strong noncovalent interactions. However, these compounds are not superior antibacterial agents compared to the ampicillin⁽¹¹⁵⁾. Ampicillin disrupt the bacterial cell wall synthesis during active replication and kill the bacterial, making it one of the chosen antibacterial agents used in medicine^(116,117). Few years later, another journal published by the same author reported on halogenated azo aspirin with additional procedures, diazotization followed by coupling reaction (Scheme 10). The presence of halogens, -Br and -I played a significant role in raising the antibacterial activities of the derived compounds compared with the aspirin and ampicillin⁽¹¹⁴⁾.



Scheme 10. The Synthesis of Azo-Aspirin by Diazotization and Coupling Reaction

The -I substituent at ortho position gave the highest inhibition with MIC value, 74 ppm against *E. coli* and 64 ppm against *S. aureus*. Surprisingly, it showed a far superior result as antibacterial agents compared to the ampicillin. Nevertheless, -Br at ortho position also gave high MIC value, 89 ppm for both *E. coli* and *S. aureus*⁽¹¹⁴⁾.

Comparing the result from previous journals, the presence of the halogens affects the inhibition of bacteria in the rate of -Cl < -F < -Br < -I (102,114). Although the -I gave the highest inhibition, it also needs to be considered whether it will affect the other cells or not. The released of -I substance need to be regulated as it can iodinate the lipids that are main component of the cell membrane, and will oxidize various cellular components. Therefore, it can be dangerous towards human skins, or cell as well if the released of the substance is not controlled. However, it does not change the fact that halogens can be strong oxidizing substances that damage the cell wall or membrane of microorganisms which contribute to the bacteriostatic effect⁽¹¹⁸⁾.

The presence of the halogen also improved the lipophilic tendency of azo-aspirin to penetrate the microorganisms' cell walls. The increasing levels of lipophilicity can enhance the ability of compounds to penetrate the cell membranes of gram-negative bacteria which are hydrophobic⁽¹¹⁹⁾. That is one the reasons on why halogens are being considered to improve antibacterial effect of drugs modification.

Meanwhile, the presence of -N=N- (azo) moieties that can be protonated under acidic condition and reacted with phosphate group on the peptidoglycan layer; can hinder the cell wall formation. Then hydrogen bonding will form between the azo-aspirin compound and the active site of the enzyme, causing the bacteriostatic effect⁽¹¹³⁾.

Table (3) Summary of the Aspirin Derivatives and Their Related Biological Activities:

Modification	Authors	Methods	Aim	Results/Finding
NO-Aspirin	Ding <i>et al.</i> ⁽²⁸⁾	-	Anticancer	<ul style="list-style-type: none"> Over 83.9 % of the tumor cells are blocked at G₀/G₁ phase
	Song <i>et al.</i> ⁽¹⁰⁾	<ul style="list-style-type: none"> MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, Annexin v/propidium iodide apoptosis assay, Colony formation assay, Tumor bioassay using mice 	Anti-Lung Cancer	<ul style="list-style-type: none"> The proliferation of the tumor cells is reduced meanwhile the frequency rate of cells' apoptosis is increased
	Chinnapaka <i>et al.</i> ⁽³⁸⁾	<ul style="list-style-type: none"> MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, Colony formation assay, Annexin V-FITC/Propidium Iodide assay, Cell cycle analysis by flow cytometry 	Anti-Prostate Cancer	<ul style="list-style-type: none"> 90% of the tumor cells are inhibited at G₀ phase
NOSH-Aspirin	Kashfi <i>et al.</i> ⁽⁴⁷⁾	<ul style="list-style-type: none"> Carrageenan-induced inflammation on rat paw 	Anti-Inflammation & Anti-Colon Cancer	<ul style="list-style-type: none"> It is 5 times more potent in targeting mouse model of colon cancer, then reduce the cell proliferation and cell cycle arrest leading it to apoptosis
	Chattopadhyay <i>et al.</i> ⁽⁵⁷⁾	<ul style="list-style-type: none"> Cell growth inhibition assay, Cell proliferation, Flow cytometry of phase distribution in the cell cycle Detection of apoptosis 	Anti-Inflammation & Anti-Pancreatic Cancer	<ul style="list-style-type: none"> It reduces gastric mucosa and arrests cells in the G₀/G₁ phase transition which caused apoptosis in vitro.
Aspirin-Thiourea	Nordin <i>et al.</i> ⁽⁶³⁾	<ul style="list-style-type: none"> Synthesis using Williamson esterification, Antibacterial screening against <i>E. Coli</i> and <i>S. Aureus</i> 	Antimicrobial/ Antibacterial	<ul style="list-style-type: none"> The presence of C=O, C=S and N-H give good inhibition, however OCH₃ and CH₃ contribute to steric hindrance, and long alkyl chain (>10) showed parabolic effect

Aspirin-Amide	Alwash <i>et al.</i> ⁽⁷⁹⁾	<ul style="list-style-type: none"> • Synthesis of N-phenylcinnamide using Claisen-Schmidt condensation linked with aspirin, • In-vitro antibacterial and antifungal screening against <i>E. coli</i>, <i>S. aureus</i> and <i>C. albicans</i> 	Antimicrobial & Antifungal	<ul style="list-style-type: none"> • -Cl substituted compound gave the highest inhibition of antibacterial and antifungal. 19 mm, 16 mm and 18 mm of <i>E. coli</i>, <i>S. aureus</i>, and <i>C. albicans</i> • -Cl substituent also found to be the most stable halogen that tolerates a steady docking on virulence-related target
Aspirin-Chalcone	Nordin <i>et al.</i> ⁽⁹⁷⁾	<ul style="list-style-type: none"> • Synthesis of hydroxylated chalcone • Synthesis of aspirin chalcone • Antibacterial screening against <i>E. coli</i> and <i>S. aureus</i> • Antioxidant evaluation using DPPH 	Antibacterial & Antioxidant	<ul style="list-style-type: none"> • Poor inhibition against bacterial and fungal activity. • Bulky structures and lack of phenolic pharmacophore contribute to poor antioxidant activity
	Lee <i>et al.</i> ⁽¹¹⁰⁾	<ul style="list-style-type: none"> • Thin-film hydration method • Cell viability assay 	Anti-Breast Cancer	<ul style="list-style-type: none"> • The cell viability of AS-DK143 against 4T1 cells reduced to 25.97%, $\pm 5.69\%$ and 11.02% $\pm 0.01\%$. • The IC₅₀ of aspirin and AS-D143 gave 4955μM and 39.61μM.
Azo-Aspirin	Ngaini and Ho ⁽¹⁰²⁾	<ul style="list-style-type: none"> • Synthesis of azo • Synthesis of aspirin-azo derivatives • Antibacterial screening against <i>E. coli</i> and <i>S. aureus</i> 	Antibacterial	<ul style="list-style-type: none"> • The ortho-fluorine gave better antibacterial activity against <i>E. coli</i>, meanwhile meta-fluorine aspirin-azo gave better results against <i>S. aureus</i>. • Larger atomic radius of -Cl atom creates larger steric hindrance than -F, making -F substituted compounds good antibacterial agent
	Ngaini and Mortadza ⁽¹¹⁴⁾	<ul style="list-style-type: none"> • Synthesis of azo • Synthesis of aspirin-azo derivatives diazotization followed by coupling reaction • Antibacterial screening against <i>E. coli</i> and <i>S. aureus</i> 	Antibacterial	<ul style="list-style-type: none"> • The -I substituent gave the highest inhibition with MIC value, 74 ppm against <i>E. coli</i> and 64 ppm against <i>S. aureus</i>. • -Br also gave high MIC value, 89 ppm for both <i>E. coli</i> and <i>S. aureus</i> • It gave superior result as antibacterial agents compared to the ampicillin.

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