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True or False? Challenges and Recent Highlights in the Development of Aspirin Prodrugs

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Keywords

Aspirin, prodrug, DMPK, gastrointestinal toxicity, cancer

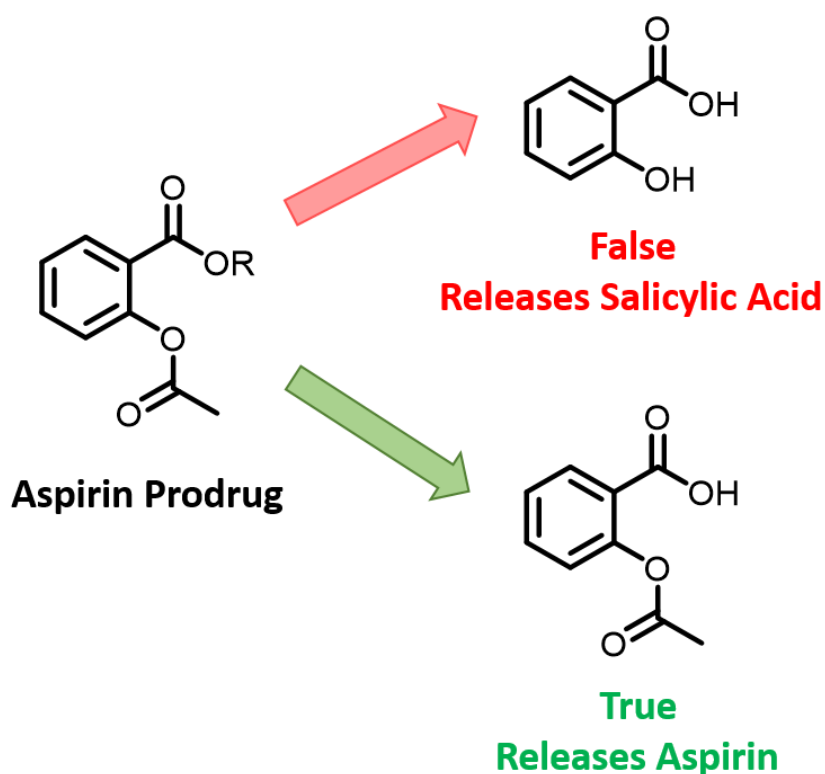
Highlights

- Aspirin use is associated with severe gastrointestinal side-effects.
- Identifying a true aspirin prodrug to overcome this limitation is challenging.
- Mutual prodrugs of aspirin have been explored to reduce toxicity.
- Mutual prodrugs of aspirin have been explored to enhance anti-cancer properties.

Abstract

Aspirin is a widely used medicine for a variety of indications. It is unique amongst non-steroidal anti-inflammatory drugs (NSAIDs) in that it causes irreversible acetylation of COX enzymes. Like all NSAIDs however, aspirin causes severe gastrointestinal side-effects, in particular with chronic administration. Prodrugs of aspirin have been proposed as a solution to these side-effects. However, identifying true prodrugs of aspirin, rather than salicylic acid, has proven challenging. This review details the challenges and highlights recent progress in the development of such prodrugs.

Graphical Abstract



Introduction

Since being marketed by Bayer in 1899, aspirin has continued to be one of the most widely used drugs in the world. It belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and has a number of therapeutic uses: analgesic, anti-inflammatory, anti-platelet and anti-pyretic [1-3].

The most serious and well-known side-effects observed with aspirin and other NSAIDs are gastrointestinal (GI) ulceration and bleeding [4-6]. They can be attributed to the systemic effects of the NSAID on prostaglandin (PG) synthesis, as well as local irritation caused by the carboxylic acid [7]. Aspirin use results in a reduction in the level of PGs that protect the membrane of the stomach from its acidic environment, making it susceptible to lesions [8]. Low-dose everyday use of aspirin is known to be beneficial in reducing risk of secondary heart-attacks [9] and preventing certain cancers [10], but the associated GI side-effects limit its usefulness [11], making a solution to this issue very desirable. COX-2 selective inhibitors (Coxibs) were subsequently developed and marketed in an attempt to reduce GI side-effects, but some have since been removed from the market due to cardiovascular safety concerns [12, 13]. Because of the cardiovascular risk, they are not compatible with chronic use.

Another approach has been to use enteric-coated (EC) aspirin tablets, however their effectiveness is controversial. While numerous studies have demonstrated that acute administration of EC-aspirin reduces GI damage [14-16], a number of meta-analyses and observational studies have found there to be no statistically significant reduction [4, 17], notably for chronic administration [11, 18]. EC-aspirin increases the exposure of the intestines to the drug by allowing it to pass through the stomach, prompting concerns that they increase irritation here instead, where symptoms are harder to detect [19]. EC-aspirin may also not be appropriate for use in the treatment of coronary heart disease [20]. The delayed absorption clearly also delays its therapeutic action. One approach considered to address these issues has been the use of prodrugs. Reviews of prodrugs of other NSAIDs have been recently published [21, 22] and this review will focus particularly on developments towards “true” prodrugs of aspirin as an especially challenging case, as well as emerging medical applications of these prodrugs.

A prodrug is an ideally inactive compound that is metabolised within the body to give the active, parent drug. Whilst prodrugs of aspirin have been reported since the 1980's [23], the difficulty in synthesising so-called ‘true prodrugs’ of aspirin lies in the fact that it contains another labile ester group which can also be hydrolysed (**Figure 1**). Furthermore, esterification of the carboxylic acid group makes this acetyl group even more susceptible to enzymatic cleavage [24]. Therefore, a true aspirin prodrug must be hydrolysed at the promoiety before hydrolysis of the acetyl group, which is inherently challenging. It is worth noting that the absolute rates of the two hydrolysis pathways are not the defining factor, but rather their ratio with respect to the other. As a result, one proposed strategy is to attempt to select a promoiety that slows the rate of deacetylation, thus promoting aspirin formation [25].

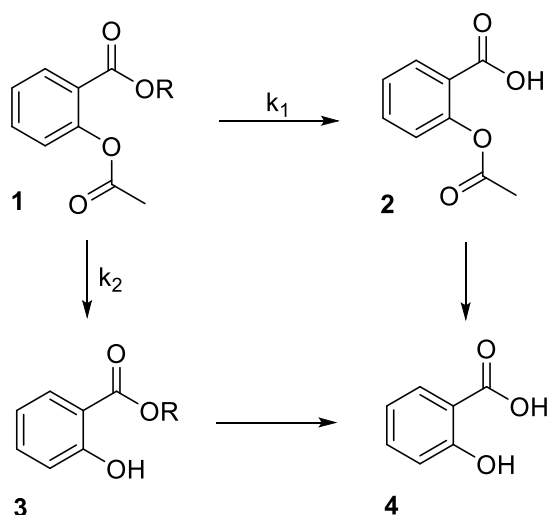


Figure 1: The two competing pathways by which an aspirin prodrug **1** can be hydrolysed to salicylic acid **4**. Hydrolysis usually occurs at the acetyl group first to give a salicylate ester **3**. For a prodrug to release aspirin **2**, k_1 must be greater than k_2 .

Towards “True” Aspirin Prodrugs

In 1979 Hussain *et al.* claimed to have synthesised a novel aspirin prodrug **5** (Figure 2) [23]. They measured the rate of hydrolysis in buffer solutions of varying pH, with detection of aspirin formation by HPLC. Their experiments found that the reaction was first order with respect to prodrug concentration, and that aspirin was formed in a quantitative conversion. Similarly, in 1989 Ankersen and Senning concluded that they had synthesised exclusive aspirin prodrugs, such as compound **6** (Figure 2) on the basis of hydrolysis experiments in buffer solutions [26]. While the buffer solutions they used are able to simulate the broad range of physiological conditions the prodrug will encounter, they only give an insight into non-enzymatic hydrolysis. To consider them as true prodrugs at this stage is therefore premature.

Loftsson *et al.* attempted to synthesise prodrugs of aspirin using methylthiomethyl-based esters as the promoiety, such as compound **7** (Figure 2) [27]. They rationalised that these protecting groups, which had previously been shown to be removable under mild conditions, could be used to temporarily mask the carboxylic acid of aspirin. Their experiments were conducted in 80% human plasma, which is rich in esterase enzymes, and the levels of aspirin, salicylate ester, and salicylic acid release was determined by HPLC analysis. Two of their candidates resulted in quantitative conversion to aspirin, while another was metabolised *via* both pathways. All three potential prodrugs were then evaluated *in vivo* in dogs. Of these, compound **7** was shown to produce high levels of aspirin in the dog, and they thus concluded that it was indeed a true prodrug. The remaining two were metabolised to salicylic acid so rapidly that they could not determine the route of their *in vivo* hydrolysis.

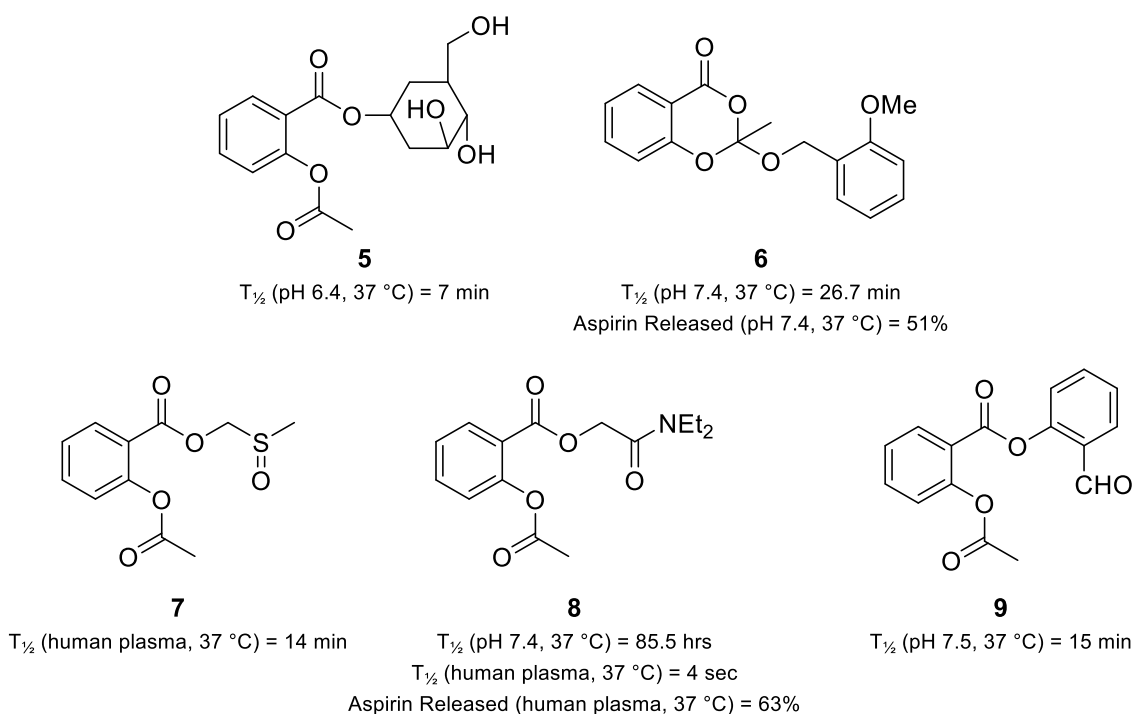


Figure 2: Structures of some of the first aspirin prodrugs synthesised, with reported stability data in various media.

Though the work of Loftsson *et al.* was far more extensive than previous studies, their results were still the subject of some debate. In 1989 Nielsen and Bundgaard conducted human plasma hydrolysis analysis of their glycolamide ester prodrugs, including compound **8** (Figure 2), in addition to analysis of prodrugs previously reported in the literature [25]. In their hands, compound **7** hydrolysed to give only 30% aspirin in plasma rather than a quantitative conversion, but no explanation was proposed for the discrepancy. Two glycolamide esters, including **8**, were found to produce around 60% aspirin. This made **8** one of the most promising true prodrugs of aspirin at that time, with a balance of stability at physiologically relevant pH ranges coupled with rapid hydrolysis to mainly aspirin in human plasma.

Bowden *et al.* reported on the alkaline and neutral hydrolysis of a series of formylphenyl esters (exemplified by compound **9**, Figure 2) of aspirin, including a study of the effect of electron withdrawing groups on the formylphenyl ester on the rate of hydrolysis. Although under alkaline conditions the prodrugs were shown to be true prodrugs of aspirin, and the prodrugs were shown to have some anti-inflammatory effects *in vivo*, evidence of systemic aspirin release *in vivo* was not provided [28, 29].

More recently, in 2008 Moriarty *et al.* claimed the most successful true aspirin prodrug yet, with almost complete conversion in human plasma [30]. Their isosorbide diaspirinate (ISDA) prodrug **10** (Figure 3) was stable across various pH ranges but underwent rapid hydrolysis in human plasma solution releasing 40-60% aspirin, as well as a complex mix of aspirin and salicylate esters [31]. It also showed sustained anti-platelet activity in dogs, characteristic of aspirin release [32]. ISDA can in theory be hydrolysed in four positions, leading to a complex mixture of aspirin, aspirinate-salicylate esters, salicylic acid and isosorbide. Through careful monitoring of metabolite formation over time they determined which metabolic route was actually responsible for productive hydrolysis and aspirin release. Two of the aspirinate-salicylate ester metabolites were then also incubated in human plasma, with compound **11** producing ~70% aspirin. It is therefore a metabolite of ISDA rather than ISDA itself that can be considered the true prodrug of aspirin. Moriarty *et al.* also confirmed ButyrylCholinesterase (BuChE) as a key esterase responsible for enzymatic hydrolysis of aspirin

prodrugs in the human plasma model, with aspirin levels produced decreasing in the presence of a BuChE inhibitor. The authors proposed that the prodrug interacted in a specific manner with BuChE so that the normal preference for acetyl hydrolysis was overcome.

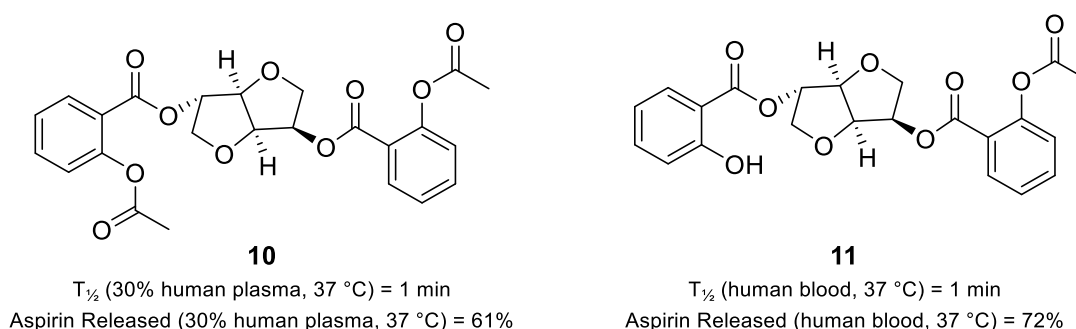


Figure 3: Structures of ISDA prodrug **10** and metabolic intermediate **11**.

In 2016, Foley *et al.* attempted to develop a PepT1 targeting prodrug of aspirin [33], building upon their earlier work with the NSAID nabumetone [34]. The hypothesis was that such transporter targeting prodrugs would be likely to retain high oral bioavailability, which can be an issue for aspirin prodrugs as esterification often reduces aqueous solubility and hence fraction absorbed. Indeed, several of the early aspirin prodrugs illustrated in **Figure 2** were assed for stability only in the presence of quite high volumes of organic co-solvents. However, whilst the nabumetone prodrugs showed affinity for and were substrates of PepT1 *in vitro*, the aspirin prodrug **12** (**Figure 4**) was too unstable in the assay buffer (pH 6.5) to be assessed further *in vitro*.

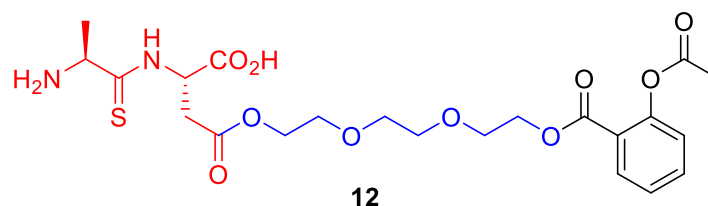


Figure 4: Transporter targeting aspirin prodrug **12**, comprising PepT1 substrate (red) and triethylene glycol spacer (blue).

NO-releasing prodrugs of aspirin

Nitric oxide (NO) is an endogenous molecule that is known to protect the GI tract by maintaining the integrity of gastric mucosa, and repair the damage induced by NSAIDs [35, 36]. Numerous attempts have therefore been made to incorporate NO-releasing groups onto NSAID structure frameworks to counteract their associated GI side-effects. In particular, one NO-releasing derivative of aspirin (NCX-4016), compound **13** (**Figure 5**), was studied extensively in the early 2000s [37-39]. It was shown to almost entirely eliminate gastric damage in healthy human volunteers while maintaining anti-platelet activity, demonstrating proof of concept for this approach [40]. It was taken on as a project by the French pharmaceutical company NicOx, though it was later discontinued when one of its metabolites **14** was found to be mutagenic [41, 42]. Rat liver microsomes were used to assess the metabolism of NCX-4016, and it was concluded that the acetyl group was the most labile, making it a prodrug of salicylic acid [43]. Efforts have since been made to develop mutual prodrugs that upon metabolism would release both aspirin and NO.

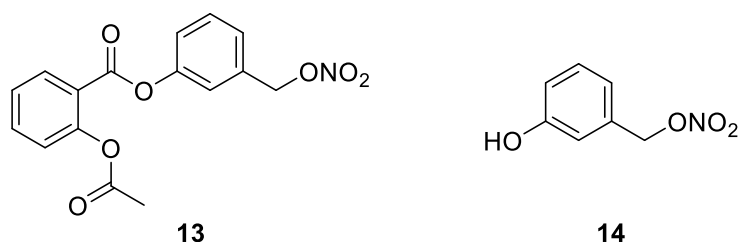


Figure 5: Structure of NCX-4016 (**13**) and its mutagenic NO-releasing metabolite (**14**).

Isosorbide derivatives were believed to be a promising starting point, based on the results obtained by Moriarty *et al.* previously [30]. Starting from the prodrug **10** previously identified (**Figure 3**), Jones *et al.* first simply substituted a nitro-oxy group in place of the salicylate ester, yielding compound **15** (**Figure 6**) [44]. Upon finding that this produced only 8% aspirin in human plasma, the same position was esterified with a wide range of substituents to elucidate their effects on aspirin formation. Nitro-oxy groups were then incorporated onto the promising candidate frameworks. Two of these compounds, including compound **16** (**Figure 6**), were shown to release 30% and 55% aspirin from human plasma by HPLC analysis. LCMS was utilised to confirm the presence of the expected metabolites, but the hydroxymethyl products which would be expected if NO was released could not be seen. They rationalised this by commenting that alkyl nitrates degrade very slowly in plasma, thus concluding that their prodrugs generate aspirin and a possible NO-releasing moiety.

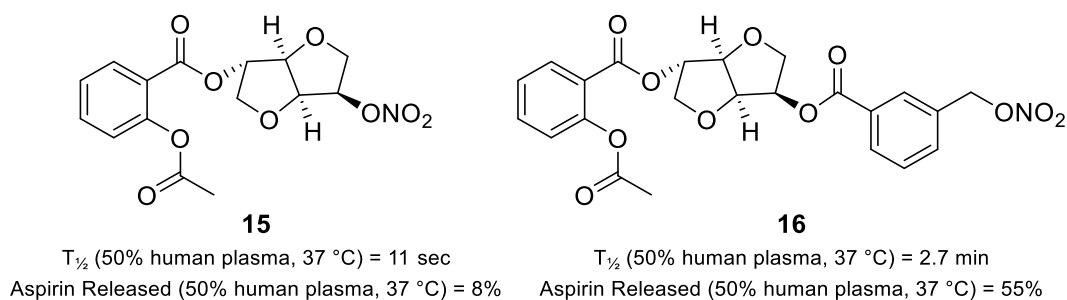
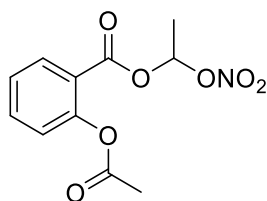


Figure 6: Initial isosorbide -based NO dual prodrug (**15**) and an optimised analogue (**16**).

Gund *et al.* claimed in 2014 to have synthesised a unique NO-releasing true prodrug of aspirin [45]. Their metabolism studies were conducted in simulated gastric and intestinal fluids, as well as 100% human plasma. Under simulated physiological conditions, aspirin was indeed generated quantitatively from the (nitro-oxy) alkyl ester prodrug **17** (**Figure 7**), demonstrating that it was hydrolysed *via* the productive route non-enzymatically, albeit extremely rapidly. However, in the plasma study only 9% aspirin was detected, suggesting that enzymatic hydrolysis was dominated by the non-productive pathway to generate the salicylate ester and then salicylic acid, indicating that in human plasma their molecule was not a true aspirin prodrug. Regardless, they also conducted an *in vivo* study to investigate gastric tolerance of the prodrug compared to parent aspirin in rats. No significant gastric lesions were observed with the prodrug, which they attribute to masking of the carboxylic acid and the function of the released NO. Their work does therefore further highlight the potential for NO-aspirin to be used to reduce gastrointestinal toxicity.



17

$T_{1/2}$ (SGF, pH 1.2, 37 °C) = < 10 min
 Aspirin Released (SGF, pH 1.2, 37 °C) = 99%
 $T_{1/2}$ (SIF, pH 6.8, 37 °C) = < 15 min
 Aspirin Released (SIF, pH 6.8, 37 °C) = 100%
 $T_{1/2}$ (human plasma, 37 °C) = < 5 min
 Aspirin Released (human plasma, 37 °C) = 9%

Figure 7: Alkyl nitrate based NO-releasing prodrug of aspirin (**17**).

Lazzarato *et al.* have synthesised a number of promising NO-releasing aspirin prodrugs [46]. They analysed the hydrolysis of a variety of aliphatic and aromatic nitro-oxy acyloyl derivatives of aspirin in physiological buffer solutions and human serum (for example compound **18**, **Figure 8**). Nearly all were stable to non-enzymatic hydrolysis at various pH ranges, but the amount of aspirin generated in serum varied considerably. Notably, all of the aliphatic derivatives were poor prodrugs of aspirin. However, five of the aromatic derivatives resulted in >58% aspirin formation, with the best candidate, compound **18** (**Figure 8**) releasing 70% aspirin. Formation of the metabolites was followed temporally by HPLC analysis, with the final metabolic products being salicylic acid and the NO-releasing moieties. Due to slow degradation of alkyl nitrates, release of the NO could not be confirmed in this way, as also found by Jones *et al.* [44]. However, they investigated the vasodilator activity of the nitro-oxy metabolites *in vitro*, which is instigated by binding of NO to the soluble guanylyl cyclase (sGC) receptor. The potencies decreased in the presence of an sGC inhibitor, confirming that activation by NO was responsible for the observed responses. These results suggest that the prodrug is capable of releasing both aspirin and NO and is a genuine mutual prodrug.

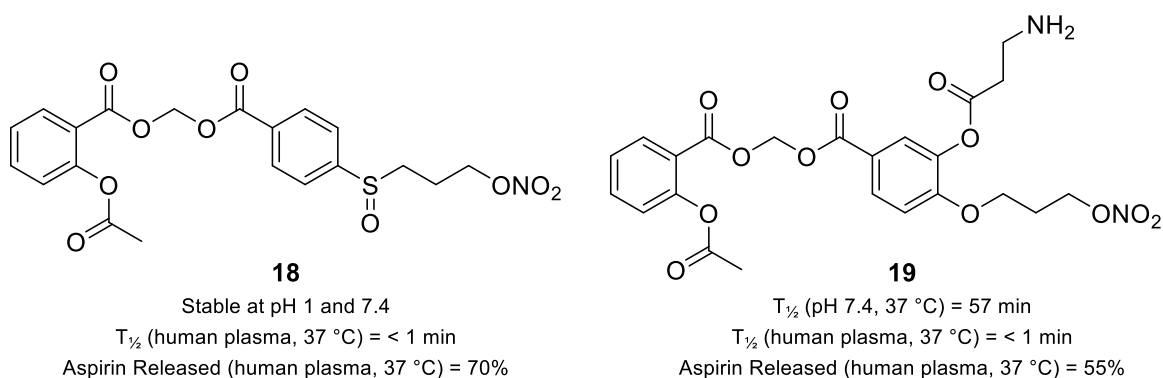


Figure 8: Structures of NO-releasing acyloyl prodrugs of aspirin. **19** was synthesised to improve aqueous solubility.

A disadvantage of the prodrugs synthesised by Lazzarato *et al.* [46] in 2009 was their poor water solubility, thus affecting their suitability for drug formulation. In order to try and develop prodrugs more suitable for clinical application, in 2013 they aimed to modify some of their structures with solubilising moieties [47]. The aromatic acyloyl derivative (**18**) previously reported had the nitro-oxy moiety at the *para* position, so they elected to functionalise at the *meta* position with aminoacyloxy groups (compound **19**, **Figure 8**). Many of these derivatives displayed greater aqueous solubility than aspirin itself, and could still act as true prodrugs in the human serum model, with 50-57% aspirin

generated. The most promising of their candidates, compound **19** (Figure 8), was analysed further and was shown to display vasodilator activity characteristic of NO release, and caused no gastric damage *in vivo* in rats. In addition, it was also found to produce anti-inflammatory and anti-platelet activity comparable to aspirin. They thus concluded that this class of prodrug was an improvement on those previously developed due to the improved aqueous solubility.

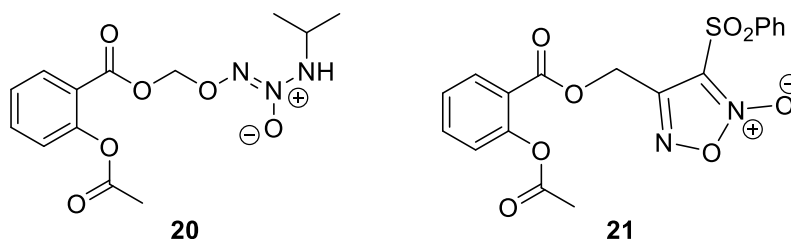


Figure 9: Alternative NO-releasing prodrugs, diazenium-diolates (**20**) or furoxans (**21**). These compounds did not release significant levels of aspirin in human plasma stability studies.

Rather than using alkyl nitrates as the NO-releasing moiety, there has also been interest in the use of diazenium-diolate groups, such as **20** (Figure 9) [48-51]. Whereas alkyl nitrates require a 3-electron reduction to release NO, the diazenium-diolates can liberate it far more easily *via* an esterase-mediated hydrolysis [50]. In addition, the efficiency of alkyl nitrate metabolism can decrease on continued use resulting in nitrate tolerance, so alternatives may be advantageous [49]. However, while they facilitate NO release, there is limited evidence to show that any of the diazenium-diolate derivatives of aspirin reported in the literature are true prodrugs. The derivatives synthesised by Basudhar *et al.* including compound **20** (Figure 9) were shown to hydrolyse at the non-productive acetyl group first [48], while Velazquez *et al.* did not perform any experiments to assess metabolism [49, 50]. Instead, they suggested that aspirin would likely be released *in vivo* based on comparable anti-inflammatory activity of the prodrugs to aspirin in the carrageenan-induced rat paw edema test. However, this could be attributed to differences in bioavailability, and it is worth noting that salicylic acid also possesses some anti-inflammatory activity.

An alternative approach to NO release employed furoxans (compound **21**, Figure 9) as the source of NO [52]. In human plasma, most of the prodrugs were rapidly degraded, but free aspirin was not detected from any prodrug, meaning they did not behave as true aspirin prodrugs. Although the compounds had anti-inflammatory activities and anti-platelet action consistent with an ability to release NO, their lack of acute gastrotoxicity was attributed principally to the masking of the acid by the ester rather than NO release in the GI tract.

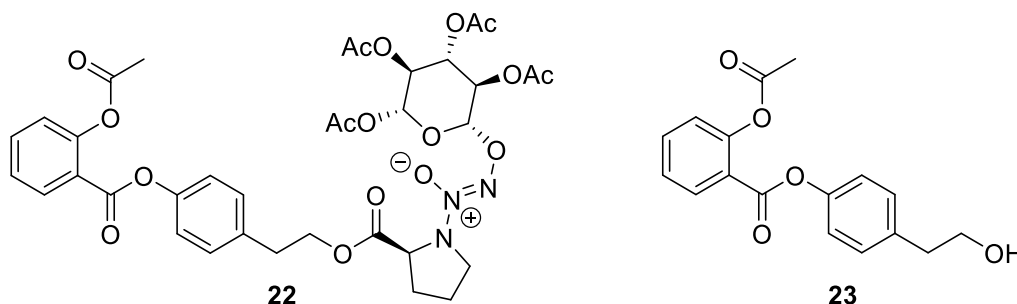


Figure 10: Planned, but not synthesised, proline-tyrosyl NO-aspirin prodrug (**22**). The intermediate (**23**) was shown to have reduced gastrotoxicity despite lacking an NO releasing moiety, however no stability data was reported for (**23**).

A debate has recently arisen in the literature as to whether NO-releasing mutual prodrugs of NSAIDs, including aspirin, offer any additional gastroprotection over simple esterification of the NSAID acid.

Originally aiming to synthesise some new NONO-NSAIDs that incorporated an NO-releasing group derived from L-proline and protected by acetylglucose, connected to an NSAID by way of a tyrosyl spacer (compound **22** **Figure 10**), Jain *et al.* first assessed the ulcerative index of the tyrosyl ester intermediate (**23** **Figure 10**) in rats [53]. They determined that this ester, lacking any NO-releasing moiety, still had gastroprotective effects. This implies that simple esterification of aspirin, provided it is a true prodrug of aspirin, could be sufficient to overcome the GI side effects of aspirin use. However, the fact that (**23**) had activity against the COX enzymes, along with the fact that the intended NO-releasing molecule (**22**, **Figure 7**) was not actually synthesised and tested alongside (**23**) as well as a lack of metabolic stability data for (**23**) makes interpretation of this observation difficult.

Despite considerable research into NO-releasing mutual prodrugs of aspirin, the difficulty of developing a true prodrug still poses a great challenge. There are also still examples in the literature of poorly supported claims of prodrugs of aspirin [45, 49]. Even when promising candidates are identified *in vitro*, their clinical utility may be hindered due to poor physicochemical properties e.g. solubility [46]. However, even though not all of the NO-releasing aspirin derivatives reported are genuine prodrugs of aspirin, they still display promise in their potential to reduce GI side-effects (e.g. NCX-4016), although it is debatable as to whether NO release or simply the masking of the acidic group is responsible for the gastroprotective effects [53]. The advancement also of NO-releasing prodrugs of other NSAIDs, such as Naproxen (compound **24**, **Figure 11**) into late-stage clinical evaluation [54] illustrates the potential application of NO-releasing aspirin prodrugs if such a compound could be identified. Indeed, in 2015 compound **24** secured orphan drug designation from the FDA as a potential treatment for Duchenne Muscular Dystrophy [55, 56].

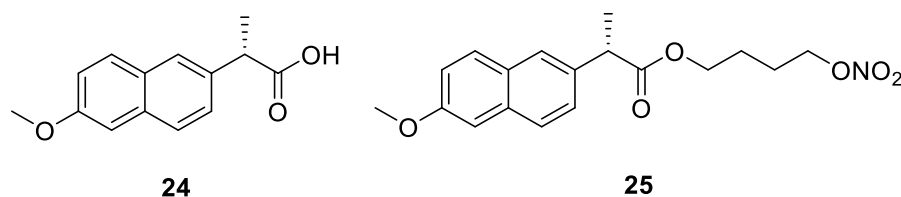


Figure 11: Structures of Naproxen (**24**) and NO-Naproxen (**25**), which advanced to Phase III clinical trials.

Prodrugs of aspirin for anti-cancer applications

Continued use of aspirin has also been reported to lower the risk of developing certain cancers, but questions remain over the long-term risk-benefit [57-63], particularly the gastrointestinal side-effects of long-term aspirin use [10, 63]. Prodrugs have therefore been proposed as a strategy to overcome this. Zhu *et al.* investigated resveratrol-based mutual prodrugs of aspirin (**26**, **Figure 12**), with the aim of capitalising on the chemopreventive properties of both moieties [64]. Resveratrol is a dietary compound found in peanuts, berries and red wine, and is reported to have gastroprotective properties [65]. It was therefore considered an ideal candidate to alleviate the side-effects of aspirin, while retaining anti-cancer and anti-inflammatory activity. These prodrugs were shown to display more cytotoxic activity *in vitro* than resveratrol and aspirin individually, as well as a combination of both compounds. The metabolism of the prodrugs was first examined *in vitro* in cancer cells, then *in vivo* in mice. The *in vitro* results indicated that deacetylation occurred first to give the salicylate ester, suggesting it is not a true prodrug of aspirin. The authors commented on the difficulty of detecting aspirin *in vivo* in mice due to its rapid hydrolysis and short half-life, meaning that its lack of detection did not necessarily confirm its absence. However, given that the salicylate ester could be detected, this in combination with the *in vitro* results suggests that if any aspirin was released it would not be in high quantity.

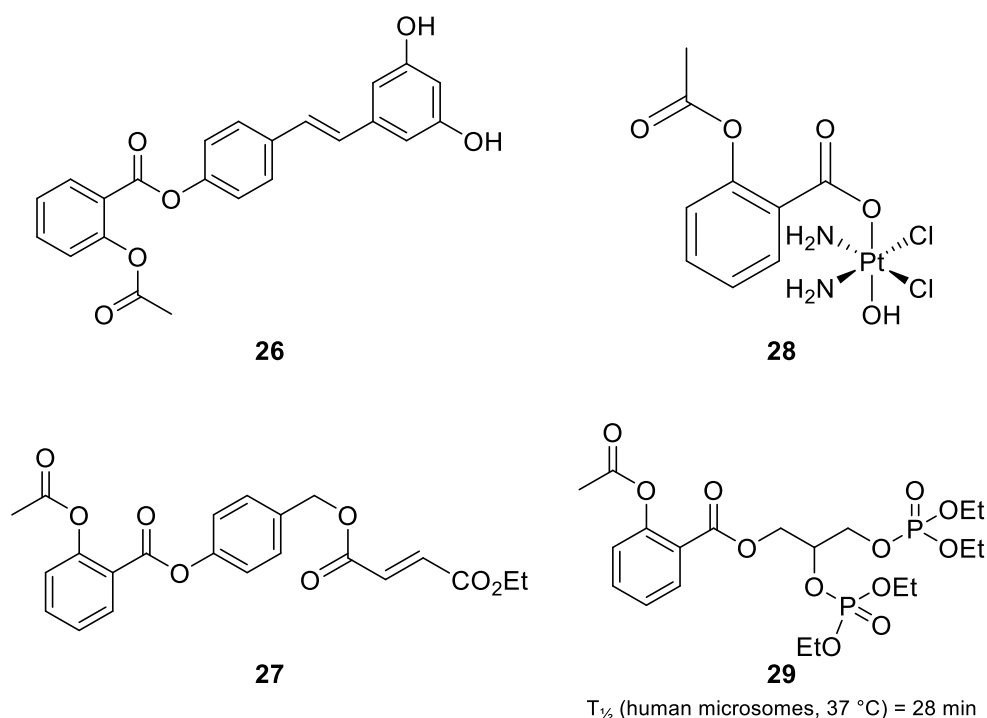


Figure 11: Structures of prodrugs of aspirin for anti-cancer applications.

High dose aspirin is reported to be able to inhibit the NF κ B signalling pathway [66, 67], which when activated can promote cancer growth and metastasis. Kastrati *et al.* investigated whether ester prodrugs of aspirin, including compound **27** (Figure 11) could be synthesised to increase their cell permeability and therefore lower the dose required, while also preventing the associated GI side-effects [68]. Their most promising candidate could potentially inhibit NF κ B signalling in breast cancer cells, but it was found that this activity was not reproducible with any of the expected metabolites, including aspirin. This indicated that the prodrug was only active in its intact form, and would be ineffective once metabolised *in vivo*. No metabolism studies were conducted to identify whether aspirin or salicylate would be released from the prodrug, so the potential of the compound to act as a gastric-sparing anti-inflammatory agent is not known.

In addition to designing prodrugs to mask the toxicity of aspirin, efforts have also been made to synthesise mutual prodrugs for anti-cancer applications. Because inflammation caused by cancer can lead to metastasis, it has been proposed that combining chemotherapy and anti-inflammatory strategies can be effective in managing aggressive cancers. Pathak *et al.* synthesised the platinum (IV) prodrug Platin-A (**28**, Figure 11), which when reduced releases both aspirin and cisplatin [69]. The release of aspirin from the prodrug was measured by HPLC and showed initial production of aspirin, followed by degradation to salicylic acid. However, this reduction experiment was conducted in a water/acetonitrile solution, so it is possible that no or very little aspirin would be seen in the presence of esterases *in vivo*. Regardless, *in vitro* studies demonstrated that Platin-A displayed better anti-cancer and anti-inflammatory activity than a combination of both drugs.

Phospho-aspirin (PA) (**29**, Figure 11), also known as MDC-22 or PA-2, has been reported as having activity in numerous *in vitro* and *in vivo* anti-cancer studies [70-72], as well as in arthritis [73]. However, the only metabolic data reported for the compound, employing liver microsomes from mice, rats and humans demonstrates that it is not a true prodrug of aspirin, with the first step being hydrolysis of the acetate group to give phospho-salicylic acid [74].

Similarly to the development of NO-releasing mutual prodrugs of aspirin, the development of prodrugs of aspirin specifically to improve its anti-cancer properties remains a challenge, with limited evidence of any true aspirin prodrug showing enhanced anti-cancer activity.

Conclusion

Reducing the gastrointestinal toxicity of NSAIDs is a challenge that still needs to be effectively addressed. It is of particular importance for aspirin, which has significant potential in cardioprotective and chemopreventive applications, but this usefulness is limited by its toxicity even at low doses. Shifting this risk/reward balance would therefore have considerable clinical value. Considering that the anti-platelet, cardioprotective activity of aspirin is derived from its unique, irreversible acetylation of COX enzymes [2, 75, 76], it is important that drug candidates for this indication are prodrugs of aspirin rather than salicylic acid.

However, developing true prodrugs of aspirin to prevent GI side-effects remains inherently challenging, due to the preference for hydrolysis at the acetyl position once the carboxylate has been esterified. Many examples exist of prodrugs that hydrolyse non-enzymatically to give aspirin as the exclusive product, but in the presence of esterases are found not to be prodrugs of aspirin at all. While there have been notable examples that hydrolyse *via* the productive pathway enzymatically and *in vivo*, these derivatised compounds have different physicochemical properties to that of parent aspirin, and thus may not be suitable for clinical application without further modification. The most promising of these compounds would appear to be compounds **8** and **10**, and synthetic routes to these molecules are shown in **Figure 12**.

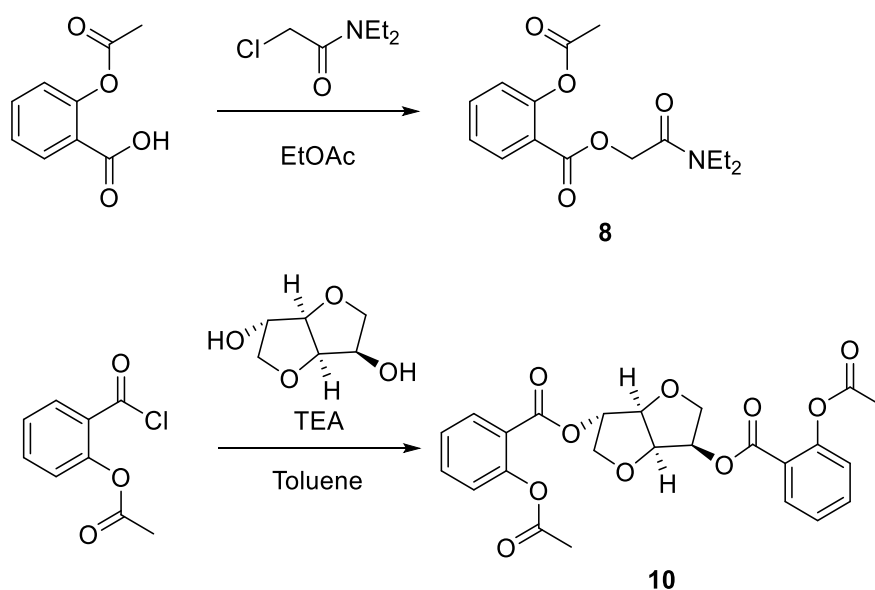


Figure 12 Synthetic routes [25, 31] to the most promising “true” aspirin prodrugs, which show good stabilities at various physiologically relevant pH ranges with rapid cleavage in human plasma to release significant quantities of aspirin. Yields were not quoted for the synthesis of **8** and **10**.

Mutual prodrugs of aspirin have also gained much attention, whereby the promoiety (e.g. NO) can directly display gastroprotective activity, although questions remain as to whether NO release or masking of the acidic functionality by the ester is the true driving force behind the gastroprotective effects. The most advanced of these compounds, NCX-4016 (**13**), progressed into clinical evaluation which was halted only because of a mutagenic metabolite. Metabolic studies also suggest that NCX-4016 is not a true aspirin prodrug.

Furthermore, the possibility of utilising aspirin in a prodrug alongside an anti-cancer agent has been explored. In this case, the anti-inflammatory effects of aspirin can potentiate the anti-cancer activity and may be useful in managing aggressive tumour growth.

A genuine, true prodrug of aspirin must undergo productive hydrolysis under both enzymatic and non-enzymatic conditions. The susceptibility of any prodrug candidate to hydrolysis should first be assessed in buffer solutions simulating physiological conditions. Metabolism should then be assessed in an enzymatic model such as human plasma, which contains a variety of esterases. Microsomal stability can also be employed, but hydrolysis of the prodrug in plasma should be preferred instead of relying on liver metabolism. Promising candidates can then be progressed to *in vivo* studies, however the choice of species chosen for study may be crucial to the detection of relevant metabolites of the prodrug, including aspirin itself. To date, very few examples exist that satisfy all of these conditions. The importance of synthesising and evaluating all of the possible metabolites of a prodrug at an early stage should not be ignored. Not only does it hold value in understanding the metabolic pathway, but it can also highlight potential toxicity pitfalls.

Though simple esterification of aspirin to form a prodrug is a valid approach to protect against its local gastric irritation, it offers no protection against the systemic effects of aspirin on PGs. It is possible that chronic administration of such prodrugs might still lead to GI side-effects, because the gastric mucosa would be subjected to long-term vulnerability as synthesis of protective PGs are inhibited. Mutual prodrugs containing a gastroprotective moiety (e.g. NO) may therefore offer more promise in this regard, but questions remain over potential carcinogenicity risks.

Despite examples reaching the later stages of clinical evaluation, there are currently no commercial prodrugs of aspirin or any of the NSAIDs. The fact that esterification of aspirin alone may be sufficient to offer a solution to the gastric side-effects of aspirin use makes it imperative to continue to search for true aspirin prodrugs. Given the uncertainty that remains around the safety of the coxib class of COX-2 inhibitors and the long-term gastroprotection of enteric formulations, there remains a potentially fruitful gap in the market to be exploited as well as considerable synthetic and DMPK challenges to be solved. A true gastro-sparing aspirin prodrug has the potential to bring real clinical benefit to chronic aspirin patients in particular.

References

1. V. Fuster, J.M. Sweeny. Aspirin. Circulation, 123 (2011), pp. 768-778.
2. G.J. Roth, P.W. Majerus. The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. J. Clin. Invest., 56 (1975), pp. 624-632.
3. J.R. Vane. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. Nature New Biology, 231 (1971), pp. 232-235.
4. L.A. García Rodríguez, S. Hernández-Díaz, F.J. de Abajo. Association between aspirin and upper gastrointestinal complications: systematic review of epidemiologic studies. Br. J. Clin. Pharmacol., 52 (2001), pp. 563-571.
5. M.M. Wolfe, D.R. Lichtenstein, G. Singh. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N. Engl. J. Med., 340 (1999), pp. 1888-1899.
6. A. Lanas, P. Serrano, E. Bajador, F. Esteva, R. Benito, R. Sainz. Evidence of aspirin use in both upper and lower gastrointestinal perforation. Gastroenterology, 112 (1997), pp. 683-689.
7. M. Abdel-Tawab, H. Zettl, M. Schubert-Zsilavec. Nonsteroidal Anti-Inflammatory Drugs: A Critical Review on Current Concepts Applied to Reduce Gastrointestinal Toxicity. Curr. Med. Chem., 16 (2009), pp. 2042-2063.
8. R.T. Schoen, R.J. Vender. Mechanisms of nonsteroidal anti-inflammatory drug-induced gastric damage. Am. J. Med., 86 (1989), pp. 449-458.

9. A.T. Collaboration. Collaborative overview of randomised trials of antiplatelet therapy Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*, 308 (1994), pp. 81-106.
10. M.J. Thun, E.J. Jacobs, C. Patrono. The role of aspirin in cancer prevention. *Nat. Rev. Clin. Oncol.*, 9 (2012), pp. 259-267.
11. H.T. Sorensen, L. Mellekjaer, W.J. Blot, G.L. Nielsen, F.H. Steffensen, J.K. McLaughlin, et al. Risk of upper gastrointestinal bleeding associated with use of low-dose aspirin. *Am. J. Gastroenterol.*, 95 (2000), pp. 2218-2224.
12. S.T. Mathew, G. Devi S, V.V. Prasanth, B. Vinod. Efficacy and Safety of COX-2 Inhibitors in the Clinical Management of Arthritis: Mini Review. *ISRN Pharmacol.*, 2011 (2011), pp. 480291.
13. S.X. Sun, K.Y. Lee, C.T. Bertram, J.L. Goldstein. Withdrawal of COX-2 selective inhibitors rofecoxib and valdecoxib: impact on NSAID and gastroprotective drug prescribing and utilization. *Curr. Med. Res. Opin.*, 23 (2007), pp. 1859-1866.
14. H.G. Dammann, F. Burkhardt, N. Wolf. Enteric coating of aspirin significantly decreases gastroduodenal mucosal lesions. *Aliment Pharmacol. Ther.*, 13 (1999), pp. 1109-1114.
15. A.B. Hawthorne, Y.R. Mahida, A.T. Cole, C.J. Hawkey. Aspirin-induced gastric mucosal damage: prevention by enteric-coating and relation to prostaglandin synthesis. *Br. J. Clin. Pharmacol.*, 32 (1991), pp. 77-83.
16. F.L. Lanza, G.L. Royer, Jr., R.S. Nelson. Endoscopic evaluation of the effects of aspirin, buffered aspirin, and enteric-coated aspirin on gastric and duodenal mucosa. *N. Engl. J. Med.*, 303 (1980), pp. 136-138.
17. J.P. Kelly, D.W. Kaufman, J.M. Jurgelon, J. Sheehan, R.S. Koff, S. Shapiro. Risk of aspirin-associated major upper-gastrointestinal bleeding with enteric-coated or buffered product. *Lancet*, 348 (1996), pp. 1413-1416.
18. S. Derry, Y.K. Loke. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. *BMJ*, 321 (2000), pp. 1183-1187.
19. N.M. Davies. Sustained release and enteric coated NSAIDs: are they really GI safe? *J. Pharm. Pharm. Sci.*, 2 (1999), pp. 5-14.
20. R. Jirmář, P. Widimský. Enteric-coated aspirin in cardiac patients: Is it less effective than plain aspirin? *Cor et Vasa*, 60 (2018), pp. e165-e8.
21. S. Sehajpal, D.N. Prasad, R.K. Singh. Prodrugs of Non-steroidal Anti-inflammatory Drugs (NSAIDs): A Long March Towards Synthesis of Safer NSAIDs. *Mini Rev. Med. Chem.*, 18 (2018), pp. 1199-1219.
22. A.M. Qandil. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. *Int. J. Mol. Sci.*, 13 (2012), pp. 17244-17274.
23. A. Hussain, J. Truelove, H. Kostenbauder. Kinetics and mechanism of hydrolysis of 1-(2'-acetoxybenzoyl)-2-deoxy-alpha-D-glucopyranose, a novel aspirin prodrug. *J. Pharm. Sci.*, 68 (1979), pp. 299-301.
24. H. Bundgaard, N.M. Nielsen, A. Buur. Aspirin prodrugs: synthesis and hydrolysis of 2-acetoxybenzoate esters of various N-(hydroxyalkyl) amides. *Int. J. Pharmaceut.*, 44 (1988), pp. 151-8.
25. N. Moerk Nielsen, H. Bundgaard. Evaluation of glycolamide esters and various other esters of aspirin as true aspirin prodrugs. *J. Med. Chem.*, 32 (1989), pp. 727-734.
26. M. Ankersen, A. Senning. Aspirin prodrugs: synthesis and hydrolysis of 2-benzyloxy-2-methyl-4H-1,3-benzodioxin-4-ones. *Acta Chem Scand.*, 43 (1989), pp. 793-798.
27. T. Loftsson, J.J. Kaminski, N. Bodor. Improved delivery through biological membranes VIII: Design, synthesis, and in vivo testing of true prodrugs of aspirin. *J. Pharmaceut. Sci.*, 70 (1981), pp. 743-749.
28. K. Bowden, A.P. Huntington, S.L. Powell. Prodrugs — Part 1. Formylphenyl esters of aspirin. *Eur. J. Med. Chem.*, 32 (1998), pp. 987-993.

29. E.A. Abordo, K. Bowden, A.P. Huntington, S.L. Powell. Prodrugs Part 3. 2-Formylphenyl esters of indomethacin, ketoprofen and ibuprofen and 6-substituted 2-formyl and 2-acylphenyl esters of aspirin. *Il Farmaco*, 53 (1998), pp. 95-101.
30. L.M. Moriarty, M.N. Lally, C.G. Carolan, M. Jones, J.M. Clancy, J.F. Gilmer. Discovery of a "true" aspirin prodrug. *J. Med. Chem.*, 51 (2008), pp. 7991-7999.
31. J.F. Gilmer, L.M. Moriarty, M.N. Lally, J.M. Clancy. Isosorbide-based aspirin prodrugs: II. Hydrolysis kinetics of isosorbide diaspirinate. *Eur. J. Pharm. Sci.*, 16 (2002), pp. 297-304.
32. J.F. Gilmer, M.A. Murphy, J.A. Shannon, C.G. Breen, S.A. Ryder, J.M. Clancy. Single oral dose study of two isosorbide-based aspirin prodrugs in the dog. *J. Pharm. Pharmacol.*, 55 (2003), pp. 1351-1357.
33. D.W. Foley, R.B. Pathak, T.R. Phillips, G.L. Wilson, P.D. Bailey, M. Pieri, et al. Thiodipeptides targeting the intestinal oligopeptide transporter as a general approach to improving oral drug delivery. *Eur. J. Med. Chem.*, 156 (2018), pp. 180-189.
34. D. Foley, P. Bailey, M. Pieri, D. Meredith. Targeting ketone drugs towards transport by the intestinal peptide transporter, PepT1. *Org. Biomol. Chem.*, 7 (2009), pp. 1064-1067.
35. A. Lanas. Role of nitric oxide in the gastrointestinal tract. *Arthritis Res. Ther.*, 10 Suppl 2 (2008), pp. S4.
36. M.D. Barrachina, J. Panes, J.V. Esplugues. Role of Nitric Oxide in Gastrointestinal Inflammatory and Ulcerative Diseases: Perspective for Drugs Development. *Curr. Pharmaceut. Des.*, 7 (2001), pp. 31-48.
37. C. Emanuelli, S.V. Linthout, M.B. Salis, A. Monopoli, P.D. Soldato, E. Ongini, et al. Nitric Oxide-Releasing Aspirin Derivative, NCX 4016, Promotes Reparative Angiogenesis and Prevents Apoptosis and Oxidative Stress in a Mouse Model of Peripheral Ischemia. *Arter. Thromb. Vasc. Biol.*, 24 (2004), pp. 2082-2087.
38. M.N. Muscará, F. Lovren, W. McKnight, M. Dickey, P. del Soldato, C.R. Triggle, et al. Vasorelaxant effects of a nitric oxide-releasing aspirin derivative in normotensive and hypertensive rats. *Br. J. Pharmacol.*, 133 (2001), pp. 1314-1322.
39. J.L. Wallace, M.N. Muscara, W. McKnight, M. Dickey, P. Del Soldato, G. Cirino. In vivo antithrombotic effects of a nitric oxide-releasing aspirin derivative, NCX-4016. *Thromb. Res.*, 93 (1999), pp. 43-50.
40. S. Fiorucci, L. Santucci, P. Gresele, R.M. Faccino, P. Del Soldato, A. Morelli. Gastrointestinal safety of NO-aspirin (NCX-4016) in healthy human volunteers: a proof of concept endoscopic study. *Gastroenterology*, 124 (2003), pp. 600-607.
41. K. Kashfi. The dichotomous role of H₂S in cancer cell biology? Déjà vu all over again. *Biochem. Pharmacol.*, 149 (2018), pp. 205-223.
42. S. Fiorucci, L. Santucci, E. Distrutti. NSAIDs, coxibs, CINOD and H₂S-releasing NSAIDs: What lies beyond the horizon. *Dig. Liver Dis.*, 39 (2007), pp. 1043-1051.
43. M. Carini, G. Aldini, M. Orioli, R. Maffei Facino. In vitro metabolism of a nitroderivative of acetylsalicylic acid (NCX4016) by rat liver: LC and LC-MS studies. *J. Pharm. Biomed. Anal.*, 29 (2002), pp. 1061-1071.
44. M. Jones, I. Inkielewicz, C. Medina, M.J. Santos-Martinez, A. Radomski, M.W. Radomski, et al. Isosorbide-Based Aspirin Prodrugs: Integration of Nitric Oxide Releasing Groups. *J. Med. Chem.*, 52 (2009), pp. 6588-6598.
45. M. Gund, P. Gaikwad, N. Borhade, A. Burhan, D.C. Desai, A. Sharma, et al. Gastric-sparing nitric oxide-releasable 'true' prodrugs of aspirin and naproxen. *Bioorg. Med. Chem. Lett.*, 24 (2014), pp. 5587-5592.
46. L. Lazzarato, M. Donnola, B. Rolando, K. Chegaev, E. Marini, C. Cena, et al. (Nitrooxyacyloxy)methyl Esters of Aspirin as Novel Nitric Oxide Releasing Aspirins. *J. Med. Chem.*, 52 (2009), pp. 5058-5068.
47. B. Rolando, L. Lazzarato, M. Donnola, E. Marini, S. Joseph, G. Morini, et al. Water-Soluble Nitric-Oxide-Releasing Acetylsalicylic Acid (ASA) Prodrugs. *ChemMedChem*, 8 (2013), pp. 1199-1209.

48. D. Basudhar, G. Bharadwaj, R.Y. Cheng, S. Jain, S. Shi, J.L. Heinecke, et al. Synthesis and chemical and biological comparison of nitroxyl- and nitric oxide-releasing diazeniumdiolate-based aspirin derivatives. *J. Med. Chem.*, 56 (2013), pp. 7804-7820.
49. C.A. Velázquez, Q.-H. Chen, M.L. Citro, L.K. Keefer, E.E. Knaus. Second-Generation Aspirin and Indomethacin Prodrugs Possessing an O²-(Acetoxymethyl)-1-(2-carboxypyrrolidin-1-yl)diazenium-1,2-diolate Nitric Oxide Donor Moiety: Design, Synthesis, Biological Evaluation, and Nitric Oxide Release Studies. *J. Med. Chem.*, 51 (2008), pp. 1954-1961.
50. C.A. Velazquez, P.N. Praveen Rao, M.L. Citro, L.K. Keefer, E.E. Knaus. O²-acetoxymethyl-protected diazeniumdiolate-based NSAIDs (NONO-NSAIDs): synthesis, nitric oxide release, and biological evaluation studies. *Bioorg. Med. Chem.*, 15 (2007), pp. 4767-4774.
51. C. Velázquez, P.N. Praveen Rao, E.E. Knaus. Novel Nonsteroidal Antiinflammatory Drugs Possessing a Nitric Oxide Donor Diazen-1-ium-1,2-diolate Moiety: Design, Synthesis, Biological Evaluation, and Nitric Oxide Release Studies. *J. Med. Chem.*, 48 (2005), pp. 4061-4067.
52. C. Cena, M.L. Lolli, L. Lazzarato, E. Guaita, G. Morini, G. Coruzzi, et al. Antiinflammatory, gastrosparring, and antiplatelet properties of new NO-donor esters of aspirin. *J. Med. Chem.*, 46 (2003), pp. 747-754.
53. S. Jain, S. Tran, M.A. El Gendy, K. Kashfi, P. Jurasz, C.A. Velazquez-Martinez. Nitric oxide release is not required to decrease the ulcerogenic profile of nonsteroidal anti-inflammatory drugs. *J. Med. Chem.*, 55 (2012), pp. 688-696.
54. T.J. Schnitzer, A. Kivitz, H. Frayssinet, B. Duquesroix. Efficacy and safety of naproxinod in the treatment of patients with osteoarthritis of the knee: a 13-week prospective, randomized, multicenter study. *Osteoarthr. Cartilage*, 18 (2010), pp. 629-639.
55. D. Miglietta, C. De Palma, C. Sciorati, B. Vergani, V. Pisa, A. Villa, et al. Naproxinod shows significant advantages over naproxen in the mdx model of Duchenne Muscular Dystrophy. *Orphanet. J. Rare Dis.*, 10 (2015), pp. 101.
56. Nicox Receives Orphan Drug Designation from FDA for Naproxinod in Duchenne Muscular Dystrophy 2015 [cited 2019 09-OCT-2019]. Available from: https://www.drugs.com/nda/naproxinod_150319.html.
57. K.K.F. Tsoi, J.M.W. Ho, F.C.H. Chan, J.J.Y. Sung. Long-term use of low-dose aspirin for cancer prevention: A 10-year population cohort study in Hong Kong. *Int. J. Cancer*, 145 (2019), pp. 267-273.
58. T.G. Simon, Y. Ma, J.F. Ludvigsson, D.Q. Chong, E.L. Giovannucci, C.S. Fuchs, et al. Association Between Aspirin Use and Risk of Hepatocellular Carcinoma. *JAMA Oncol.*, 4 (2018), pp. 1683-1690.
59. M.E. Barnard, E.M. Poole, G.C. Curhan, A.H. Eliassen, B.A. Rosner, K.L. Terry, et al. Association of Analgesic Use With Risk of Ovarian Cancer in the Nurses' Health Studies. *JAMA Oncol.*, 4 (2018), pp. 1675-1682.
60. D.A. Drew, Y. Cao, A.T. Chan. Aspirin and colorectal cancer: the promise of precision chemoprevention. *Nature Rev. Cancer*, 16 (2016), pp. 173-186
61. P.M. Rothwell, M. Wilson, C.-E. Elwin, B. Norrving, A. Algra, C.P. Warlow, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet*, 376 (2010), pp. 1741-1750.
62. T.W. Johnson, K.E. Anderson, D. Lazovich, A.R. Folsom. Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer. *Cancer Epidemiol. Biomarkers Prev.*, 11 (2002), pp. 1586-1591.
63. J. Cuzick, F. Otto, J.A. Baron, P.H. Brown, J. Burn, P. Greenwald, et al. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. *Lancet Oncol.*, 10 (2009), pp. 501-507.
64. Y. Zhu, J. Fu, K.L. Shurknight, D.N. Soroka, Y. Hu, X. Chen, et al. Novel Resveratrol-Based Aspirin Prodrugs: Synthesis, Metabolism, and Anticancer Activity. *J. Med. Chem.*, 58 (2015), pp. 6494-6506.
65. A. Dey, P. Guha, S. Chattopadhyay, S.K. Bandyopadhyay. Biphasic activity of resveratrol on indomethacin-induced gastric ulcers. *Biochem. Biophys. Res. Comm.*, 381 (2009), pp. 90-95.

66. M.J. Yin, Y. Yamamoto, R.B. Gaynor. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature*, 396 (1998), pp. 77-80.
67. E. Kopp, S. Ghosh. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science*, 265 (1994), pp. 956-959.
68. I. Kastrati, V.A. Litosh, S. Zhao, M. Alvarez, G.R. Thatcher, J. Frasor. A novel aspirin prodrug inhibits NFkappaB activity and breast cancer stem cell properties. *BMC Cancer*, 15 (2015), pp. 845.
69. R.K. Pathak, S. Marrache, J.H. Choi, T.B. Berding, S. Dhar. The prodrug platin-A: simultaneous release of cisplatin and aspirin. *Angew. Chem. Int. Ed.*, 53 (2014), pp. 1963-7.
70. G. Mattheolabakis, I. Papayannis, J. Yang, B.M. Vaeth, R. Wang, J. Bandovic, et al. Phospho-Aspirin (MDC-22) Prevents Pancreatic Carcinogenesis in Mice. *Cancer Prev. Res.*, 9 (2016), pp. 624.
71. L. Huang, C.C. Wong, G.G. Mackenzie, Y. Sun, K.W. Cheng, K. Vrankova, et al. Phospho-aspirin (MDC-22) inhibits breast cancer in preclinical animal models: an effect mediated by EGFR inhibition, p53 acetylation and oxidative stress. *BMC Cancer*, 14 (2014), pp. 141.
72. L. Huang, C.C. Wong, K.W. Cheng, B. Rigas. Phospho-Aspirin-2 (MDC-22) Inhibits Estrogen Receptor Positive Breast Cancer Growth Both In Vitro and In Vivo by a Redox-Dependent Effect. *PLoS One*, 9 (2014), pp. e111720.
73. L. Huang, G. Mackenzie, N. Ouyang, Y. Sun, G. Xie, F. Johnson, et al. The novel phospho-non-steroidal anti-inflammatory drugs, OXT-328, MDC-22 and MDC-917, inhibit adjuvant-induced arthritis in rats. *Br. J. Pharmacol.*, 162 (2011), pp. 1521-1533.
74. G. Xie, C.C. Wong, K.W. Cheng, L. Huang, P.P. Constantinides, B. Rigas. In vitro and in vivo metabolic studies of phospho-aspirin (MDC-22). *Pharm. Res.*, 29 (2012), pp. 3292-3301.
75. M.J. Lucido, B.J. Orlando, A.J. Vecchio, M.G. Malkowski. Crystal Structure of Aspirin-Acetylated Human Cyclooxygenase-2: Insight into the Formation of Products with Reversed Stereochemistry. *Biochemistry*, 55 (2016), pp. 1226-1238.
76. M. Lecomte, O. Laneuville, C. Ji, D.L. DeWitt, W.L. Smith. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *J. Biol. Chem.*, 269 (1994), pp. 13207-13215.