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THE 21ST GADDUM MEMORIAL LECTURE

Building a better aspirin: gaseous solutions to a century-old problem

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The gastrointestinal adverse effects of nonsteroidal anti-inflammatory drugs (NSAIDs) have been recognized since shortly after the introduction of aspirin to the marketplace over a century ago. However, the underlying pathogenesis of NSAID-induced gastropathy remains incompletely understood. Advances in understanding some of the factors that contribute to the mucosal injury have provided clues for the development of safer NSAIDs. The inhibitory effects of nitric oxide (NO) on NSAID-induced leukocyte adherence were exploited in the development of NO-releasing NSAIDs. As well as eliciting less gastrointestinal damage than conventional NSAIDs, these drugs do not elevate blood pressure and show anti-inflammatory effects, additional to those of the parent drugs. Modification of other drugs in a similar manner (i.e., NO-releasing derivatives) has similarly resulted in more effective drugs. More recently, hydrogen sulphide-releasing derivatives of NSAIDs and of other drugs, have been developed, based on the observed ability of H_2S to reduce inflammation and pain in experimental models. H_2S-releasing NSAIDs produce negligible gastric damage and exhibit enhanced anti-inflammatory potency as compared to the parent drugs. The NO-NSAIDs and H_2S-releasing NSAIDs represent examples of new anti-inflammatory drugs with greatly reduced toxicity and improved therapeutic activity, both created through the concept of exploiting the beneficial effects of endogenous gaseous mediators.
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Abbreviations: COX, cyclooxygenase; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug

The clinical conundrum

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain the first-line therapy for osteoarthritis and rheumatoid arthritis, despite many advances in the development of 'disease-modifying' agents (such as anti-cytokine therapies) and despite a reasonably high incidence of significant adverse effects. The world market for NSAIDs exceeds £4 billion per year. The market expanded following the introduction of selective cyclooxygenase (COX)-2 inhibiting NSAIDs, but has also been steadily increasing because of ageing populations throughout the developed world (with the concomitant increase in prevalence of age-related diseases, such as arthritis). Use of the original NSAID, aspirin, has also been on the rise because of its perceived beneficial effects in attenuating the incidence of serious cardiovascular events (such as strokes and myocardial infarctions).

As the use of NSAIDs increases, so does the prevalence of NSAID-related adverse events. Most common among these are gastrointestinal (GI) bleeding and ulceration. The incidence of 'clinically significant' GI adverse events with conventional NSAIDs has been estimated at between 2 and 4% (Steen *et al*., 2001). With selective COX-2 inhibitors, the results of large 'outcomes studies' suggest that this rate may be reduced by ~50–70% (Bombardier *et al*., 2000; Schnitzer *et al*., 2004), although the magnitude of the benefit continues to be debated (Juni *et al*., 2002; Hippisley-Cox *et al*., 2005).

Historical perspective

Aspirin entered the marketplace in 1898. It was developed as a better-tasting alternative to salicylate for the treatment of rheumatic conditions, but of course, it turned out to be distinct from salicylate in many ways. An association between its use and dyspepsia was quickly recognized, as it had been with the use of salicylate. However, direct evidence that aspirin caused bleeding in the stomach was not provided until 1938 (Douthwaite and Lintott, 1938).

In the decades that followed, numerous other studies confirmed that aspirin had the ability to induce damage in

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the stomach, and partly in response to this, dozens of new NSAIDs entered the marketplace. In general, these were more potent analgesic and anti-inflammatory agents than aspirin, but shared with aspirin the ability to promote ulceration and bleeding in the GI tract. The mechanism through which these agents elicited the mucosal injury, however, was not known. It was the discovery that NSAIDs inhibited prostaglandin synthesis (Vane, 1971) that provided an essential clue to the pathogenesis of NSAID-induced GI damage. The pioneering studies of Robert and colleagues in the early 1970s established the crucial importance of prostaglandins as mediators of mucosal defence against injury (Robert *et al*., 1976, 1979). Indeed, they provided the basis for the development of prostaglandin analogues as a prophylactic or curative therapy for NSAID-induced GI damage.

Prostaglandins are produced by the gastric mucosa and appear to mediate many of the components of what has been termed 'gastric mucosal defence' (Wallace and Granger, 1996). This includes the maintenance of gastric blood flow during exposure to a noxious substance, secretion of bicarbonate and mucus by the surface epithelial cells, and the rapid repair of superficial injury through the process of epithelial restitution. Studies in the 1980s helped to define better the mechanism through which prostaglandins contributed to mucosal defence and the mechanisms through which NSAIDs impaired the ability of the GI mucosa to resist and respond to damage (reviewed by Wallace, 1997).

While suggestions of the existence of multiple forms of prostaglandin synthase, or COX, date back to 1972 (Flower and Vane, 1972), it was in 1991 that separate discoveries by two groups confirmed the existence of two distinct isoforms of COX (Kujubu *et al*., 1991; Xie *et al*., 1991). This triggered an enormous investment by pharmaceutical companies into the development of selective inhibitors of COX-2. This activity was based on the premise that the prostaglandins mediating inflammation were derived from this isoform, while the prostaglandins involved in protecting the GI tract were derived from COX-1. This has proven to be somewhat an over-simplification, although it is the case that COX-1 is the primary source of prostaglandin synthesis in the normal GI mucosa, and COX-2 appears to be the major source of prostaglandin synthesis at sites of inflammation. However, both COX-1 and COX-2 contribute significantly to GI mucosal defence, and both isoforms must be inhibited to generate mucosal injury in the absence of pre-existing injury (Wallace *et al*., 2000; Tanaka *et al*., 2001). Selective COX-2 inhibitors, such as rofecoxib and lumiracoxib, produce severe GI complications less frequently than conventional (non-selective) NSAIDs. The 'residual' GI damage seen with these agents may be a consequence of the fact that COX-2 is rapidly expressed in response to GI injury (even when quite subtle), and contributes significantly to mucosal defence and repair in these circumstances (Davies *et al*., 1997b; Tanaka *et al*., 2002; Wallace and Devchand, 2005). Other adverse effects of selective COX-2 inhibitors, such as in the renal and cardiovascular systems, continue to be a significant limitation to their use (Mitchell and Warner, 2006; Zarraga and Schwarz, 2007). However, these adverse effects are unlikely to be produced only by selective COX-2 inhibitors. Indeed, there is good evidence that the same adverse effects are associated with the use of conventional NSAIDs (Kearney *et al*., 2006). Thus, although the development of selective COX-2 inhibitors has represented an important advance in addressing the GI toxicity of NSAIDs, it must be viewed only as an incremental advance given that GI and cardiovascular toxicity remain as important limitations to the use of these drugs.

The NO solution

In the 1970s and 1980s, the 'gastroprotective' effects of prostaglandins were the focus of a great deal of research. In the late 1980s and early 1990s, nitric oxide (NO) emerged as another important mediator of mucosal defence. Application to the stomach of a solution of NO or of a NO donor significantly protected the mucosa from injury (MacNaughton *et al*., 1989; Kitagawa *et al*., 1990). However, it was another observation pertaining to the biology of NO that led to a novel approach to the development of GI-sparing NSAIDs.

In the early 1990s, we provided evidence that NSAID-induced acute gastric mucosal damage was a neutrophil-dependent process. Rats that had been immuno-depleted of their circulating neutrophils developed very little gastric damage when given NSAIDs at doses that, in normal rats, caused widespread haemorrhagic lesions (Wallace *et al*., 1990). Moreover, interfering with the adherence of neutrophils to the vascular endothelium, through administration of monoclonal antibodies directed against leukocyte or endothelial adhesion molecules, also greatly reduced the severity of NSAID-induced gastric damage (Wallace *et al*., 1991, 1993). Various NSAIDs were found to trigger leukocyte adherence to the vascular endothelium, and this effect could be reversed by administration of prostaglandins (Asako *et al*., 1992a, b). In the same period of time, NO was demonstrated to be an important modulator of adhesive interactions between leukocytes and the vascular endothelium (Kubes *et al*., 1991). This raised the possibility that protective effects of NO that had been observed in experimental models of gastric damage might be in part due to its ability to inhibit leukocyte–endothelial adhesion. Thus, we postulated that if NO could be delivered in small amounts (that is, not causing systemic hypotension) over a prolonged period of time, NSAID-induced leukocyte adherence should be prevented, as well as the usual reduction of gastric blood flow caused by NSAIDs and, thus, there should be a marked reduction in the severity of gastric mucosal injury (Figure 1). Testing this hypothesis was achieved through collaborative efforts that eventually led to the formation of NicOx S.A., a company with a mission to produce improved therapeutics by linking a NO-releasing moiety to existing anti-inflammatory drugs (an example is shown in Figure 2). We found that so-called 'NO-NSAIDs' (also referred to as 'COX-inhibiting NO donors') produced substantially less GI damage than the parent NSAIDs, despite markedly inhibiting gastric prostaglandin synthesis (Wallace *et al*., 1994a, b). This profile was observed with many different types of NSAIDs, including aspirin (Wallace *et al*., 1995). The presence of the NO-releasing moiety was essential for the GI safety of these derivatives (Wallace *et al*., 2004). The NO-NSAIDs did not

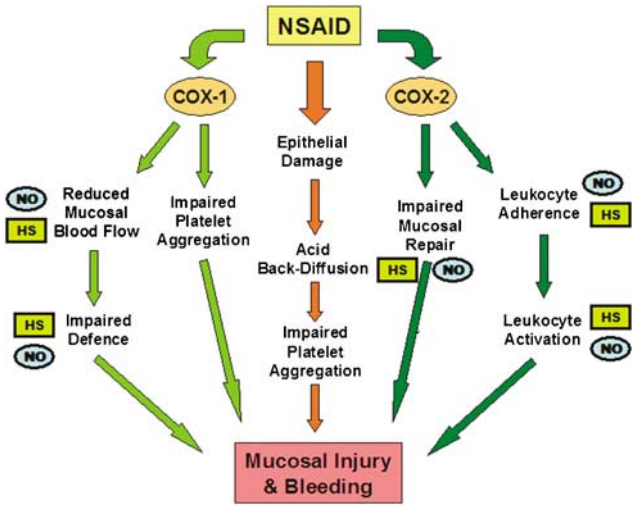

Figure 1 Key steps in the pathogenesis of NSAID-induced gastric mucosal injury and bleeding. The steps where NO- and H_2S-releasing drugs may exert beneficial effects are marked by NO (on blue) and HS (on yellow), respectively. COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug.

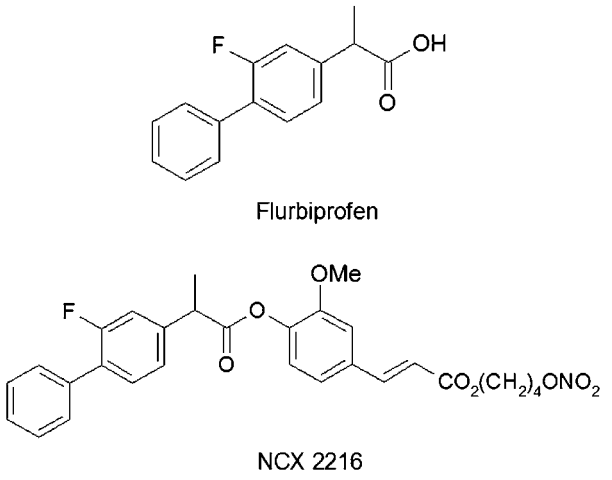

Figure 2 Structures of flurbiprofen (conventional NSAID) and a NO-releasing derivative of flurbiprofen (NCX-2216). NSAID, nonsteroidal anti-inflammatory drug.

cause the decrease in gastric mucosal blood flow that was observed in rats following administration of the parent NSAID (Wallace *et al*., 1994a). As predicted, NO-NSAIDs did not cause the increase in leukocyte–endothelial adherence that occurred when conventional NSAIDs were administered to rats (Wallace *et al*., 1994b). Furthermore, unlike conventional NSAIDs, the NO-NSAIDs did not interfere with the healing of pre-existing gastric ulcers, and in some cases, accelerated experimental gastric ulcer healing (Elliott *et al*., 1995; Ma *et al*., 2002; Brzozowska *et al*., 2004). The reduced gastric injury that had been observed with NO-NSAIDs in experimental models was confirmed in human studies (Hawkey *et al*., 2003; Fiorucci *et al*., 2003b, c). Moreover, the use of nitrovasodilators in combination with NSAIDs was shown to reduce significantly the incidence of GI bleeding associated with the latter (Lanas *et al*., 2000).

One of the surprising findings in studies of NO-NSAIDs was an increase in the anti-inflammatory and analgesic potency of these drugs (Davies *et al*., 1997a). Several mechanisms have been suggested as an explanation for these observations. First, NO itself can exhibit anti-inflammatory effects, such as inhibition of leukocyte adherence to the vascular endothelium (Kubes *et al*., 1991). NO has also been shown to exert analgesic actions (Ferreira, 1993) and to reduce the release of pro-inflammatory mediators from a number of cells, including mast cells (Hogaboam *et al*., 1993). Second, small amounts of NO have been shown to reduce expression of the inducible NO synthase (Cirino *et al*., 1996), which produces supra-physiological concentrations of NO that can contribute to inflammation and tissue injury. Third, NO can inhibit the activity of various caspases, thereby reducing synthesis of several pro-inflammatory cytokines (that is, interleukin (IL)-1, IL-18) and preventing apoptosis of endothelial cells (Fiorucci *et al*., 1999a, b).

The positive results from studies with NO-releasing NSAIDs triggered the evaluation of NO-releasing derivatives of a number of other types of drugs, in an attempt to either reduce toxicity, boost potency, or both (Table 1). Thus, NO-releasing derivatives of glucocorticoids were shown to exhibit significantly less detrimental effects on bone (Paul-Clark *et al*., 2002), but to be more potent that the parent drugs in various inflammatory models (Paul-Clark *et al*., 2000, 2002; Fiorucci *et al*., 2002a; Turesin *et al*., 2003; Wallace *et al*., 2004). NO-releasing acetaminophen was shown to exhibit enhanced analgesic and anti-inflammatory effects, and to cause significantly less hepatic injury than the parent drug (Futter *et al*., 2001; Fiorucci *et al*., 2002a). NO-releasing derivatives of aspirin were found to be significantly more potent than aspirin in models of *in vivo* and *in vitro* models of colon cancer (Bak *et al*., 1998; Williams *et al*., 2001; Rigas and Williams, 2002). NO-aspirin also exhibited additional cardioprotective and antithrombotic effects, compared to those of aspirin (Wallace *et al*., 1995, 2002; Momi *et al*., 2000; Rossoni *et al*., 2000, 2001; Gresele *et al*., 2007). A NO-releasing derivative of mesalamine produced substantially greater beneficial effects in experimental colitis than the parent drug (Wallace *et al*., 1999).

It is the cardiovascular safety of NO-NSAIDs that is now seen as particularly attractive with respect to their prospects of gaining regulatory approval for the treatment of arthritis. This, of course, is in part a consequence of the increasing awareness of the cardiovascular toxicity associated with conventional and COX-2-selective NSAIDs. Animal studies demonstrated that a NO-releasing naproxen derivative did not exhibit the hypertensive effects of naproxen (Muscara *et al*., 1998, 2000, 2001). Phase III clinical trials of 'naproxcinod', Nicox's lead drug, are currently underway, and aimed particularly at further demonstrating a safer cardiovascular profile of this drug as compared to older NSAIDs and selective COX-2 inhibitors. In studies completed thus far, the toxicity associated with NO-NSAIDs has mainly been attributable to the NSAID moiety (Lohmander *et al*., 2005), although in one study there were reports of orthostatic hypotension and dizziness experienced by some patients taking naproxcinod (likely to be related to the NO release from this compound) (Michael Hill *et al*., 2006).

Table 1 Improvement of pharmacological profile of compounds through NO-releasing modification

Drug class	*Parent drugs*	*Added benefit*	*Key reviews and references*
NSAID	Naproxen, diclofenac, flurbiprofen, ketoprofen, aspirin	↓ Gastrointestinal toxicity ↑ Anti-inflammatory potency and/or efficacy ↓ Hypertension ↑ Efficacy and GI safety in experimental Alzheimer's disease ↑ Cardioprotective and antithrombotic ↑ Chemoprevention in colon cancer models	Wallace *et al.* (2002); Wallace and Del Soldato (2003); Muscara *et al.* (2000); Jantzen *et al.* (2002); Gresele *et al.* (2007); Rigas and Williams (2002)
Glucocorticoid	Prednisolone, flunisolide, hydrocortisone	↑ Anti-inflammatory potency/efficacy ↓ Adverse effects on bone	Paul-Clark *et al.* (2000); Fiorucci *et al.* (2002b); Hyun *et al.* (2004); Wallace *et al.* (2004)
Analgesic	Paracetamol (acetaminophen)	↑ Anti-inflammatory and analgesic potency/efficacy ↓ Adverse effects on liver	Futter *et al.* (2001); Fiorucci *et al.* (2002a)
	Gaba-pentin	↑ Potency/efficacy	Wu *et al.* (2004)
Mesalamine	5-Aminosalicylic acid	↑ Anti-inflammatory potency/efficacy	Wallace *et al.* (1999)
Vasodilator	Ursodeoxycholic acid	↑ Potency/efficacy in portal hypertension	Fiorucci *et al.* (2003)
Statin	Pravastatin	↑ Anti-thrombotic efficacy	Rossiello *et al.* (2005)

SHH—another solution

As was the case for NO before the 1980s, hydrogen sulphide (H_2S) has until recently been better known as an industrial pollutant than as an endogenous mediator of numerous physiological processes (Wang, 2002; Fiorucci *et al.*, 2006). Nevertheless, the notion that H_2S may have beneficial effects is not new. For centuries, bathing in H_2S-rich mineral springs has been perceived to relieve pain and reduce inflammation, modulate the immune system and improve blood flow. In recent years, scientific evidence to support many of these contentions has been generated. H_2S is produced through a number of pathways, the most common being related to the metabolism of L-cysteine, cystine and homocysteine (Wang, 2002). Endogenous levels of H_2S have been reported to be as high as 160 μM in the brain, with serum levels of 30–100 μM (Wang, 2002). Even after administration of H_2S donors in doses that produce pharmacological effects, the concentration of H_2S in plasma seldom exceeded the normal range, or did so for a very brief period of time (Li *et al.*, 2005; Distrutti *et al.*, 2006b), because of the highly efficient systems for metabolizing, scavenging and sequestering H_2S (Wang, 2002; Fiorucci *et al.*, 2006). Toxic effects of H_2S are observed in the lung with concentrations in excess of 250 μM, and coma and death can occur with concentrations above 1000 μM (Milby and Baselt, 1999).

In various models of tissue injury, production of H_2S is markedly enhanced (Bhatia *et al.*, 2005; Yusuf *et al.*, 2005). Whether H_2S is primarily detrimental or beneficial in such circumstances is not yet clear. As is the case with NO, physiological concentrations of H_2S are likely to be beneficial, while concentrations many times greater than the physiological range would tend to be detrimental. The highest levels of H_2S in the body are found in the lumen of the colon (low millimolar concentrations) (Magee *et al.*, 2000), but in this case the primary source of this gaseous mediator is the commensal flora. Recent studies suggest that H_2S is an inorganic substrate for mammalian mitochondria, and the mitochondria of colonic epithelial cells may be particularly well adapted for the use of the substrate (Goubern *et al.*, 2007).

H_2S can relax vascular smooth muscle, possibly via activation of ATP-sensitive K^+ channels (K^+_{ATP}) (Wang, 2002; Tang *et al.*, 2005). Recently, potent anti-inflammatory effects of H_2S were demonstrated. For example, H_2S donors suppressed leukocyte adherence to the vascular endothelium and reduced leukocyte extravasation and oedema formation (Zanardo *et al.*, 2006). Inhibition of endogenous H_2S synthesis, on the other hand, triggered leukocyte adherence to the vascular endothelium and enhanced oedema formation (Zanardo *et al.*, 2006). These actions of H_2S also appeared to be mediated via activation of K^+_{ATP} channels. H_2S has been shown to induce apoptosis of neutrophils (Mariggio *et al.*, 1998), which could contribute to resolution of inflammation (Gilroy *et al.*, 2004). H_2S has also been reported to inhibit oxidative damage to tissue, in part through scavenging of peroxynitrite (Whiteman *et al.*, 2004, 2005). H_2S donors have been shown to decrease endotoxin-induced cytokine expression and NO production (Hu *et al.*, 2007; Li *et al.*, 2007). H_2S has also been shown to reduce visceral pain perception, which may also be mediated via activation of K^+_{ATP} channels (Distrutti *et al.*, 2006a). Indeed, analgesic effects of NSAIDs have been suggested to be mediated, at least in part, via K^+_{ATP} channels (Ortiz *et al.*, 2001).

H_2S is also produced by the gastric mucosa, and like NO, contributes to the ability of this tissue to resist damage induced by luminal substances (Fiorucci *et al.*, 2005). Surprisingly, H_2S synthesis was found to be significantly reduced following administration of NSAIDs, apparently through suppression of the expression of cystathionine-γ-lyase, one of the key enzymes for conversion of L-cysteine into H_2S (Fiorucci *et al.*, 2005). Thus, suppression of mucosal H_2S synthesis may represent another mechanism, aside from suppression of COX activity, through which NSAIDs produce GI damage. Administration of H_2S donors could prevent the decrease in gastric blood flow induced by NSAIDs, as well as diminishing NSAID-induced leukocyte adherence. It also

decreased the NSAID-induced accumulation of leukocytes in the gastric mucosa, as well as expression of endothelial and leukocyte adhesion molecules (Fiorucci *et al*., 2005; Zanardo *et al*., 2006; Wallace *et al*., 2007). Several of these H_2S-induced effects were inhibited by glibenclamide and/or mimicked by pinacidil, suggesting that they were produced via activation of K^+_{ATP} channels.

Building on these observations, we explored the effects of several H_2S-releasing derivatives of NSAIDs (synthesized by Antibe Therapeutics, Toronto, Canada), to determine if release of small amounts of this mediator could counteract the detrimental effects of the NSAID moiety. Indeed, it appeared from the studies described above that H_2S had the potential to interfere with many of the key events in the pathogenesis of NSAID-induced mucosal injury in similar fashion to NO (Figure 1). As was the case with NO-NSAIDs, the H_2S-releasing NSAIDs were substantially better tolerated, in terms of gastric damage, than the parent drugs. At single doses of more than five times the ED_{50} for anti-inflammatory activity in the carrageenan paw oedema model in rats, no gastric damage was detected with an H_2S-releasing derivative of diclofenac (Figure 3) (Wallace *et al*., 2007). With administration of this same dose of the compound three times over 24 h, very low levels of intestinal damage were observed, at least 90% less than that observed in rats given diclofenac at an equimolar dose (Wallace *et al*., 2007). Moreover, there was no change in haematocrit in the rats treated with the H_2S-releasing derivative, while diclofenac administration resulted in a decrease in haematocrit of 50%, consistent with the widespread bleeding that was evident in the intestine (Wallace *et al*., 2007).

The benefit provided by the H_2S-releasing moiety was not only in terms of reduced GI toxicity. We also observed a 2- to 3-fold increase in anti-inflammatory potency of the H_2S-diclofenac compound as compared to diclofenac (Wallace *et al*., 2007). This increase in potency has also been observed with other H_2S-releasing compounds that we and others have evaluated (Fiorucci *et al*., 2007; Li *et al*., 2007). The increased anti-inflammatory potency may be related to more potent suppression of COX activity. Alternatively, suppression of pro-inflammatory cytokine synthesis by H_2S-releasing NSAIDs may explain their increased anti-inflammatory activity (Fiorucci *et al*., 2005; Li *et al*., 2007).

The anti-inflammatory and visceral analgesic effects of H_2S have also been exploited recently in the design of novel derivatives of mesalamine for the treatment of inflammatory bowel disease (IBD). Mesalamine is the first-line therapy for IBD, but it is a weak drug, so doses of up to 6 g/day are necessary. Moreover, mesalamine is only effective in mild-to-moderate cases of IBD. A mesalamine derivative that includes an H_2S-releasing moiety (ATB-429) was recently shown to exhibit markedly enhanced anti-inflammatory actions in experimental colitis (Fiorucci *et al*., 2007). The H_2S-releasing moiety alone did not produce significant beneficial effects in this model of colitis (Fiorucci *et al*., 2007). Unlike mesalamine, the H_2S-releasing derivative significantly suppressed the expression of several pro-inflammatory cytokines in the colon, as well as reducing granulocyte levels in colonic tissue to those of healthy

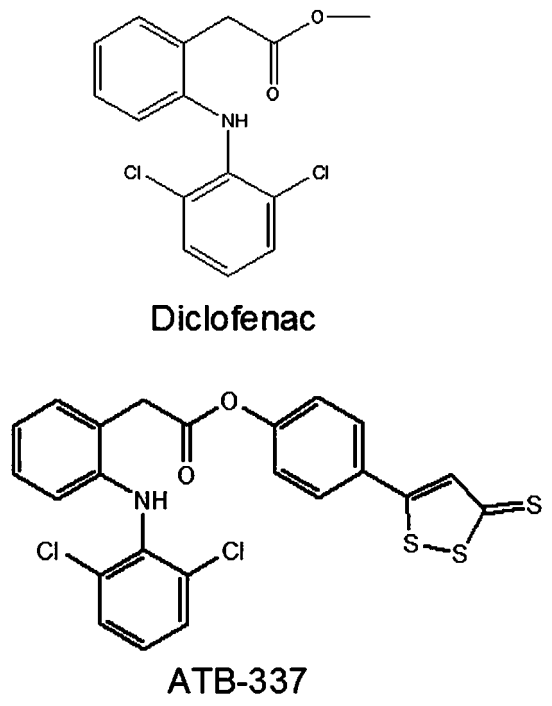

Figure 3 Structures of diclofenac (conventional NSAID) and a H_2S-releasing derivative of diclofenac (ATB-337). NSAID, nonsteroidal anti-inflammatory drug.

controls (Fiorucci *et al*., 2007). Moreover, the drug exhibited significant analgesic effects in a rat model of visceral pain (Distrutti *et al*., 2006b). This may have particular clinical significance given that abdominal pain is one of the most common and debilitating symptoms of IBD.

On the horizon

The great challenge for those attempting to develop safer NSAIDs is shifting from a focus on the GI toxicity to the increasingly more appreciated cardiovascular toxicity. While selective COX-2 inhibitors represented an important advance in anti-inflammatory therapy, there is plenty of room for improvement. NO-NSAIDs and several other NO-releasing drugs (Table 1) are in advanced clinical trials, so in the near future the degree to which these drugs represent significant advances in a clinical setting will become clearer. While the development of H_2S-releasing anti-inflammatory drugs is in its infancy, the preclinical data available thus far provide cause for optimism.

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Conflict of interest

The author holds shares in Antibe Therapeutics Inc., a company focused on hydrogen sulphide-releasing drugs.

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