

# Synthesis and evaluation of mutual azo prodrug of 5-aminosalicylic acid linked to 2-phenylbenzoxazole-2-yl-5-acetic acid in ulcerative colitis

Jamal A Jilani<sup>1</sup>  
Maha Shomaf<sup>2</sup>  
Karem H Alzoubi<sup>3</sup>

<sup>1</sup>Department of Medicinal Chemistry and Pharmacognosy, Jordan University of Science and Technology, Irbid, Jordan; <sup>2</sup>Department of Pathology, Jordan University, Amman, Jordan; <sup>3</sup>Department of Clinical Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

**Abstract:** In this study, the syntheses of 4-aminophenylbenzoxazol-2-yl-5-acetic acid, (an analogue of a known nonsteroidal anti-inflammatory drug [NSAID]) and 5-[4-(benzoxazol-2-yl-5-acetic acid)phenylazo]-2-hydroxybenzoic acid (a novel mutual azo prodrug of 5-aminosalicylic acid [5-ASA]) are reported. The structures of the synthesized compounds were confirmed using infrared (IR), hydrogen-1 nuclear magnetic resonance (<sup>1</sup>H NMR), and mass spectrometry (MS) spectroscopy. Incubation of the azo compound with rat cecal contents demonstrated the susceptibility of the prepared azo prodrug to bacterial azoreductase enzyme. The azo compound and the 4-aminophenylbenzoxazol-2-yl-5-acetic acid were evaluated for inflammatory bowel diseases, in trinitrobenzenesulfonic acid (TNB)-induced colitis in rats. The synthesized diazo compound and the 4-aminophenylbenzoxazol-2-yl-5-acetic acid were found to be as effective as 5-aminosalicylic acid for ulcerative colitis. The results of this work suggest that the 4-aminophenylbenzoxazol-2-yl-5-acetic acid may represent a new lead for treatment of ulcerative colitis.

**Keywords:** benzoxazole acetic acid, azo prodrug, colon drug delivery

## Introduction

5-aminosalicylic acid (5-ASA) is widely used for the treatment of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease.<sup>1,2</sup> The drug is also useful for the treatment of colorectal cancer.<sup>2-4</sup> However, its absorption in the upper gastrointestinal tract (GIT) after oral administration leads to unwanted systemic effects and lower bioavailability at the site of action.<sup>5,6</sup> Therefore, colon delivery systems were developed for 5-ASA, which include controlled formulations, through coating of the 5-ASA with suitable polymer.<sup>7</sup> Another controlled delivery method is the use of prodrugs of 5-ASA. This can be achieved through utilizing an azo linkage between 5-ASA and other agents. Sulfasalazine, olsalazine and balsalazine represent examples of azo prodrugs.<sup>8-10</sup> Although these prodrugs improve the therapeutic utility of 5-ASA, their use is associated with side effects of their own.<sup>11-13</sup> The need for 5-ASA to be safer and more effective stimulates researchers to continue their efforts to optimize the efficacy of 5-ASA.<sup>14-16</sup>

Finding a carrier of 5-ASA that itself has anti-inflammatory or anticancer activities would represent a significant improvement in the treatment of colorectal cancer or IBD. Search of the literature reveals that a benzoxazole derivative may be a suitable target carrier for 5-ASA. Arylbenzoxazoles are reported to have a

Correspondence: Jamal A Jilani  
Department of Medicinal Chemistry  
and Pharmacognosy, Faculty of Pharmacy,  
Jordan University of Science and  
Technology, PO box 3030, Irbid, Jordan  
Tel +962 2 720 1000  
Fax +962 2 720 1075  
Email jilani@just.edu.jo

wide range of pharmacological activities that includes anti-inflammatory<sup>17,18</sup> and anticancer activities.<sup>19–21</sup> Also, a recent study showed that arylbenzoxazoles have the potential to treat IBD by acting as an antagonist at the serotonin subtype 3 receptor.<sup>22</sup> Based on the above argument, 4-aminophenylbenzoxazol-2-yl-5-acetic acid was selected for synthesis and evaluated as a carrier of 5-ASA via azo linkage. The compound represents a novel analogue of two known nonsteroidal anti-inflammatory drugs (NSAIDs) namely, benoxaprofen and flunoxaprofen.

Benoxaprofen and flunoxaprofen are well known for their anti-inflammatory properties. They belong to the class of benzoxazoleacetic acid derivatives.<sup>23,24</sup> Benoxaprofen has been recognized to be an NSAID with a spectrum of activity that is different from other NSAIDs. It shows both lipoxigenase- and cyclooxygenase-inhibition activities.<sup>25,26</sup> This dual inhibition has encouraged researchers to evaluate benoxaprofen activity in the treatment of UC and psoriasis.<sup>27–29</sup> In UC, benoxaprofen showed an accelerated healing effect in a rat IBD model.<sup>30</sup> In this study, the syntheses of 4-aminophenylbenzoxazol-2-yl-5-acetic acid (an analogue of a known NSAID) and 5-[4-(benzoxazol-2-yl-5-acetic acid) phenylazo]-2-hydroxybenzoic acid (as a novel mutual azo produg of 5-ASA) are reported. Additionally, the anti-UC activity of the compounds are investigated.

## Materials and methods

The 5-ASA was obtained from the Jordanian Pharmaceutical Manufacturing Co, PLC (JPM), Naour, Jordan. Trinitrobenzenesulfonic acid (TNB) was purchased from Sigma-Aldrich Corp, St Louis, MO, USA. All other reagents were obtained from commercially available sources. The melting points were determined using a Gallenkamp capillary melting point apparatus (model MPD 350 BM 2.5; SANYO Gallenkamp PLC, Loughborough, UK). <sup>1</sup>H NMR spectra were obtained using a Varian Unity 300 Spectrometer (Varian Medical Systems, Inc, Palo Alto, CA, USA), and chemical shifts ( $\delta$ ) were reported as parts per million (ppm) relative to the internal standard, tetramethylsilane. IR spectra were obtained with a Nicolet Impact 410 (Nicolet Instrument Corp, Fitchburg, WI, USA). MS data were obtained by VG7070 mass spectrometer (M-Scan Inc, West Chester, PA, USA). The ultraviolet (UV)-visible spectra were recorded using a Shimadzu UV-1800 UV-VIS Spectrophotometer (Shimadzu Corp, Kyoto, Japan). Thin layer chromatography (TLC) was conducted using Silica gel 60 GF<sub>245</sub> precoated sheets (E Merck KG, Darmstadt, Germany) and was visualized by UV-lamp at wavelength 254 nm.

## Chemistry

### Methyl-4-nitrophenylbenzoxazol-2-yl-5-acetate (5)

A solution of methyl-3-amino-4-hydroxyphenylacetate (20.0 g, 0.109 mol) and 4-nitrobenzaldehyde (17.0 g, 0.113 mol) in absolute ethanol was heated under reflux for 4 hours. Evaporation of ethanol gave a thick product, which was dissolved in hot glacial acetic acid (250 mL). To the formed solution, lead tetraacetate (14.25 g) was added, and the formed mixture was allowed to cool to room temperature, yielding 28 g (82%) of compound 5, a pure solid precipitate, which was collected by filtration. Melting point (mp) 195°C–196°C. IR: (KBr) 1731 cm<sup>-1</sup>, 1516 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>),  $\delta$  3.5 (s, 3H, OCH<sub>3</sub>), 3.6 (s, 2H, CH<sub>2</sub>), 7.3 (d, 1H, ArH, J = 8.43 Hz), 7.6 (d, 1H, ArH, J = 8.4 Hz), 7.75 (s, 1H, ArH), 8.4 (m, 4H, ArH). MS m/z 313 (MH<sup>+</sup>).

### Methyl-4-aminophenylbenzoxazol-2-yl-5-acetate (6)

The amount 4.5 g (0.014 mol) of methyl-4-nitrophenylbenzoxazol-2-yl-5-acetate and 7.0 g of tin were suspended in 300 mL methanol. Hydrochloric acid (HCl) gas was introduced to the mixture until all of the tin was consumed, and the color changed. The formed solution was concentrated to 50 mL, yielding 3.5 g of the HCl salt upon cooling, which was collected as an orange precipitate. The formed salt was dissolved in 50 mL deionized water, forming a solution that was adjusted to pH 8.0 by adding sodium hydroxide (NaOH) solution. Stirring for 10 minutes, followed by filtration, gave a solid precipitate, which was washed with water. The solid was collected and recrystallized from ethyl acetate, yielding 2.75 g (70%) of 6, mp 185°C–186°C, <sup>1</sup>H NMR (CDCl<sub>3</sub>),  $\delta$  3.65 (s, 3H, OCH<sub>3</sub>); 3.75 (s, 2H, CH<sub>2</sub>); 4.0 (s, 2H, NH<sub>2</sub>), 6.75 (d, 2H, ArH, J = 8.5 Hz), 7.15 (dd, 1H, ArH, J = 1.2, 8.3 Hz), 7.45 (d, 1H, ArH, J = 8.3 Hz); 7.6 (d, 1H, J = 1.2 Hz, ArH), 8.05 (d, 2H, ArH, J = 8.5 Hz); MS m/z 283 (MH<sup>+</sup>).

### 4-aminophenylbenzoxazol-2-yl-5-acetic acid (1)

A solution of 6 (0.5 g, 0.002 mol) and NaOH (1.4 g, 0.035 mol) in 90% ethanol (EtOH) (30 mL) was stirred at room temperature for 4 hours. A solid precipitate was formed, collected, washed with Acetone, and dissolved in H<sub>2</sub>O (70 mL). The aqueous solution was acidified with HCl (2 mL), giving a solid precipitate, which was collected and purified by recrystallization from HOAc (acetic acid) to give 0.3 g of 1, mp 220°C–224°C. IR: (KBr) cm<sup>-1</sup> 1705, <sup>1</sup>H NMR (DMSO-d<sub>6</sub>),  $\delta$  3.8 (s, 2H, CH<sub>2</sub>), 3.95 (s, 2H, NH<sub>2</sub>); 6.75 (d, 2H, ArH, J = 8.5 Hz); 7.2 (d, 1H, ArH, J = 8.5 Hz); 7.55 (s, 1H, ArH), 7.6 (d, 1H, ArH, J = 8.5 Hz); 7.95 (d, 2H, ArH, J = 8.5 Hz), MS m/z 269 (MH<sup>+</sup>).

## 2-hydroxy-5-[4-(benzoxazol-2-yl-5-acetic acid) phenylazo]benzoic acid (4)

The amount of 16.0 g (0.053 mol) of HCl salt of 4-amino-phenylbenzoxazole-5-acetic acid 1 was suspended in 250 mL 6 N HCl and cooled to 0°C. To this suspension, a precooled (0°C) solution of sodium nitrite (5.0 g in 50 mL H<sub>2</sub>O) was added dropwise within 15–20 minutes. The temperature of the mixture was kept between 0°C–5°C. In another flask, 8.0 g (0.057 mol) of salicylic acid was dissolved in 200 mL of 25% NaOH solution, forming a solution, to which the diazonium salt solution was added dropwise within 40–60 minutes, while keeping the temperature at 0°C–5°C and the pH above 8.0 (by adding NaOH solution). The formed mixture was stirred at 0°C–5°C for 30 minutes, and the pH was adjusted to about 7.0, then filtered. A red-to-orange color powder was collected, washed with water, and dissolved in 200 mL boiling 5% NaOH solution. The formed solution was filtered; and the filtrate was adjusted to a pH of 2.0. Upon cooling, a precipitate was formed, which was collected, yielding 8.0 g (38%) of 4 as a pure, colored solid. Mp: decomposition at 250°C; IR: (KBr) 1720 cm<sup>-1</sup>, 1485 cm<sup>-1</sup> (N = N) stretching. <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ 3.73 (s, CH<sub>2</sub>), 6.79 (d, 1H, ArH, J = 8.82 Hz), 7.34 (dd, 1H, ArH, J = 1.37, 8.37 Hz), 7.75 (d, 2H, ArH, J = 8.7 Hz), 7.84 (dd, 1H, ArH, J = 2.63, 8.8), 7.98 (d, 2H, ArH, J = 8.56 Hz), 8.25 (m, 2H, ArH). MS m/z 418 (MH<sup>+</sup>).

## Animals

Female Wistar rats (200–250 g) were utilized in this study. The animals were housed in metallic cages (maximum five rats per cage) under hygienic conditions and maintained at room temperature, with free access to food and water. Animals were housed on a 12-hour light/dark cycle (light on 7 am), at 25°C. All experimental procedures were performed during the light cycle and were in accordance with international laboratory animals care and use procedures.

## In vitro study

The stability study of the azo prodrug under acidic conditions was performed by dissolving a sufficient quantity of the prodrug in HCl buffer (pH 1.2). The resulting solution was incubated at 37°C, with stirring. The stability of the prodrug was monitored by measuring the absorbance at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 hours.

On the other hand, the susceptibility of the azo bond to reduction by the bacterial azo reductase enzyme was evaluated according to a previously reported method.<sup>31</sup> In a group of test tubes flushed with N<sub>2</sub> gas, 1 g of rat fecal

matter was placed. To each tube, 5 mL of 8 µg/mL solution of the prodrug in phosphate buffer (pH 7.4) was added. The formed suspensions were incubated in a shaking water bath at 37°C, under anaerobic conditions. At predetermined time intervals (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 hours), aliquots were taken, filtered and monitored using the UV spectrophotometer.

## In vivo study

The synthesized compounds were evaluated for IBD activity using a TNB-induced UC rat model, as previously described.<sup>32,33</sup> In brief, inflammation of the colon was induced in a pre-anesthetized rat by a single intracolonic administration of TNB solution. Rats (n = 24) were randomly grouped in groups A, B, C, and D. Group A was a positive control, and each of the other three groups received predetermined equimolar doses of the compounds under testing. Briefly, animals were fasted for 12 hours. Thereafter, all animals received a solution (0.25 mL) of methylcellulose solution (50% methylcellulose in absolute ethanol) containing 20 mg of TNB. The TNB solution was administered by intracolonic route, using a syringe and rubber cannula. The cannula was inserted into the lumen of the colon via the anus. The cannula was advanced so that its tip was located at 8 cm from the anus. Six hours after administering the TNB solution, the compound and placebo suspensions were given orally twice daily to each animal as follows: group A was administered 0.5 mL of the suspension containing 2.5 mg of methylcellulose solution, twice daily; group B was administered 0.5 mL of the methylcellulose suspension containing 5 mg of 5-ASA, twice daily; group C was administered 0.5 mL of the methylcellulose suspension containing 18 mg of compound 4, twice daily; and group D was administered 0.5 mL of the methylcellulose suspension containing 12 mg of compound 1, twice daily. The treatments continued for 8 days, and on the ninth day, the animals were sacrificed and opened along their abdomen for evaluation. The morphological and histological changes in the rat colons were assessed. A gross inspection of the intestine, before opening, was carried out. For the histological evaluation, the distal colons were excised, and each was examined under a stereomicroscope (SMZ 445, Nikon, Tokyo, Japan) by an observer who was unaware of the treatment. Then, samples of inflamed colons were excised and fixed in 10% formalin and processed by routine technique that included sectioning at 5 µm thickness, mounting on glass slides, and staining with hematoxylin and eosin. The sections were examined under the light microscope, and the extent of inflammation and damage was assessed with respect to three

parameters: the presence of mucosal ulcers, the extent of wall thickness, and the transmural inflammation. Each parameter was graded (grade 1 = normal; grade 2 = very minimal; grade 3 = mild; grade 4 = moderate; and grade 5 = severe). Then, the overall score of damage was considered as the sum of the grades of each parameter and divided as the following (score 1 = normal, sum of the grades 3; score 2 = mild damage, sum of the grades 4–7; score 3 = moderate damage, sum of the grades 8–11; and score 4 = severe damage, sum of the grades 12–15).

## Results

The preparation of 4-aminophenylbenzoxazol-2-yl-5-acetic acid 1 (Figure 1) was done by coupling 4-nitrobenzaldehyde with methyl-3-amino-4-hydroxyphenyl acetate in the presence of lead tetraacetate.<sup>34,35</sup> Once the nitro group of the resulted compound was reduced, the ester function then hydrolyzed to give the target compound 1, as shown in Figure 2. On the other hand, the diazo compound 4 (Figure 3) was synthesized by diazotization of the aminophenylbenzoxazole, followed by the attack of salicylic acid (Figure 4). The structures of the compounds were proven by <sup>1</sup>H NMR, IR, and MS.

The stability study of the prepared prodrug 4 in acidic conditions – similar to those of the upper GIT – showed that the compound is stable during the time of the study, which was 5 hours. The absorbencies at 650 nm were 0.041, 0.042, 0.041, 0.042, 0.039, 0.040, 0.043, 0.041, and 0.040 at zero time, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0, at 5.0 hours, respectively. The release of 5-ASA from the prodrug in rat fecal matter showed a continuous increase in 5-ASA concentration, as reflected by the increase in UV absorbance, which reached a plateau phase at the third hour and continued to the end of the study (at 5.0 hours). In the *in vivo* studies, the administration of TNB solution resulted in severe inflammation. In group A, the intestines were swollen from the cecum to rectum, the colon was rigid and full of stool in contrast to normal, and the

intestine was almost completely damaged. On the other hand, the changes in the treated groups (B, C, and D) were much less evident, swelling was mild, only a small amount of stool was present, and the damage was minimal. Histopathological examination of the intestines of group A showed inflammation and ulcerations in the distal colon. The inflammation was severe, with multiple superficial ulcers present, and there was a marked increase in wall thickness due to fibrosis and transmural extension of the inflammation, which was prominent in most of the animals (Figure 5A). The overall score of the damage was 3–4. In groups B, C, and D, section of the colons showed only occasional superficial ulcerations, and there was no significant increase in wall thickness or transmural inflammation (Figure 5B–D). There were no significant differences in changes among the treated groups, whereas the differences between the three treated groups and the positive control (group A) were remarkable (Table 1).

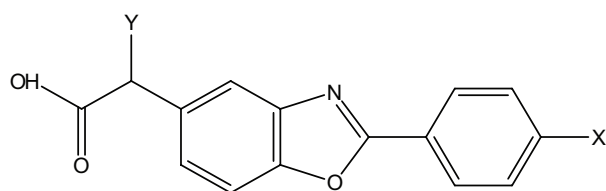
## Discussion

UC is a major gastrointestinal disease for which current treatments are still lacking. This study reports the synthesis of a novel mutual azo prodrug of 5-ASA prodrug with pharmacological activity both *in vitro* and *in vivo*. This prodrug could have the potential to reduce 5-ASA adverse effects in human, especially those related to its absorption in the upper GIT.

The activity of 4-aminophenylbenzoxazole acetic acid was shown to be similar to that of 5-ASA, in the treatment of TNB-induced colitis. Similarly, previous studies have shown that arylbenzoxazoles possess anti-inflammatory,<sup>17,18</sup> and anticancer activities.<sup>19–21</sup> In fact, it has been recently shown that arylbenzoxazoles have potential anti-UC properties.<sup>22</sup> In addition, the presence of the amino group, together with the carboxylate in compound 1, possibly decreases the absorption due to zwitterion formation. Thus, this study suggests that 4-aminobenzoxazole 1 may represent a new lead for the treatment of IBD.

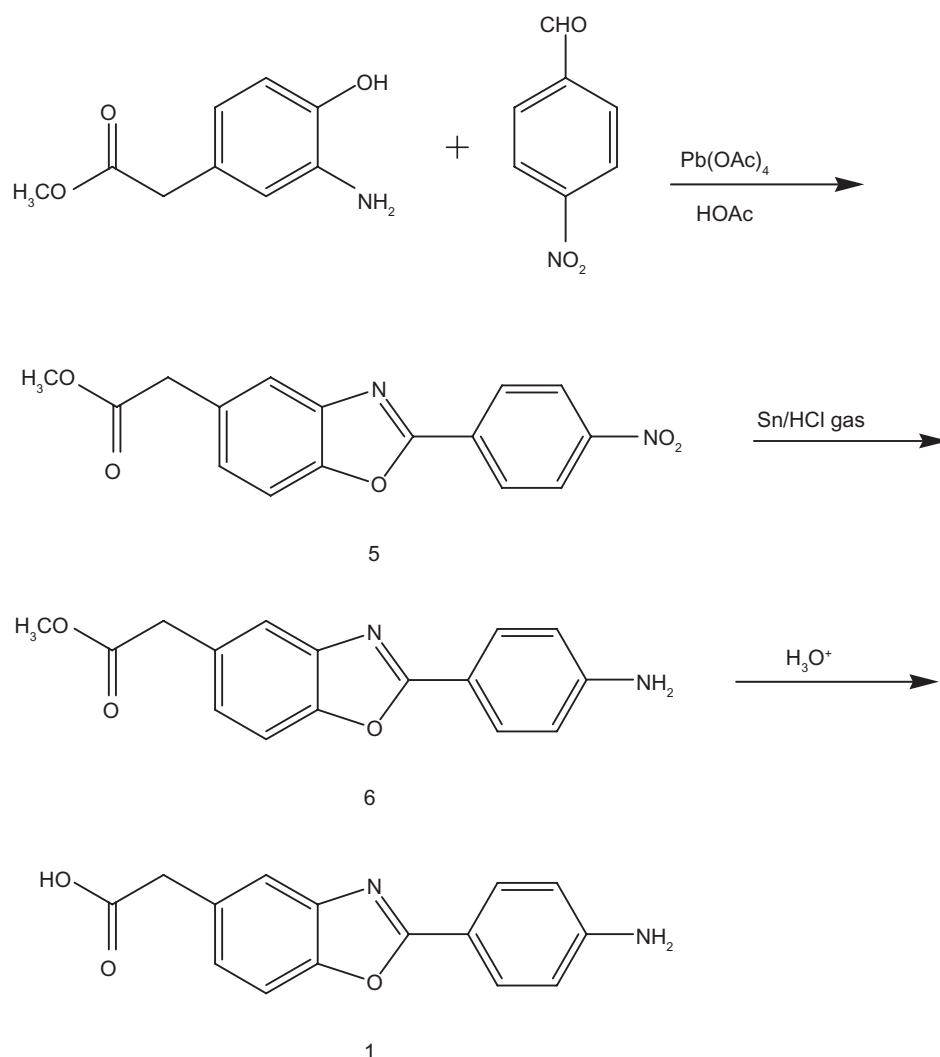
Concerning the diazo compound 4, current results showed that it has similar activity to the 4-aminophenylbenzoxazole acetic acid compound 1 and 5-ASA. The similar potency between the diazo compound 4 and both 5-ASA and 1 could be explained based on poor solubility and/or incomplete diazo reduction in the large intestine. However, since the absorption of this compound is expected to be poor, it could represent a promising colon delivery system for both 5-ASA and the aminobenzoxazole 1. This matter requires further future studies.

The diazo compound reported in the current study represents a novel mutual azo prodrug that should deliver 5-ASA as well as the aminobenzoxazole 1. The aminobenzoxazole 1



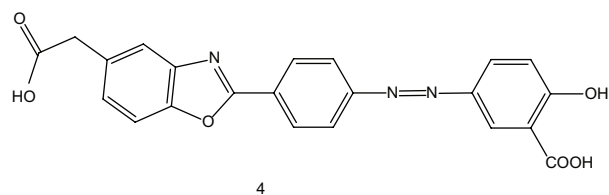
- 1: X = NH<sub>2</sub>, Y = H  
 2: X = Cl, Y = CH<sub>3</sub>  
 3: X = F, Y = CH<sub>3</sub>

**Figure 1** Chemical structures of compounds 1, 2, and 3.



**Figure 2** Synthesis of compound 1.

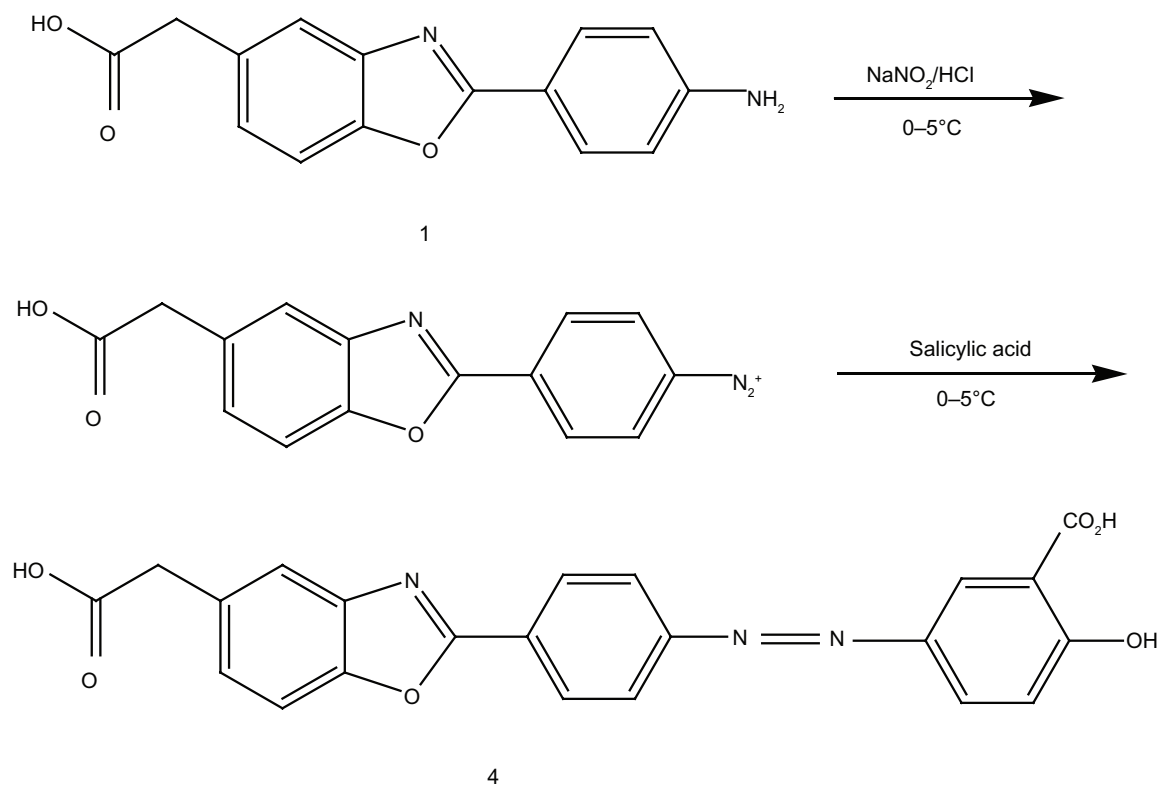
is an analogue of benoxaprofen and flunoxaprofen, which are known NSAIDs with anti-inflammatory properties.<sup>23,24</sup> In fact, benoxaprofen has been shown to promote accelerated healing in a rat UC model of inflammatory bowel disease, which could be explained by its action in leukotriene B4 inhibition.<sup>30</sup> The reported toxicities of benoxaprofen and flunoxaprofen, which should be taken in to consideration in this study, are due to their systemic effect after absorption. The prodrug under investigation is expected to exhibit very



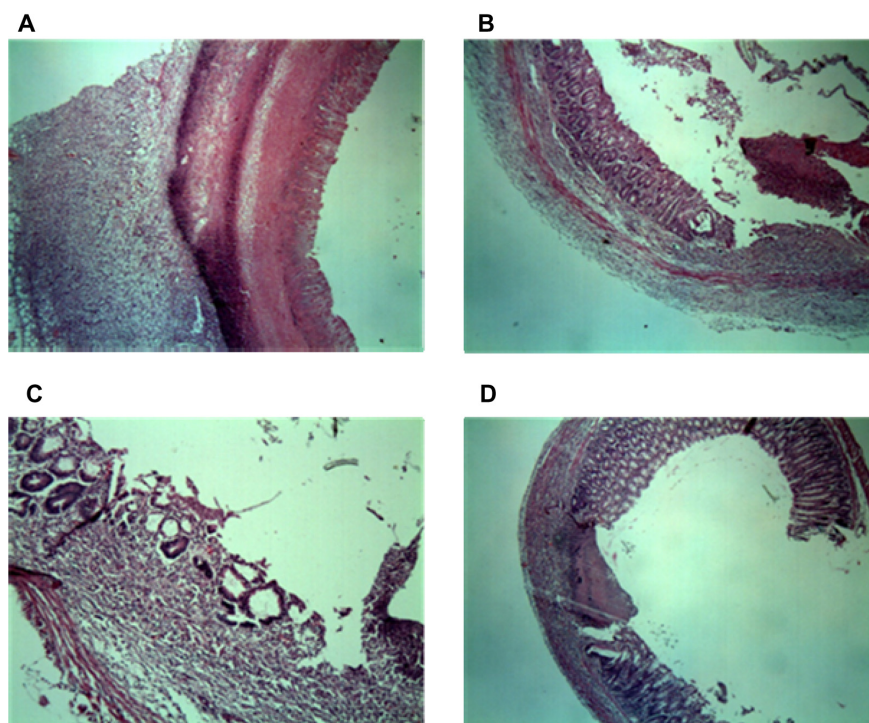
**Figure 3** Chemical structure of compound 4.

minimal absorption, as it is a highly charged compound with low solubility. In a previous study, olsalazine, which is an azo dimer of 5-ASA, showed an oral bioavailability of only 3%.<sup>36</sup> Yet, studying the pharmacokinetic and pharmacological profile of the currently reported prodrug and related compound could be a recommended future study.

Results of this study showed that the prepared azo prodrug is stable under acidic conditions similar to those of the upper GIT, which ensures its ability to reach the lower intestine intact. Also, the *in vitro* study showed that the azo prodrug was susceptible to bacterial azo reductase. When this is correlated with the anti-UC capacity of the synthesized compound, it indicates that bacteria within the large intestine will very likely digest the azo component of the synthesized compound, leading to a release of the 5-ASA and aminobenzoxazole from the moieties. Both of these possess anti-inflammatory and anti-UC activities,



**Figure 4** Synthesis of compound 2.



**Figure 5** Representative histopathology pictures from rat colons treated with synthesized prodrugs: **(A)** a representative picture of group A (negative control) showing severe mucosal and transmural inflammation, in addition to increased wall thickness; **(B)** a representative picture of group B (treated with 5-ASA), shows that mucosal inflammation and ulceration were minimal and no increase in wall thickness was present; **(C)** a representative picture of group C (treated with compound 4) showing mucosal ulceration with no transmural inflammation or increase of wall thickness; and **(D)** a representative picture of group D (treated with compound 1) shows mucosal ulceration with no transmural inflammation and that wall thickness was minimally increased.

**Notes:** All pictures show staining with hematoxylin and eosin. Pictures A and C are at 40 $\times$ , pictures B and D are at 10 $\times$ .

**Table I** Inflammation scores of synthesized prodrug-treated rat colons, by histopathological examination

Group-Rat #	Mucosal ulceration	Wall thickness	Transmucosal inflammation	Overall score of inflammation
A1	3	3	4	3
A2	4	4	5	4
A3	4	4	5	4
A4	–	–	–	Died
A5	3	4	4	3
A6	4	4	4	4
B1	1	1	1	1
B2	1	1	1	1
B3	2	3	1	2
B4	2	3	3	3
B5	1	1	1	1
B6	1	1	1	1
C1	1	1	1	1
C2	3	2	1	2
C3	3	1	1	2
C4	3	1	1	2
C5	3	2	1	2
C6	3	2	1	2
D1	3	3	3	3
D2	3	1	1	2
D3	3	3	3	3
D4	1	1	1	1
D5	3	3	3	3
D6	3	3	1	2

**Notes:** Groups were: A was the negative control; B was treated with 5-ASA-treated; C was treated with compound 4; and D was treated with compound I. The inflammation scores were: 1 = normal; 2 = very minimal; 3 = mild; 4 = moderate; and 5 = severe.

yet, we do not exactly know which one of these compounds primarily manifest the UC-related activity. The exact determination of the mechanism of action of our newly synthesized compound requires more investigations. Once this is known, further work could result in modified diazo derivatives with augmented anti-UC activities compared with 5-ASA and the currently reported diazo model prodrug.

In conclusion, the current results describe the synthesis of a novel diazo prodrug derivative 4-aminophenylbenzoxazol-2-yl-5-acetic acid and 5-[4-(benzoxazol-2-yl-5-acetic acid) phenylazo]-2-hydroxybenzoic acid. These two compounds were shown to be effective and promising for the treatment of UC.

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Gordon M, Naidoo K, Thomas AG, Akobeng AK. Oral 5-aminosalicylic acid for maintenance of surgically-induced remission in Crohn's disease. *Cochrane Database Syst Rev*. 2011:CD008414.
- Baan B, Dihal AA, Hoff E, et al. 5-Aminosalicylic acid inhibits cell cycle progression in a phospholipase D dependent manner in colorectal cancer. *Gut*. 2012;61(12):1708–1715.
- Koelink PJ, Hawinkels LJ, Wiercinska E, et al. 5-Aminosalicylic acid inhibits TGF-beta1 signalling in colorectal cancer cells. *Cancer Lett*. 2010;287(1):82–90.
- Velayos FS, Terdiman JP, Walsh JM. Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: a systematic review and metaanalysis of observational studies. *Am J Gastroenterol*. 2005;100(6):1345–1353.
- Bonner GB, and Ruderman WB. 5-Aminosalicylic acid preparations in the treatment of inflammatory bowel disease. In: *Inflammopharmacology*. Norwell, Mass. Kluwer Academic, 1993;247–262.
- Brzezinski A, Rankin GB, Seidner DL, Lashner BA. Use of old and new oral 5-aminosalicylic acid formulations in inflammatory bowel disease. *Cleve Clin J Med*. 1995;62(5):317–323.
- Fernandez-Becker NQ, Moss AC. Improving delivery of aminosaliculates in ulcerative colitis: effect on patient outcomes. *Drugs*. 2008;68(8):1089–1103.
- Brown JP, McGarraugh GV, Parkinson TM, Wingard RE Jr, Onderdonk AB. A polymeric drug for treatment of inflammatory bowel disease. *J Med Chem*. 1983;26(9):1300–1307.
- Campbell DE, Berglinth T. Pharmacology of olsalazine. *Scand J Gastroenterol Suppl*. 1988;148:7–12.
- Riley SA, Turnberg LA. Sulphasalazine and the aminosaliculates in the treatment of inflammatory bowel disease. *Q J Med*. 1990; 75(278):551–562.
- Mahmud N, Kamm MA, Dupas JL, et al. Olsalazine is not superior to placebo in maintaining remission of inactive Crohn's colitis and ileocolitis: a double blind, parallel, randomised, multicentre study. *Gut*. 2001;49(4):552–556.
- Rao SS, Read NW, Holdsworth CD. Influence of olsalazine on gastrointestinal transit in ulcerative colitis. *Gut*. 1987;28(11):1474–1477.
- Sutherland LR, Roth DE, Beck PL. Alternatives to Sulfasalazine: A Meta-analysis of 5-ASA in the Treatment of Ulcerative Colitis. *Inflamm Bowel Dis*. 1997;3(2):65–78.

14. Epifano F, Genovese S, Carlucci G, Locatelli M. Novel prodrugs for the treatment of colonic diseases based on 5-aminosalicylic acid, 4'-geranyloxyferulic acid, and auraptene: biological activities and analytical assays. *Curr Drug Deliv*. 2012;9(2):112–121.
15. Dhaneshwar SS, Gairola N, Kandpal M, Bhatt L, Vadnerkar G, Kadam SS. Synthesis, kinetic studies and pharmacological evaluation of mutual azo prodrug of 5-aminosalicylic acid with D-phenylalanine for colon specific drug delivery in inflammatory bowel disease. *Bioorg Med Chem Lett*. 2007;17(7):1897–1902.
16. Wiwattanapatapee R, Lomlim L, Saramunee K. Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. *J Control Release*. 2003;88(1):1–9.
17. Dunwell DW, Evans D. Synthesis and antiinflammatory activity of some 2-aryl-6-benzoxazoleacetic acid derivatives. *J Med Chem*. 1977;20(6):797–801.
18. Bekhit AA, Khalil MA. Non-steroidal anti-inflammatory agents: synthesis of novel benzopyrazolyl, benzoxazolyl and quinazoliny derivatives of 4(3 H)-quinazolinones. *Pharmazie*. 1998;53(8):539–543.
19. Kumar D, Jacob MR, Reynolds MB, Kerwin SM. Synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1. *Bioorg Med Chem*. 2002;10(12):3997–4004.
20. Huang ST, Hsei IJ, Chen C. Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoxazoles), and benzothiazoles. *Bioorg Med Chem*. 2006;14(17):6106–6119.
21. Oksuzoglu E, Tekiner-Gulbas B, Alper S, et al. Some benzoxazoles and benzimidazoles as DNA topoisomerase I and II inhibitors. *J Enzyme Inhib Med Chem*. 2008;23(1):37–42.
22. Yang Z, Fairfax DJ, Maeng JH, et al. Discovery of 2-substituted benzoxazole carboxamides as 5-HT3 receptor antagonists. *Bioorg Med Chem Lett*. 2010;20(22):6538–6541.
23. Cashin CH, Dawson W, Kitchen EA. The pharmacology of benoxaprofen (2-[4-chlorophenyl]-alpha-methyl-5-benzoxazole acetic acid), LRCL 3794, a new compound with antiinflammatory activity apparently unrelated to inhibition of prostaglandin synthesis. *J Pharm Pharmacol*. 1977;29(6):330–336.
24. Dunwell DW, Evans D, Hicks TA. Synthesis and antiinflammatory activity of some 2-heteroaryl-alpha-methyl-5-benzoxazoleacetic acids. *J Med Chem*. 1975;18(11):1158–1159.
25. Dawson W. The comparative pharmacology of benoxaprofen. *J Rheumatol Suppl*. 1980;6:5–11.
26. Egan RW, Gale PH. Comparative biochemistry of lipoxygenase inhibitors. In: Bailey JM, editor. *Prostaglandins, Leukotrienes and Lipoxins (Contemporary Issues in Risk Analysis)*. New York, NY: Springer; 1985:593–607.
27. Allen BR, Littlewood SM. The aetiology of psoriasis: clues provided by benoxaprofen. *Br J Dermatol*. 1983;109 Suppl 25:126–129.
28. Bosman B. Testing of lipoxygenase inhibitors, cyclooxygenase inhibitors, drugs with immunomodulating properties and some reference antipsoriatic drugs in the modified mouse tail test, an animal model of psoriasis. *Skin Pharmacol*. 1994;7(6):324–334.
29. Hawkey CJ, Rampton DS. Benoxaprofen in the treatment of active ulcerative colitis. *Prostaglandins Leukot Med*. 1983;10(4):405–409.
30. Wallace JL, MacNaughton WK, Morris GP, Beck PL. Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease. *Gastroenterology*. 1989;96(1):29–36.
31. Uekama K, Minami K, Hirayama F. 6A-O-[(4-biphenyl)acetyl]-alpha-, -beta-, and -gamma-cyclodextrins and 6A-deoxy-6A-[(4-biphenyl)acetyl]amino]-alpha-, -beta-, and -gamma-cyclodextrins: potential prodrugs for colon-specific delivery. *J Med Chem*. 1997;40(17):2755–2761.
32. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology*. 1989;96(3):795–803.
33. Morris GP, Rebeiro L, Herridge MM, Szewczuk M, Depew W. An animal model of chronic granulomatous inflammation of the stomach and colon. *Gastroenterology*. 1984;86:1188.
34. Stephens FF, Bower JD. 626. The preparation of benzimidazoles and benzoxazoles from Schiff's bases. Part I. *J Chem Soc*. 1949;1949:2971–2972.
35. Stephens FF, Bower JD. 348. The preparation of benzimidazoles and benzoxazoles from Schiff's bases. Part II. *J Chem Soc*. 1950;1950:1722–1726.
36. Wadworth AN, Fitton A. Olsalazine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in inflammatory bowel disease. *Drugs*. 1991;41(4):647–664.

## Drug Design, Development and Therapy

### Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which

Submit your manuscript here: <http://www.dovepress.com/drug-design-development-and-therapy-journal>

Dovepress

has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.