Received Date: 29-Sep-2016
Revised Date: 25-Dec-2016
Accepted Date: 28-Dec-2016
Article Type: Full Paper

Synthesis and *in-vitro* bioactivity evaluation of new galactose and fructose ester derivatives of 5-aminosalicylic acid

Samira Yousefi [a] *), **Saadi Bayat**[a]) , **Mohd Basyaruddin Abdul Rahman**[a,b,c]), **Zalikha Ibrahim**[a]), and **Emilia Abdulmalek**[a,b]*)

[a]) Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia, (syousefi12@yahoo.com and emilia@science.upm.edu.my)

[b]) Enzyme and Microbial Technology Centre, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

[c]) Structural and Synthetic Biology Research Center, Malaysia Genome Institute, 43600 Bangi, Selangor, Malaysia

Inflammatory bowel disease (IBD) is the main risk factor for developing colorectal cancer which is common in patients of all ages. 5-aminosalicylic acid (5-ASA), structurally related to the salicylates, is highly active in the treatment of IBD with minor side effects. In this study, the synthesis of galactose and fructose esters of 5-ASA was planned to evaluate the role of glycoconjugation on the bioactivity of the parent drug. The antibacterial activity of the new compounds were evaluated against two Gram-negative and two Gram-positive species of bacteria, with a notable effect observed against *Staphylococcus aureus* and *Escherichia coli in* comparisons with the 5-ASA. Cytotoxicity testing over HT-29 and 3T3 cell lines indicated that the toxicity of the new products against normal cells was significantly reduced compared with the original drug, whereas their activity against cancerous cells was slightly decreased. The anti-inflammatory activity test in RAW264.7 macrophage cells indicated that the inhibition of nitric oxide by both of the monosaccharide conjugated derivatives was slightly improved in comparison with the non-conjugated drug.

Keywords: 5-ASA • Galactose • Fructose • Biological evaluation

Introduction

One of the principal risk factors for developing colorectal cancer is a personal history of inflammatory bowel disease (IBD), an umbrella term which also includes ulcerative colitis and Crohn's disease. IBD is a highly prevalent group of diseases which can affect patients across a wide age range [1-3]. 5-aminosalicylic acid (5-ASA) is one particular pharmaceutical that has long been favoured for the first-stage treatment of IBD because it causes relatively few side effects and is well tolerated by the majority of patients [4-6]. 5-ASA is also a proven antioxidant agent, displaying scavenging effects on reactive nitrogen and oxygen species (known as free radicals) [5]. Experimental and epidemiological studies have revealed that, in long-term usage, 5-ASA represses gastrointestinal toxicity [1] and acts as a chemopreventive agent, [6, 7] particularly against cancers of the intestine and colon [8]. Up to the present time, diverse derivatives of this drug have been prepared to optimize the properties and applications of 5-ASA. As an example, 5-ASA which has been linked to amino acids indicated better stability of the drug in the stomach and small intestine of the test animals due to the selective cleavage by intestinal brush-borders [9]. In addition, metal complexes of 5-ASA have been reported to have increased antimicrobial activity compared to 5-ASA [10]. Nanoparticle oral formulations of 5-ASA have allowed the drug to be released more selectivity in the colon thus reducing drug dosages [11]. To release higher concentrations of intact 5-ASA in the colon a number of researchers have focused on the location of IBD in the gastrointestinal tract and have since designed different location-specific colon delivery systems to achieve this outcome [12, 13].

Despite the remarkable properties of 5-ASA which have led to the creation of numerous synthetically-designed derivatives of the drug, the problem of its poor stability in the intestinal tract [14] and low water-solubility is yet to be resolved [13, 15]. Unfortunately, a high proportion of 5-ASA is absorbed in the small intestine [2] requiring an increased dose and a prolonged treatment period are required to ensure that a sufficient concentration of the drug reaches the colon [4, 16].

Sugar drug derivatives have opened a new gateway in the medical and pharmaceutical field and, currently, a great number of the drugs commonly in use rely on linkage of carbohydrates [17]. Glycosidic bonds are known to improve the water-solubility, stability, and biocompatibility of drugs [18, 19]. Glycosidic conjugation also leads to better absorption [20] and penetration of drugs into the cell membranes, thus aiding delivery of drugs to the targeted site [21]. For example, the anticancer activity and water solubility of glucose-aspirin conjugate were improved eight to nine-fold and seven-fold, respectively, in comparison with unlinked aspirin [22]. The ibuprofen-fructose ester showed extended stability in aqueous solution, and fewer adverse effects, enhanced tissue uptake in comparison with ibuprofen alone [23]. Many carbohydrate-based compounds have also demonstrated antimicrobial activity comparable to currently available antimicrobials [24]. For instance, galactose and fructose laurates have demonstrated excellent antibacterial effects against both Gram-positive and Gram-negative bacteria when compared with alternative analogues of synthesized fatty acid-carbohydrate esters [25]. Likewise, fructose derivatives of 1,2,3-triazole have displayed antibacterial activity toward initial compound [26].

Because the conjugation of drugs with saccharides presents multiple opportunities to improve bioavailability and pharmaceutical properties [19, 22], monosaccharide derivatives of 5-ASA were prepared in this study to investigate the role of the glycoside conjugation on the biological properties of this drug, [27]. Since the ester linkage is known to be stable in acidic environments such as the stomach, yet readily hydrolyses in the basic environment of the lower alimentary tract [28], the ester derivatives of 5-ASA were prepared with the intention of permitting the active moiety of the drug to be released only when it arrives at the colon.

Results and Discussion

Synthesis

To prepare monosaccharide ester derivatives of 5-ASA, before initiating the esterification process it is necessary to protect the multiple hydroxyl groups of sugars and the amino group of 5-ASA, in order to limit their involvement in the reaction. Since the amino group is more reactive than the phenolic OH and carboxylic acid groups, *N*-acylation readily occurs in the presence of acetic anhydride and *N*-acetyl-5-aminosalicylate (**1**) was obtained with a yield of 67.5%.

The selective protection of hydroxyl groups of galactose and fructose was performed using the *O*-isopropylidenation technique. The biological advantages of *O*-isopropylidene derivatives include low toxicity, anti-inflammatory activity and antipyretic activity, and their convenient application in synthetic studies continue to encourage researchers to apply them as key starting materials in many biochemical studies. [29]. *O*-isopropylidenation provides selective protection of the carbohydrate hydroxyl groups under mild conditions in a single step, therefore, reducing the number of steps. Also, the acetal protecting groups are relatively stable at room temperature, yet are readily removed under mildly acidic or basic conditions [29-31]. Adding Lewis acid metal salt catalysts such as $CuSO_4$, $FeCl_3$, or $ZnCl_2$ to the reaction decreases the yield of mono-*O*-isopropylidenation which leads to an increase in selectivity and increases the yield of di-*O*-isopropylidenation [30]. This is because they generate a far superior leaving group than acid catalysts. After protection of monosaccharides 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (**2**) and 1,2:4,5-Di-*O*-isopropylidene-D-fructopyranose (**3**) were obtained with yields of 63.8% and 58.3%, respectively.

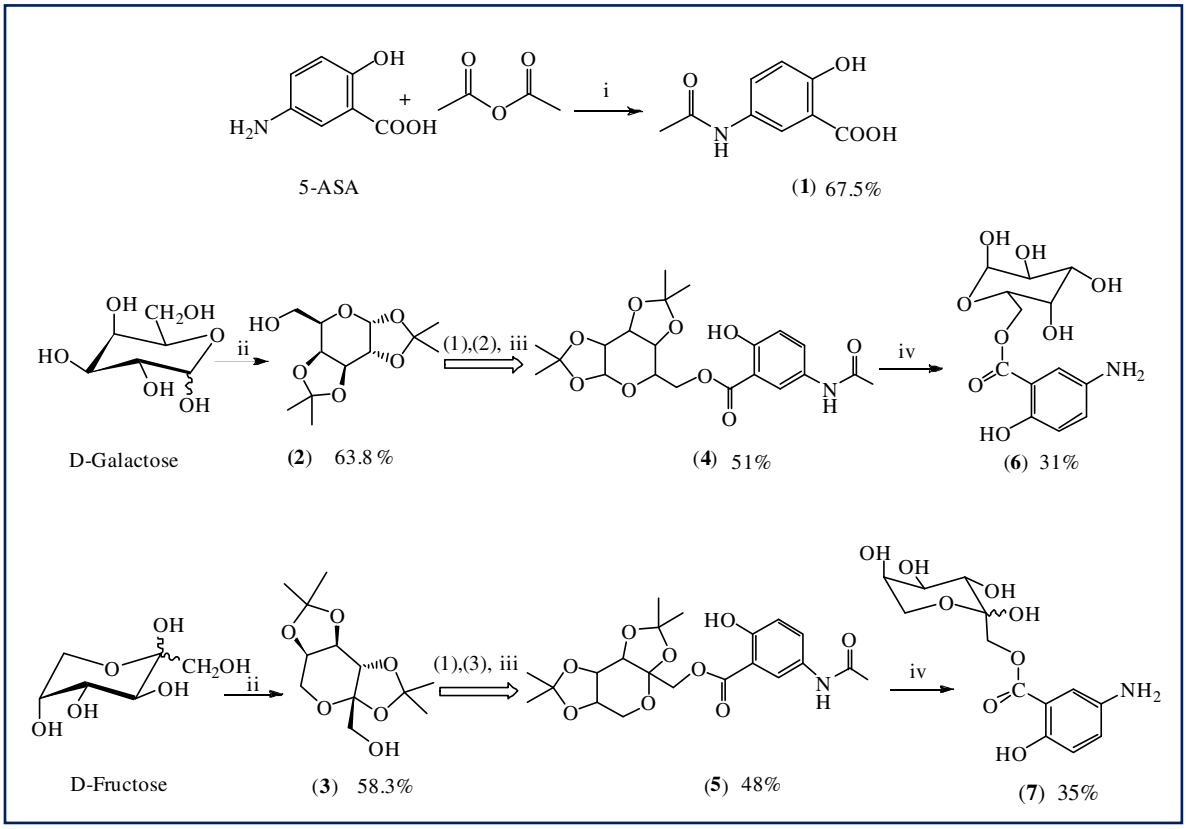
Recent advances in utilising coupling reagents in organic synthesis, particularly carbodiimides in the presence of an organic base, have been steadily progressing since they are broadly applicable across a wide range of reactions such as esterification, amidation and peptide synthesis. Using coupling reagents has major advantages, including the fact that the carboxyl component and the alcohol are directly combined and the subsequent treatment with activating agents can be immediate; the reaction performs at room temperature using relatively non-toxic solvents in neutral to mildly basic (pH 7-8) conditions; the coupling reagents are inexpensive and commercially available and a relatively small equimolar of them (1.5-2 eq.) is needed.

Therefore, esterification of *N*-acetyl-5-aminosalicylate with the protected sugars was carried out via a one-step procedure which avoid necessitating harsh reaction conditions by using the coupling reagents *N,N'*-Dicyclohexylcarbodiimide (DCC) and 4-Dimethylaminopyridine (DMAP). From the esterification reaction 6-*O*-*N*-acetyl-5-aminosalicylate-1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (**4**) and 1-*O*-*N*-acetyl-5-aminosalicylate-2,3:4,5-Di-*O*-isopropylidene-D-fructopyranose (**5**) were obtained in a moderate yield of 51% and 48%, respectively.

The disparate solubilities of the reaction products were advantageously utilised to facilitate purification. Dicyclohexylurea (DHU) is insoluble in the final mixture and was readily eliminated via filtration; DCC is extremely nonpolar and was easily separated using the nonpolar solvent, hexane; whereas DMAP is relatively water-soluble and was removed via an aqueous workup.

Finally, deprotection of **4** and **5** was achieved under mildly acidic conditions in the presence of acetic acid and 5-ASA monosaccharide ester derivatives were successfully obtained, including 6-*O*-5-aminosalicylate-α-D-galactopyranose (**6**) and 1-*O*-5-aminosalicylate-D-fructopyranose (**7**). The multistep process for preparing ester derivatives and the subsequent purification caused a significant loss in yield amount of the final products, and resulted in low yields of **6** and **7**, with 31% and 35% in respective yields.

Synthesis steps designed to prepare monosaccharide ester derivatives of 5-ASA are illustrated in Scheme 1. The yields of all the produced compounds were measured after purification and their structures were fully characterized by spectroscopic techniques, (^{1}H NMR, ^{13}C NMR, FT-IR and DIMS).



Scheme 1. Synthetic route for preparing 5-ASA galactose and fructose ester derivatives (**6** and **7**). Reagents and conditions: (i) H_3PO_4, 80C° ; (ii) acetone, $ZnCl_2$, H_3PO_4, 5-7 h ; (iii) DMAP, DCM, DCC, 24 h, *r.t* ; (iv) acetic acid 85%, reflux under N_2 gas, 5-7 h. Yields were reported in purified compounds.

Antibacterial activity

Although 5-ASA is not an antibiotic agent, the antibacterial activity of **6** and **7** were investigated in comparison with the initial drug to explore the potential of glycosidic conjugation against microorganisms for future study and interest. The antibacterial test was carried out via the disc diffusion technique against two Gram-positive bacteria (*Salmonella choleraesuis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Bacillus subtilis* and *Escherichia coli*). The antibacterial activity of **6** and **7** shown not to be as effective as streptomycin (an antimicrobial agent), ($p < 0.05$). Also, no activity was detected against the *Salmonella choleraesuis* and *Bacillus subtilis* bacteria for new derivatives; however, conjugation of 5-ASA to monosaccharides has somewhat activated the derivatives against the two bacterial strains *Staphylococcus aureus* (*S. aureus*) and as well as *Escherichia coli* (*E. coli*), in comparison with the parent drug which is devoid of any antibacterial properties, (Table 1).

An average inhibition zone of 15(±1) mm was determined for both **6** and **7** against *S. aureus* and an average inhibition zone of 10(±1) mm and 11(±1) mm was measured against *E. coli* for **6** and **7**, respectively (Table 1). The inhibition activity of antibacterial agents depends on their permeability into the ribosomes of microorganisms or microbial cells [10]. Although, no significant difference was determined between the antibacterial activities of **6** and **7** ($p > 0.05$), based on our observations with naked eyes, both esters had greater inhibition zones against Gram-positive (*E. coli*) bacteria compared to Gram-negative ones (*S. aureus*). This is due to the fact that most Gram-negative

bacteria have a thicker cell wall than Gram-positive bacteria and are thus more difficult to penetrate into their cells [24, 32].

MIC (Minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) of **6** and **7** were determined using the macro broth serial dilution method [33] only in the bacteria that displayed sensitivity to test compounds in the disc diffusion assay. As shown in Table 2, against *S. aureus* bacteria, almost no visible turbidity was observed at concentrations of 1.92×10^3 μg mL^{-1} and 1.60×10^3 μg mL^{-1} for **6** and **7**, respectively, whereas the parent drug showed a MIC value of 3.33×10^3 μg mL^{-1}.

Also, against *E. coli*, both **6** and **7** showed a lower MIC value of 2.77×10^3 μg mL^{-1} and 2.31×10^3 μg mL^{-1}, respectively, than 5-ASA which maintained its turbidity at a concentration of 3.33×10^3 μg mL^{-1}. (Table 2).

The MBC test on a nutrition agar medium resulted in a lack of growth of microbial colonies of *S. aureus* bacteria at concentrations of 3.33×10^3 μg mL^{-1} and 2.77×10^3 μg mL^{-1} for **6** and **7**, respectively. However, a slight growth of *E. coli* bacteria was observed for both of them even at a 3.33×10^3 μg mL^{-1} concentration (Table 2). The lower MIC/MBC values obtained for **6** and **7** against *S. aureus* compared to *E. coli* support our observations in the disc diffusion assay that the new derivatives are more effective against Gram-positive bacteria than Gram-negative bacteria.

Table 1. Antibacterial inhibition area (mm) of test compounds [a].

Sample	Microbes			
	Diameter of inhibition zone in millimeter (±1mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	S. choleraesuis	E. coli	B. subtilis
5-ASA	NA	NA	NA	NA
Streptomycin	35	NA	27	NA
Ethanol	NA	NA	NA	NA
6	15	NA	10	NA
7	15	NA	11	NA

[a] *NA*: No activity found at the tested concentration. Streptomycin and ethanol were used as positive and negative controls, respectively. The test was duplicated and the average inhibition zones were recorded. *S. choleraesuis*: Salmonella choleraesuis, *S. aureus*: Staphylococcus aureus, *B. subtilis*: Bacillus subtilis and *E. coli*: Escherichia coli. For statistical analysis of the data Student's *t* test was employed, ($p > 0.05$ compared between two species of bacteria, $p < 0.05$ versus positive control). *P*-value < 0.05 was considered as statistically significant from initial drug, 5-ASA.

Table 2. MIC and MBC of test compounds [a].

Sample	Microbes			
	MIC/MBC (μg mL^{-1})			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	S. choleraesuis	E. coli	B. subtilis
5-ASA	3330/>3330	NT/NT	>3330/NT	NT/NT
6	1920/3330	NT/NT	2770/>3330	NT/NT
7	1600/2770	NT/NT	2310/>3330	NT/NT
Streptomycin*	16.74/24.11	NT/NT	24.11/28.93	NT/NT

[a] *NT*: Not tested. Streptomycin *: Positive control. *S. choleraesuis*: Salmonella choleraesuis, *S. aureus*: Staphylococcus aureus, *B. subtilis*: Bacillus subtilis and *E. coli*: Escherichia coli.

Anti-inflammatory activity

Inflammation is the body's immune system defense mechanism in dealing with external threats such as infection, tissue damage and other harmful foreign stimuli. It responds to these threats by secreting proinflammatory mediators. An excess of these mediators is characterized by symptoms such as pain and swelling; however, long-term exposure contributes towards serious problems such as cardiovascular disorders and cancer. One of the key inflammatory factors is nitric oxide (NO), which is secreted from nitric oxide synthase (iNOS) during the process of inducing macrophage activation within inflammation, [34, 35].

In this work, the NO inhibition activity of **6** and **7** was evaluated in IFN-γ/LPS-stimulated RAW264.7 macrophages and compared with the initial drug. Although the newly-synthesized esters and 5-ASA at a 50 µM test concentration were found to be less effective than curcumin (positive control) and a less than 50% NO inhibition rate was achieved, the conjugation of 5-ASA to galactose and fructose enhanced the NO inhibition (%) activity of 5-ASA from 9.0 to 15.7 (approximately 1.7-fold) and to 12.7 (almost 1.4-fold) for **6** and **7**, respectively ($p < 0.05$ for both compounds from the 5-ASA). Furthermore, the cytotoxicity test confirmed that the NO inhibition at a 50 µM concentration was not due to the toxicity of the samples, (Table 3).

Table 3. *In vitro* NO suppression activity and cytotoxicity of test compounds on RAW 264.7 cells [a].

Sample	NO inhibition (%) ±S.E.M	NO inhibition IC_{50} (µM) ± S.E.M	Cytotoxicity IC_{50} (µM)
Curcumin	99.3 ± 0.2	14.7 ± 0.2	>100
5-ASA	9.0 ± 1.1	ND	>100
6	15.7 ± 1.2*	ND	>100
7	12.7 ± 2.3*	ND	>100

[a] *ND*: Not detected. *S.E.M*: Standard error of the mean (n = 6). Curcumin: Positive control. Tested concentration: 50 µM. For statistical analysis of the data Student's *t* test was employed. **P*-value < 0.05 was considered as statistically significant from initial drug, 5-ASA.

Cytotoxicity evaluation

During the course of cancer treatment, the main purpose is to reduce the toxicity of drugs towards normal cells while maintaining their anticancer properties towards cancerous cells.

The cytotoxicity study against cancerous cells (HT-29 cell line) showed that the cytotoxic activity of **6** (IC_{50}: 12.2 µg mL^{-1}) and **7** (IC_{50}: 10.9 µg mL^{-1}) were somewhat decreased toward the original drug (IC_{50}: 5.1 µg mL^{-1}). However, they were much less toxic towards normal cells (3T3 cell line) than the parent drug, because 50% of 3T3 cells were inhibited by approximately 221.4 µg mL^{-1} of ester **6** and 311.9 µg mL^{-1} of ester **7** while only 6.1 µg mL^{-1} of 5-ASA suppressed 50% of normal cells. As a result, despite the cytotoxicity activity of the new esters (**6** and **7**) over both HT-29 and 3T3 cell lines being slightly less than 5-ASA, their reduced toxicity towards normal cells made them less harmful in higher dosages of the drug, (Table 4 and Fig. 1).

Table 4. *In vitro* cytotoxicity activity of test compounds against 3T3 and HT-29 cell lines [a].

Sample	HT-29 cells IC_{50} (µg mL^{-1}) ± SD	3T3 cells IC_{50} (µg mL^{-1}) ± SD
5-ASA	5.1 ± 0.01	6.1 ± 0.008
6	12.2 ± 0.08	221.4 ± 0.020
7	10.9 ± 0.02	311.9 ± 0.010

[a] SD: Standard deviation, (n = 6).

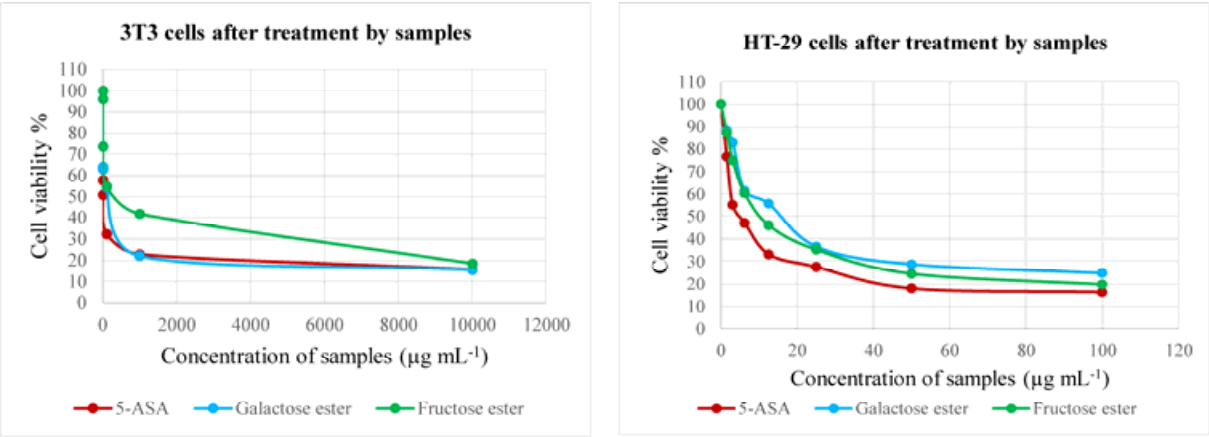


Fig. 1. Graphs were plotted for HT-29 and 3T3 cell lines after treatment by test compounds via MTT assay which measured cell viability by percentage against their respective concentrations (µg mL^{-1}).

Conclusion

Galactose and fructose esters of 5-aminosalicylic acid were successfully prepared in average yields. The structures of all of the compounds produced were characterized by spectroscopic techniques. Bioactivity evaluations demonstrated that esterification of 5-ASA with monosaccharide have somewhat activated the derivatives toward the *E. coli* and *S. aureus* bacteria. Also, they appeared to be more effective against nitric oxide inflammatory factors than the parent drug. Although the anticancer properties of the new sugar ester derivatives were slightly lower than the parent drug, their toxicities towards normal cells were significantly reduced. These findings may be a step forward in optimizing the properties of 5-ASA sugar esters and provide encouragement for further studies in the future.

Experimental Part

General

All materials purchased for used in this project had analytical grade and have been used without further purification, unless specifically stated. Merck silica gel 60 (70–230 mesh) was used for the flash chromatography. Analytical thin layer chromatography (TLC) was carried out by using Merck 60 F254 precoated silica gel plate (0.2 mm thickness). 5-ASA, DCC, DMAP and molecular sieves 3Å (8 to 12 mesh) were purchased from ACROS. D-galactose and D-fructose were purchased from Fisher Scientific. All solvents were purchased from J.T. Baker. Nutrient agar and nutrient broth were purchased from Sigma Aldrich. Bacterial strain subcultures were prepared at the Department of

Microbiology, Faculty of Medicine and Health Sciences, UPM. RAW 264.7 murine macrophages cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA).

NMR data were obtained on a 500 MHz for ^{1}H NMR JEOL spectrometer and a 125.8 MHz for ^{13}C NMR JEOL spectrometer. Detection of functional groups was conducted by Fourier Transform Infrared Spectroscopy (FT-IR, Perkin Elmer Spectrum 100). Direct infusion mass spectrometry (DIMS) was applied for mass characterization using a Single Quadrupole Mass Detector. Mass of compound N-acetyl-5-aminosalicylate was recorded on a GCMS QP2010 Plus SHIMADZU. Optical rotations of corresponding products were measured by a JASCO P-2000 Polarimeter. Melting points were measured by a Barnstead Electrothermal instrument.

Preparation of *N*-acetyl-5-aminosalicylate (1)

The synthesis of *N*-acetyl-5-aminosalicylate was conducted in accordance with the previously described procedures [36]. Under the fume hood, 5-ASA (3 g, 19.6 mmol) in a flask placed in a warm water bath (80°C) was dissolved in acetic anhydride (20 mL) and 5 to 10 drops of phosphoric acid (85%) were added to the mixture, which was then stirred for about 15 to 30 minutes. When the mixture had been dissolved completely, it was removed from the warm water bath and 10 to 20 drops of distilled water were cautiously added to the warm mixture. Subsequently, the flask was placed in an ice bath and 50 mL of cool distilled water was added to the mixture; crystallization was induced by scratching the container's walls with a glass rod. Afterwards, the obtained crystals were filtered using a Buchner funnel and were washed several times with the cold distilled water to remove any excess acid. Ethanol (95%) was used for recrystallization to yield pure *N*-acetyl-5-aminosalicylate (**1**), (Scheme 1). The product was initially monitored with TLC (mobile phase 8:2, ethyl acetate/methanol) and visualized under a UV lamp.

Preparation of 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (2) and 1,2:4,5-Di-*O*-isopropylidene-D-fructopyranose (3)

Hydroxyl groups of galactose and fructose were protected based on the previously discussed methods [26, 37] with some modification. Briefly, in a flask fitted with an anhydrous calcium chloride guard tube monosaccharide (1 g, D-galactose or D-fructose; 5.5 mmol), freshly fused anhydrous $ZnCl_2$ (0.77 g, 5.7 mmol) and acetone (100 mL) were mixed together in the presence of 5 to 10 drops of H_3PO_4 and molecular sieves (2% w/w). The mixture was stirred vigorously for 5-7 hours at room temperature. The product was initially monitored by TLC (mobile phase 9:1, ethyl acetate/hexane). After that, the mixture was filtered and gradually neutralized with $NaHCO_3$ (10%), dried with $MgSO_4$, and acetone was removed under the reduced pressure. Afterwards, the mixture was dissolved in hexane (5 × 50 mL) and precipitations were separated from the hexane by decanting and collecting the hexane. Once hexane evaporated under reduced pressure, the precipitate was collected. Further purification was carried out via flash chromatography with the same mobile phase applied for TLC to obtain 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (diacetone galactose, **2**) and/or 1,2:4,5-Di-*O*-isopropylidene-D-fructopyranose (diacetone fructose, **3**) (Scheme 1).

Preparation of *N*-acetyl-5-aminosalicylate diacetone-monosaccharide ester derivative

In a flask placed on an ice bath, *N*-acetyl-5-aminosalicylate, (0.1 g, 0.5 mmol), diacetone-monosaccharide (0.2 g, ~0.7mmol) and DMAP (0.09 g, 0.7 mmol) were dissolved in dichloromethane

(DCM). After that, DCC (0.15 g, 0.7 mmol) was added batch-wise (over 15-20 minutes) to the mixture and it was then stirred for 24 hours at room temperature. After monitoring of the product by TLC (eluent solvent 8:2, ethyl acetate/hexane) DHU was separated by filtration and the solvent was removed under high vacuum. Next, the mixture was dissolved in cold hexane (5 × 50 mL) to remove the remaining DCC. Later, hexane was removed by decanting and precipitations were collected. After that, DMAP was separated from precipitations by a liquid-liquid extraction method using NH_4Cl (20%) and DCM. Subsequently, the organic layer was collected and dried with $MgSO_4$ and DCM was evaporated under reduced pressure. Further purification was achieved through column chromatography (mobile phase 8:2, ethyl acetate/hexane) to obtain 6-*O*-*N*-acetyl-5-aminosalicylate-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**4**) and/or 1-*O*-*N*-acetyl-5-aminosalicylate-2,3:4,5-di-*O*-isopropylidene-D-fructopyranose (**5**), (Scheme 1).

Preparation of *O*-5-aminosalicylate monosaccharide esters

In a round bottom flask, **4** or **5**; (0.1 g, 0.22 mmol) and acetic acid (85%, 10 mL) were combined together and refluxed under nitrogen gas for 5-7 hours to form a clear solution. The mixture was then concentrated under reduced pressure and then neutralized by $NaHCO_3$ (5%). Purification was performed with column chromatography (9:1 ethyl acetate/methanol) to yield 6-*O*-5-aminosalicylate-α-D-galactopyranose and/or 1-*O*-5-aminosalicylate-D-fructopyranose, (Scheme 1).

***N*-acetyl-5-aminosalicylate or 5-acetamido-2-hydroxy benzoic acid (1)**

White, needle-shape crystals. **M.p**: 228-230 °C. **Yield**: 67.5%. **FT-IR** (ν): 3561, 3239, 3064, 2818, 2699, 2573, 2490, 1796, 1674, 1618, 1537, 1486, 1208, 1018 cm^{-1}. **^{1}H NMR** (500 MHz, DMSO-d_6): δ (ppm) = 2.08 (3H, s, C**H$_3$**), 6.83 (1H, d, *J*= 7.8 Hz, Ar-C**H**), 7.55 (1H, d, *J*= 7.8 Hz, Ar-C**H**), 8.05 (1H, s, Ar-C**H**), 10.41 (1H, s, COO**H**). **^{13}C NMR** (125.8 MHz, DMSO-d_6) δ = 24.2 (**C**H$_3$), 113.8, 114.4, 120.9, 129.7; (4C, Ar-**C**H), 130.4 (Ar-**C**-NH), 156.7 (Ar-**C**-OH), 165.9 (**C**=O), 169.8 (**C**OOH). **Ms:** GC-MASS m/z: 195 ([M]$^+$, $C_9H_9NO_4^+$; calc. 195.05).

1,2:3,4-di-*O*-isopropylidene-D-galactopyranose or diacetone-galactose (2)

A colourless viscous Liquid. **B.p**: 117-118°C. $[\alpha]_D^{24}$ = -0.57, (c=0.4, acetone). **Yield**: 63.8%. **FT-IR** (ν): 3491, 2990, 2925, 1706, 1456, 1379, 1214, 1169, 1070, 1002 cm^{-1}. **^{1}H NMR** (500 MHz, acetone-d_6): δ (ppm) = 1.25 (6H, s, 2 C**H$_3$**), 1.34 (3H, s, C**H**$_3$), 1.43 (3H, s, C**H**$_3$) 2.90 (1H, s, O**H**), 3.57-3.60 (1H, m, **H$_5$**), 3.64-3.68 (2H, m, **H$_6$**), 3.80 (1H, t, *J*=12.5 Hz, **H$_3$**), 4.25 (1H, d, *J*=9.4 Hz, **H$_4$**), 4.57 (1H, d, *J*=8.1 Hz, **H$_2$**), 5.42 (1H, d, *J*= 5.06 Hz, **H$_1$**). **^{13}C NMR** (125.8 MHz, acetone-d_6) δ = 23.9, 24.6, 25.5, 25.5; (4C, **C**H$_3$), 60.9 (**C$_6$**), 61.0, 68.6, 70.7; (**C$_2$**, **C$_3$**, **C$_4$**), 70.98 (**C$_1$**), 96.3 (**C$_5$**), 108.0, 108.6; (2C, **C**-$(CH_3)_2$). **MS**: DIMS m/z: 283.115 ([M+Na]$^+$, $C_{12}H_{20}$ Na O_6^+; calc. 283.116).

2,3:4,5-di-*O*-isopropylidene-D-fructopyranose or diacetone-fructose (3)

White crystals. **M.p**: 94-96°C. $[\alpha]_D^{24}$ =-0.42, (c=0.4, acetone). **Yield**: 58.3%. **FT-IR** (ν): 3389, 2984, 2967, 1702, 1455, 1370, 1242, 1212, 1194, 1009 cm^{-1}. **^{1}H NMR** (500 MHz, acetone-d_6): δ (ppm) = 1.31 (6H, s, 2 C**H_3**), 1.43 (6H, s, 2 C**H_3**), 2.40 (1H, s, O**H**), 3.62-3.71 (2H, m, **H_{1a}**, **H_{6a}**), 3.80-3.93 (2H, m, **H_{1b}**, **H_{6b}**), 4.28-4.37 (2H, m, **H_3**, **H_4**), 4.69 (1H, dd, *J*=11.4 Hz, 5.7 Hz, **H_5**). **^{13}C NMR** (125.8 MHz, acetone-d_6): δ = 23.8, 24.2, 25.8; (4C, **C**H_3), 60.9, 61.0; (2C, **C_1**, **C_6**), 68.7, 71.7: (**C_4**, **C_5**), 74.4 (**C_3**), 103.5 (**C_2**), 104.0 110.6; (2C, **C**-$(CH_3)_2$). **MS**: DIMS m/z: 283.114 ($[M+Na]^+$, $C_{12}H_{20}$ Na O_6^+; calc. 283.116).

6-*O*-*N*-acetyl-5-aminosalicylate-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (4)

Colorless crystals. **M.p**: 206-208°C. **Yield**: 51%. **FT-IR** (ν): 3391, 3191, 2920, 2856, 1720, 1642, 1455, 1377, 1271, 1128, 1056 cm^{-1}. **^{1}H NMR** (500 MHz, methanol-d_4): δ (ppm) = 1.28 (12H, br s, 2 C**H_3**-C-C**H_3**), 2.07 (3H, s, C**H_3**-C=O), 4.17-4.29 (4H, m, **H_2**, **H_3**, **H_4**, **H_{6a}**), 4.43-4.49 (2H, m, **H_1**, **H_5**), 5.01 (1H, s, O**H**), 5.27 (1H, d, *J*= 4.2 Hz, **H_{6b}**), 6.92 (1H, d, *J*= 8.2 Hz, Ar-C**H**), 7.29 (1H, d, *J*= 8.2 Hz, Ar-C**H**), 8.09 (1H, s, Ar-C**H**), 8.28 (1H, s, N**H**). **^{13}C NMR** (125.8 MHz, methanol-d_4) δ = 25.9 (O=C-**C**H_3), 26.3, 26.5; (4C, **C**H_3), 63.4 (**C_2**), 70.7, 70.8; (**C_3**, **C_4**), 72.2 (**C_6**), 76.5 (**C_5**), 94.5 (**C_1**), 106.3 (Ar-**C**-COO), 111.1, 116.3; (2C, Ar-**C**H), 117.2, 122.1; (2C, **C**-$(CH_3)_2$), 127.6 (Ar-**C**H), 130.4 (Ar-**C**-NH), 157.7 (Ar-**C**-OH), 171.9 (**C**OO), 172.1 (O=**C**-CH_3). **Ms**: DIMS m/z: 460.159 ($[M+Na]^+$, $C_{21}H_{27}N$ Na O_9^+; calc. 460.158).

1-*O*-*N*-acetyl-5-aminosalicylate-2,3:4,5-di-*O*-isopropylidene-D-fructopyranose (5)

White crystals. **M.p**: 162-164°C. **Yield**: 48%. **FT-IR** (ν): 3358, 3292, 2920, 2852, 1719, 1636, 1568, 1462, 1337, 1273, 1135 cm^{-1}. **^{1}H NMR** (500 MHz, methanol-d_4): δ (ppm) = 1.30 (6H, s, 2 C**H_3**), 1.50 (6H, s, 2 C**H_3**), 2.07 (3H, s, C**H_3**-C=O), 3.59 (2H, dd, *J*= 10.2 Hz, 4.9 Hz, **H_{6a}**, **H_{6b}**), 3.87-3.92 (2H, m, **H_4**, **H_5**), 4.29 (1H, d, *J*= 7.6 Hz, **H_3**), 4.41-4.55 (2H, m, **H_{1a}**, **H_{1b}**), 5.48 (1H, s, O**H**), 7.30 (1H, d, *J*= 7.9 Hz, Ar-C**H**), 7.63 (1H, d, *J*= 7.9 Hz, Ar-C**H**), 7.89 (1H, s, Ar-C**H**), 8.05 (1H, s, N**H**). **^{13}C NMR** (125.8 MHz, methanol-d_4) δ = 20.9 (O=C-**C**H_3), 21.6, 23.5, 26.9: (4C, **C**H_3), 62.0, 62.6; (2C, **C_1**, **C_6**), 70.0, 70.3; (**C_4**, **C_5**) 76.2 (**C_3**), 101.8 (**C_2**), 103.9, 111.0, 116.2; (3C, Ar-**C**H), 116.7, 121.2 (2C, **C**-$(CH_3)_2$), 122.4 (Ar-**C**H), 130.0 (Ar-**C**-NH), 156.4 (Ar-**C**-OH), 167.9 (**C**OO), 168.1 (O=**C**-CH_3). **MS**: DIMS m/z: 460.157 ($[M+Na]^+$, $C_{21}H_{27}N$ Na O_9^+; calc. 460.158).

6-*O*-5-aminosalicylate-α-D-galactopyranose (6)

White crystals. **M.p**: 222-224°C. **Yield**: 31%. **FT-IR** (ν): 3584, 3468, 3396, 3102, 2923, 2889, 1745, 1655, 1525, 1482, 1308, 1197, 1032 cm^{-1}. **^{1}H NMR** (500Hz, methanol-d_4): δ (ppm) = 2.10 (4H, s, 4 O**H**), 3.51-3.60 (1H, m, **H_4**), 3.67 (2H, dd, *J*=6.5 Hz, 4.5 Hz, **H_2**, **H_3**), 3.91 (2H, s, N**H_2**), 4.10-4.18 (1H, m, **H_5**), 4.37-4.46 (2H, m, C**H_2**), 5.03 (1H, s, Ar-O**H**), 5.47 (1H, d, *J*=4.5 Hz, **H_1**), 6.81 (1H, d, *J*=8.1 Hz, Ar-C**H**), 6.91 (1H, d, *J*=8.1 Hz, Ar-C**H**), 7.64 (1H, s, Ar-C**H**). **^{13}C NMR** (125.8 MHz, methanol-d_4) δ = 62.2 (**C_6**), 70.6, 71.3, 74.1 (**C_2**, **C_3**, **C_4**) 76.5; (**C_5**), 94.4 (**C_1**), 115.5 (Ar-**C**H), 116.2 (Ar-**C**-COO), 116.5, 122.0; (2C, Ar-**C**H), 129.7 (Ar-**C**-NH), 151.8 (Ar-**C**-OH), 165.9 (**C**OO). **MS**: DIMS m/z: 338.085 ($[M+Na]^+$, $C_{13}H_{17}NNaO_8^+$; calc. 338.085).

1-*O*-5-aminosalicylate-D-fructopyranose (7)

Light brown crystals. **M.p**: 173-175°C. **Yield**: 35%. **FT-IR** (ν): 3553, 3466, 3120, 2941, 2891, 1745, 1655, 1562, 1426, 1262, 1070, 1047 cm^{-1}. **^{1}H NMR** (500Hz, methanol-d_4): δ (ppm) = 2.88 (1H, s, O**H**), 2.91 (3H, s, 3 O**H**), 3.50-3.56 (4H, m, **H_3**, **H_4**, **H_5**, **H_{6a}**), 3.64-3.67 (1H, m, **H_{6b}**), 4.12 (2H, s, N**H_2**), 4.38 (2H, dd, *J*=14.4 Hz, 9.6 Hz, **H_{1a}**, **H_{1b}**), 5.08 (1H, s, Ar-O**H**), 6.59 (1H, d, *J*= 7.6 Hz, Ar-C**H**), 6.67 (1H, d, *J*= 7.6 Hz, Ar-C**H**), 6.98 (1H, s, Ar-C**H**). **^{13}C NMR** (125.8 MHz, methanol-d_4) δ = 62.3, 62.4; (2C, **C_1**, **C_6**), 72.0, 72.1(**C_3**, **C_5**), 76.6 (**C_4**), 78.5 (**C_2**), 116.1 (Ar-**C**H), 116.6 (Ar-**C**-COO), 118.2, 122.1; (2C, Ar-**C**H), 148.5 (Ar-**C**-NH), 151.8 (Ar-**C**-OH), 168.0 (**C**OO). **MS**: DIMS m/z: 338.084 ([M+Na]$^+$, $C_{13}H_{17}NNaO_8^+$; calc. 338.085).

Anti-bacterial activity (*Disc diffusion assay*)

In-vitro antibacterial activity of the new compounds was evaluated against two pathogenic Gram-negative bacteria; *Escherichia coli* and *Bacillus subtilis* and two Gram-positive bacteria, *Staphylococcus aureus* and *Salmonella choleraesuis* via a disc diffusion method and compared with the initial drug at a test concentration of 2×10^3 µg mL^{-1}. The i*n-vitro* antibacterial test was carried out in keeping with the recommended standards of the National Committee for Clinical Laboratory Standards based on the determination of the inhibition zone in millimetres (mm) in nutrient agar (NA), [38, 39]. The microbe cultures were standardized to the 0.5McFarland standard which is approximately 10^8 cells. Pure ethanol which was employed to dissolve samples was used as a negative control and streptomycin was applied as a positive control (100 µg mL^{-1} concentration).

In brief, test compounds which had been previously sterilized under UV, were inoculated onto 5 mm diameter paper discs which were then positioned on the NA surface of the plates on which microbial growth took place. The plates were inverted and incubated at 30-37°C for 18-24 hours until sufficient growth occurred. After incubation, by ruler, (naked eyes), from the back of the inverted petri plates, the diameters of the zones around the samples which indicated the amount of inhibition were measured in millimetre.

Determination of MIC and MBC

The lowest concentration of the test compound at which no turbidity or visible growth of organism were observed (MIC) and the minimum level of compound concentration at which no growth of microbial colonies was produced on NA medium (MBC) were determined through the macro broth serial dilution method according to the reported works with a minor modification [33, 39].

5-ASA, **6** and **7** were serially diluted into eight consecutive tubes containing nutrient broth which were pre-inoculated with bacteria adjusted to the 0.5 Mcfarland standard concentration 10^8 cfu mL^{-1} to obtain a concentration range of (0.93-3.33) $\times 10^3$ µg mL^{-1}. Also, a concentration range of 11.62-41.66 µg mL^{-1} was prepared for streptomycin. Furthermore, two additional tubes; containing a sample without inoculum as well as containing the growth medium and inoculum were built-up for each test batch as control tubes. MIC was recorded after 24 hours of incubation at 37°C. Following

that, MBC was determined by sub-culturing the test dilution on NA after further incubation for 18-24 hours.

Anti-inflammatory activity (NO suppression assay)

NO suppression activity of test compounds was carried out in accordance with the described protocols [34]:

Cell culture: Murine macrophages RAW 264.7 cells were grown in 50 µL of DMEM (Dulbecco's Modified Eagle's Medium) including 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin for 24 hours at 37°C in a 95% air and 5% CO_2 atmosphere.

Nitrite determination: Murine macrophages RAW 264.7 cells were detached at 90%–95% confluency and seeded (50,000 cells/well) into a 96-well culture plate. DMEM (50 µL) were added into each well and the plate was incubated for 24 hours. The cells were then induced in 5×10^3 µg mL^{-1} of LPS (Escherichia coli, serotype 0111:B4) and 1 ng mL^{-1} of interferon-gamma (IFN-γ) in the presence or absence of test compounds for 17 hours. Nitrite concentration was measured by reacting 50 µL of cell culture supernatant with 50 µL of Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid) at room temperature. After 5 minutes of incubation at room temperature, the optical density was recorded with a microplate reader.

Cell Cytotoxicity determination on macrophages RAW 264.7 cells (MTT Assay): The supernatant in each well was removed and DMEM (100 µL) was added to wells. Following that 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5×10^3 µg mL^{-1} in D-PBS (physiological balanced solution) filtered by 0.22 µm sterilized filter) was added and the plate was then incubated in a 95% air and 5% CO_2 atmosphere at 37 °C for 4 hours. The mixture of culture media and MTT in all wells was removed and dissolved in dimethyl sulfoxide (DMSO) to show the formed purplish formazan crystals and was then incubated at room temperature for a further 15 minutes.. The optical density (OD) was then calculated at 570 nm at room temperature with a microplate reader. Cell viability (%) was calculated by Equation 1 and the IC_{50} value was considered as the concentration of a drug that is required for 50% inhibition.

Equation 1: Cell viability (%) = (OD of Sample/OD of Control) ×100

Cytotoxicity evaluation on normal cells (3T3 cells) and colon cells (HT-29) (MTT Assay)

Cell viability assessment was performed against HT-29 and 3T3 cell lines via *in-vitro* MTT test. The HT-29 cell line was obtained from cultured human colon cancer; the 3T3 cell line is the standard fibroblast cell line and is originally acquired from mouse embryo tissue and they are mainly used as a representative of intestinal cells and normal cells, respectively for *in-vitro* studies.

5-ASA and compounds **6** and **7** were prepared at concentrations of 0, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg mL^{-1} for HT-29 cell lines. Since 5-ASA and its derivatives are expected to be less effective on normal cells, therefore, higher concentrations of samples were set for 3T3 cells compared to HT-29 cells. For 3T3 cell lines, the concentrations used were 0, 0.1, 1, 100, 1000 and 10000 µg mL^{-1}. For

both cells a concentration of zero was used for untreated control cells, and 5-ASA was also applied as a reference drug. Previously prepared cells were treated with test compounds in their appropriate concentrations and incubated for 48 hours. After incubation, the MTT assay was carried out in accordance with the above-mentioned method for cell viability determination of macrophage cells. The MTT test for each compound was performed in triplicate and assayed in duplicate.

Acknowledgements

The authors express their deepest appreciation towards University Putra Malaysia (UPM) for providing financial support for this work by ERGS (KPM), with Grant No. 5527054. We are also grateful to Majid Froghi for his cooperation in this research.

Author Contribution Statement

SY carried out the synthesis of all the products, prepared the manuscript and assisted in performing bioassays. SY, SB and EA designed the study and collaborated in the results and discussion sections. The entire work was conducted under the supervision of EA. MBAR and ZI contributed towards the elucidation of experiments and analysis of the data. All authors have read and approved the final manuscript.

References

[1] D. R. Friend, 'New oral delivery systems for treatment of inflammatory bowel disease', *Adv. Drug Delivery Rev.* **2005**, *57*, 247-265.

[2] E. Giannini, S. Kane, R. Testa, V. Savarino, '5-ASA and colorectal cancer chemoprevention in inflammatory bowel disease: Can we afford to wait for 'best evidence'?', *Dig Liver Dis* **2005**, *37*, 723-731.

[3] A. Tursi, R. E. Joseph, P. Streck, 'Expanding applications: the potential usage of 5-aminosalicylic acid in diverticular disease', *Dig. Dis. Sci.* **2011**, *56*, 3112-3121.

[4] C. Williams, R. Panaccione, S. Ghosh, K. Rioux, 'Optimizing clinical use of mesalazine (5-aminosalicylic acid) in inflammatory bowel disease', *Therap Adv Gastroenterol* **2011**, *4*, 237–248.

[5] D. Couto, D. Ribeiro, M. Freitas, A. Gomes, J. L. Lima, E. Fernandes, 'Scavenging of reactive oxygen and nitrogen species by the prodrug sulfasalazine and its metabolites 5-aminosalicylic acid and sulfapyridine', *Redox Rep* **2010**, *15*, 259-267.

[6] S. Ahmad, R. F. Tester, A. Corbett, J. Karkalas, 'Dextran and 5-aminosalicylic acid (5-ASA) conjugates: synthesis, characterisation and enzymic hydrolysis', *Carbohydr. Res.* **2006**, *341*, 2694-2701.

[7] A. Lopez, L. Peyrin-Biroulet, '5-Aminosalicylic acid and chemoprevention: does it work?', *Dig Dis* **2012**, *31*, 248-253; S. R. Ritland, J. A. Leighton, R. E. Hirsch, J. D. Morrow, A. L. Weaver, S. J. Gendler, 'Evaluation of 5-aminosalicylic acid (5-ASA) for cancer chemoprevention: Lack of efficacy against nascent adenomatous polyps in the ApcMin mouse', *Clin. Cancer Res.* **1999**, *5*, 855-863.

[8] C. Campregher, M. G. Luciani, P. Biesenbach, R. Evstatiev, A. Lyakhovich, C. Gasche, 'The position of the amino group on the benzene ring is critical for mesalamine's improvement of replication fidelity', *Inflamm Bowel Dis* **2010**, *16*, 576-582.

[9] Y. J. Jung, J. S. Lee, Y. M. Kim, 'Colon‐specific prodrugs of 5‐aminosalicylic acid: Synthesis and in vitro/in vivo properties of acidic amino acid derivatives of 5‐aminosalicylic acid', *J. Pharm. Sci.* **2001**, *90*, 1767-1775.

[10] M. H. Soliman, G. G. Mohamed, 'Cr (III), Mn (II), Fe (III), Co (II), Ni (II), Cu (II) and Zn (II) new complexes of 5-aminosalicylic acid: spectroscopic, thermal characterization and biological activity studies', *Spectrochim Acta A Mol Biomol Spectrosc* **2013**, *107*, 8-15.

[11] D. Pertuit, B. Moulari, T. Betz, A. Nadaradjane, D. Neumann, L. Ismaïli, B. Refouvelet, Y. Pellequer, A. Lamprecht, '5-amino salicylic acid bound nanoparticles for the therapy of inflammatory bowel disease', *J. Control. Release* **2007**, *123*, 211-218.

[12] Q. X. Cai, K. J. Zhu, D. Chen, L. P. Gao, 'Synthesis, characterization and in vitro release of 5-aminosalicylic acid and 5-acetyl aminosalicylic acid of polyanhydride – P(CBFAS)', *Eur. J. Pharm. Biopharm.* **2003**, *55*, 203-208; S. Travis, D. Jewell, 'Salicylates for ulcerative colitis—their mode of action', *Pharmacol. Ther.* **1994**, *63*, 135-161.

[13] C. Aguzzi, P. Capra, C. Bonferoni, P. Cerezo, I. Salcedo, R. Sánchez, C. Caramella, C. Viseras, 'Chitosan–silicate biocomposites to be used in modified drug release of 5-aminosalicylic acid (5-ASA)', *Appl. Clay Sci.* **2010**, *50*, 106-111.

[14] H. Abdu-Allah, A. Abdel-Alim, S. Abdel-Moty, A. El-Shorbagi, 'Synthesis of trigonelline and nicotinamide linked prodrugs of 5-aminodalicylic acid (5_ASA) with analgestic and anti-inflammatory effects', *Bull. Pharm. Sci.* **2005**, *28*, 237.

[15] C. Aguzzi, A. Ortega, M. Bonferoni, G. Sandri, P. Cerezo, I. Salcedo, R. Sánchez, C. Viseras, C. Caramella, 'Assessement of anti-inflammatory properties of microspheres prepared with chitosan and 5-amino salicylic acid over inflamed Caco-2 cells', *Carbohydr. Polym.* **2011**, *85*, 638-644; M. Zou, H. Okamoto, G. Cheng, X. Hao, J. Sun, F. Cui, K. Danjo, 'Synthesis and properties of polysaccharide prodrugs of 5-aminosalicylic acid as potential colon-specific delivery systems', *Eur. J. Pharm. Biopharm.* **2005**, *59*, 155-160.

[16] D. R. Friend, 'Colon-specific drug delivery', *Adv. Drug Delivery Rev.* **1991**, *7*, 149-199; H. J. Freeman, 'Medical Management of Ulcerative Colitis with a Specific Focus on 5-Aminosalicylates', *Clin Med Insights Gastroenterol* **2012**, *5*, 77.

[17] J. Quan, Z. Chen, C. Han, X. Lin, 'The synthesis of amphipathic prodrugs of 1, 2-diol drugs with saccharide conjugates by high regioselective enzymatic protocol', *Bioorg. Med. Chem.* **2007**, *15*, 1741-1748.

[18] J. Quan, J.-M. Xu, B.-K. Liu, C.-Z. Zheng, X.-F. Lin, 'Synthesis and characterization of drug–saccharide conjugates by enzymatic strategy in organic media', *Enzyme Microb. Technol.* **2007**, *41*, 756-763; D. S. Kohane, M. Lipp, R. C. Kinney, D. C. Anthony, D. N. Louis, N. Lotan, R.

Langer, ‘Biocompatibility of lipid‐protein‐sugar particles containing bupivacaine in the epineurium’, *J Biomed Mater Res A* **2002**, *59*, 450-459.

[19] R. K. Uhrig, M. A. Picard, K. Beyreuther, M. Wiessler, ‘Synthesis of antioxidative and anti-inflammatory drugs glucoconjugates’, *Carbohydr. Res.* **2000**, *325*, 72-80.

[20] T. Mizuma, K. Ohta, M. Hayashi, S. Awazu, ‘Intestinal active absorption of sugar-conjugated compounds by glucose transport system: implication of improvement of poorly absorbable drugs’, *Biochem. Pharmacol.* **1992**, *43*, 2037-2039.

[21] B. L. Sorg, W. E. Hull, H.-C. Kliem, W. Mier, M. Wiessler, ‘Synthesis and NMR characterization of hydroxyurea and mesylglycol glycoconjugates as drug candidates for targeted cancer chemotherapy’, *Carbohydr. Res.* **2005**, *340*, 181-189.

[22] J. N. Jacob, M. J. Tazawa, ‘Glucose–aspirin: Synthesis and in vitro anti-cancer activity studies’, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3168-3171.

[23] M. K. Mahmmod, ‘Synthesis of the new carbohydrate Ibuprofen ester as possible prodrug’, *Journal of kerbala University* **2008**, *6* 4-10.

[24] A. Smith, P. Nobmann, G. Henehan, P. Bourke, J. Dunne, ‘Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives’, *Carbohydr. Res.* **2008**, *343*, 2557-2566.

[25] P. Nobmann, A. Smith, J. Dunne, G. Henehan, P. Bourke, ‘The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against Listeria spp. and food spoilage microorganisms’, *Int J Food Microbiol* **2009**, *128*, 440-445; T. Watanabe, S. Katayama, M. Matsubara, Y. Honda, M. Kuwahara, ‘Antibacterial carbohydrate monoesters suppressing cell growth of Streptococcus mutans in the presence of sucrose’, *Curr. Microbiol.* **2000**, *41*, 210-213; N. H. Noaman, A. Fattah, M. Khaleafa, S. H. Zaky, ‘Factors affecting antimicrobial activity of Synechococcus leopoliensis’, *Microbiol. Res* **2004**, *159*, 395-402.

[26] A. I. Mohammed, N. H. Mansoor, J. H. Mohammed, ‘Copper (I) Catalyzed Synthesis and Antibacterial activity of 1, 2, 3-Triazoles Based on D-Fructose’, *Kerbala Journal of Pharmaceutical Sciences* **2013**, *6*, 179-195.

[27] V. K Kapoor, A. Kaur, ‘Drug-Glycosidation and Drug Development’, *Mini. Rev. Med. Chem.* **2013**, *13*, 584-596; A. L. Raymond, P. Levene, ‘Synthetic Hexosephosphates and their Phenylhydrazine Derivatives’, *J. Biol. Chem.* **1929**, *83*, 619-630.

[28] Y. Rajesh, O.P. Mahatma, ‘Ester Prodrug of 5-Aminosalicylic Acid for Colon Specific Drug Delivery: Synthesis, Kinetics, Hydrolysis and Stabilities studies’, *J. Pharm. Sci. & Res.* **2011**, *3(1)*, 966-972.

[29] A. T. Khan, M. Musawwer Khan, ‘A simple and convenient synthetic protocol for O-isopropylidenation of sugars using bromodimethylsulfonium bromide (BDMS) as a catalyst’, *Carbohydr. Res.* **2010**, *345*, 154-159.

[30] Y. W. Rong, Q. H. Zhang, W. Wang, B. L. Li, ‘A Simple and Clean Method for O-Isopropylidenation of Carbohydrates’, *Notes* **2014**, *35*, 2165.

[31] D. C. Baker, D. Horton, C. G. Tindall, ‘Large-scale preparation of D-allose: observations on the stereoselectivity of the reduction of 1, 2: 5, 6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose hydrate’, *Carbohydr. Res.* **1972**, *24*, 192-197.

[32] J. J. Kabara, D. M. Swieczkowski, A. J. Conley, J. P. Truant, 'Fatty acids and derivatives as antimicrobial agents', *Antimicrob. Agents Chemother.* **1972**, *2*, 23-28.

[33] S. Yousefi, S. Bayat, M. B. A. Rahman, I. S. Ismail, E. Saki, E. Abdulmalek, 'Synthesis and in vitro bioactivity evaluation of new glucose and xylitol ester derivatives of 5-aminosalicylic acid', *RSC Advances* **2015**, *5*, 97295-97307; C. V. Nakamura, T. Ueda-Nakamura, E. Bando, A. F. N. Melo, D. A. G. Cortez, B. P. Dias Filho, 'Antibacterial activity of Ocimum gratissimum L. essential oil', *Memórias do Instituto Oswaldo Cruz* **1999**, *94*, 675-678.

[34] S. W. Leong, S. M. M. Faudzi, F. Abas, M. F. F. M. Aluwi, K. Rullah, L. K. Wai, M. N. A. Bahari, S. Ahmad, C. L. Tham, K. Shaari, 'Synthesis and Sar Study of Diarylpentanoid Analogues as New Anti-Inflammatory Agents', *Molecules* **2014**, *19*, 16058-16081.

[35] F. Mellou, H. Loutrari, H. Stamatis, C. Roussos, F. N. Kolisis, 'Enzymatic esterification of flavonoids with unsaturated fatty acids: effect of the novel esters on vascular endothelial growth factor release from K562 cells', *Process Biochem. (Amsterdam, Neth.)* **2006**, *41*, 2029-2034; R. Kowti, R. Harsha, M. Ahmed, A. Hareesh, S. Thammanna Gowda, R. Dinesha, B. Satish Kumar, M. Irfan Ali, 'Antimicrobial activity of ethanol extract of leaf and flower of Spathodea campanulata P. Beauv', *Res. j. pharm. biol. chem. sci.* **2010**, *3*, 691-698.

[36] E. Pastorini, M. Locatelli, P. Simoni, G. Roda, E. Roda, A. Roda, 'Development and validation of a HPLC-ESI-MS/MS method for the determination of 5-aminosalicylic acid and its major metabolite N-acetyl-5-aminosalicylic acid in human plasma', *J. Chromatogr. B* **2008**, *872*, 99-106; M. Nobilis, Z. Vybiralova, K. Sladkova, M. Lisa, M. Holčapek, J. Květina, 'High-performance liquid-chromatographic determination of 5-aminosalicylic acid and its metabolites in blood plasma', *J. Chromatogr. A* **2006**, *1119*, 299-308.

[37] M. M. Midland, J. J. Beck, J. L. Peters, R. A. Rennels, G. Asirwatham, 'Synthesis and Conformational Analysis of 1, 2: 3, 4-Di-O-isopropylidene-alpha-D-galactopyranose: A Nuclear Magnetic Resonance and Molecular Modeling Experiment', *J. Chem. Educ.* **1994**, *71*, 897; A. R. Rufino, F. C. Biaggio, J. C. Santos, H. F. de Castro, 'Chemoenzymatic synthesis: a strategy to obtain xylitol monoesters', *J. Chem. Technol. Biotechnol.* **2009**, *84*, 957-960.

[38] R. P. Samy, S. Ignacimuthu, 'Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India', *J. Ethnopharmacol.* **2000**, *69*, 63-71; R. Khandanlou, M. B. Ahmad, K. Shameli, E. Saki, K. Kalantari, 'Studies on Properties of Rice Straw/Polymer Nanocomposites Based on Polycaprolactone and Fe3O4 Nanoparticles and Evaluation of Antibacterial Activity', *International journal of molecular sciences* **2014**, *15*, 18466-18483.

[39] A. Chanwitheesuk, A. Teerawutgulrag, J. D. Kilburn, N. Rakariyatham, 'Antimicrobial gallic acid from Caesalpinia mimosoides Lamk', *Food Chem.* **2007**, *100*, 1044-1048.

Entry for the Table of Contents