Medicinal chemistry

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5-Aminosalyclic Acid (5-ASA): A Unique Anti-Inflammatory Salicylate

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Abstract

Salicylic acid (SA) derivatives are widely used for treatment of various diseases. Acetylsalicylic acid represents the most widely used drug in the world, 4-Aminosalicylic acid (4-ASA) was historically used as a systemic anti-tuberculosis drug as well as diflunisal is a strong pain killer and antipyretic. 5-Aminosalicylic acid (5-ASA) which had been synthesized at the end of 19th century and employed first for the production of azo dyes, was then identified as a very valuable medicinal agent as well as part of many biologically active agents. 5-ASA is not metabolized to salicylic acid for pharmacological activity. It is not considered a true salicylate. In contrary to other salicylates, 5-ASA doesn't induce upper gastrointestinal (GI) side effects. Moreover, It was found, especially, useful for treatment of inflammatory bowel diseases (IBD). It is unique among salicylates and has a broad specrum of biological activities including, anti-inflammatory, analgesic, neuroprotective and antitumor. Since we are interested in this compound and its derivatives, we prepared this review to give insight into its chemistry, anti-inflammatory activity, in particular, for treatment of IBD. Different approaches for colonic targeting of 5-ASA will be covered with emphasis on chemical methods as well as its proposed mechanisms of action.

Keywords: 5-Aminosalyclic acid; 4-Aminosalicylic acid; Salicylate; Anti-inflammatory activity

Chemical and Physical Properties of 5-ASA

The structural unit; 5-ASA is part of numerous antimicrobial agents [1,2], colorectal cancer chemopreventive [3], antitumors [4-8], neuroprotective [9,10,88] and anti-inflammatory [11]. Since the discovery that 5-ASA is the active moiety of sulfasalazine (SASP) in the treatment of IBD; several new 5-ASA based drugs have been developed to improve its pharmacokinetic and pharmacodynamic properties.

Chemically, 5-ASA has extremely low solubility in water (1 mg/ml at 20°C) and the saturated aqueous solution has a pH about 4.1 [11]. It's easily oxidized and its stability in solution is influenced by temperature, light exposure and pH. The compound is expected to undergo oxidative degradation, because of its 4-aminophenol structure this may lead to stability problems in pharmaceutical preparations [11].

The autooxidation of 5-ASA has been studied [12] and it was found that 5-ASA is susceptible to autooxidative transformation resulting in polymeric species of 5-ASA, from which a trimeric species could be synthesized by oxidation of 5-ASA with hypochlorite anion. The instability of 5-ASA in biological samples has also been reported [13]. It is significant to note that the N-acetyl derivative of 5-ASA is stable under conditions causing rapid decomposition of 5-ASA. This indicates that the amine function plays an essential role in 5-ASA degradation [14]. Although the presence of the amino group in 5-ASA may not be an important determinant of GI anti-inflammatroy activity, yet its presence does appear to be important since salicylic acid does'nt possess such activity in IBD. The extremely low solubility and instability of 5-ASA are disadvantages with respect to its medical utilization. In addition, when 5-ASA is ingested orally in, a non linked and non protected form, is rapidly and extensively absorbed from the proximal part of the gastrointestinal tract [15] before reaching the ileum and colon causing low drug bioavailability and low efficiency for IBD. Minimizing the rate and extent of absorption of 5-ASA would not only reduce the occurrence of adverse effects, but also maintain a high concentration of the drug directly at the diseased intestinal site for an extended period of time.

5-ASA and Inflammatory Bowel Diseases

Inflammatory bowel diseases and treatments

IBD include ulcerative colitis (UC) and Crohn's disease (CD), which are serious disorders of the lower part of GIT involving tissue damage and inflammation leading to bowel impairment. The more common ulcerative colitis involves inflammation of the lining of the colon, whereas Crohn's disease affects all layers of the intestinal wall. Both disorders are chronic and progressive diseases associated with considerable pain, abdominal cramping, persistent diarrhea, nausea, vomiting, gastrointestinal bleeding, anaemia, fever, and weight loss, as well as secondary infections [16]. Although an explanation for the etiopathogenesis has not emerged, the inflammatory process itself is well understood. The final common pathway of immune activation in IBD is the local influx of monocytes, macrophages and polymorphonuclear neutrophils (PMNs). The process that accounts for the recruitment of these cells includes cytokine generation, complement activation, and eicosanoid biosynthesis. Once the influx of PMNs and macrophages occurs, the production of prostagandins, platelet activating factors (PAF), and leukotrienes in parcticular LTB_4 incease, leading to scecondary amplification of the inflammatory response, which produces the clinical manifestations of IBD [16]. Currently established therapies consist mainly of 5-aminosalicylates and glucocorticoids. Although glucocorticoids are effective for an attack therapy of ulcerative colitis, yet they are disappointing when used in moderate doses as maintenance therapy to prevent relapses. On the otherhand, larger doses cannot be justified for long term treatment because of the risk of serious side effects such as osteoporosis, hypertension, fluid retension, increased susceptability to infection, and glycosuria [17].

5-ASA for treatment of IBD: Historical background

The origin of 5-ASA derivatives was SASP (1), which was introduced in the early 1940's for the treatment of rheumatoid arthritis. However, since this initial use, numerous clinical studies have provided clear
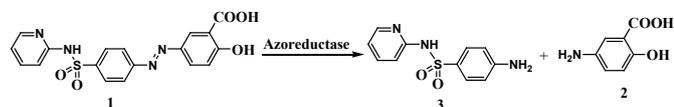
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evidence of the drug's efficacy in the short and long term treatment of IBD and has become the most widely prescribed agent for these diseases over 60 years [18]. SASP (1) is an azo dye consisting of 5-ASA (2) and sulfapyridine (SP, 3) moieties.

Unlike many other drugs, sulfasalazine was not a chance find, but was synthesized in an attempt to combine the therapeutic properies of both salicylates and sulfonamides with the aim of creating an effective treatment for rheumatoid arthritis [19].

The disposition and metabolism of the drug, in man, have been well studied [15]. When orally administered, about 30% of the intact drug is absorbed from the small intestine. The absorbed portion undergoes enterohepatic circulation with 2-10% excreted intact in the urine, and the remainder returned to the gut by the bile [15]. SASP travels in the gut to the lower bowel where anaerobic bacteria reductively cleave the azo bond producing 5-ASA, and SP. 5-ASA remains largely in the colon and about 70% of it is excreted unchanged in the feaces [20]. The rest is partly absorbed and partly excreted in the urine as an N-acetyl derivative. Most SP is absorbed from the colon and excreted in urine in the free form or as N-acetyl and glucuronide derivatives. The reduction of SASP to its constituent moieties is carried out by intestinal bacteria as established by investigations in conventional and germ free rats [21]. This was also confirmed by anaerobic incubation studies with cultures of bacteria isolated from the gut of experimental animals and man [21]. The bacterial azo reductase system catalyzing the reaction is located within the bacterial cells and requires anaerobic conditions for activity [22]. Indirect evidence supporting a role of gut flora in the metabolism of the drug, in humans, comes from several pharmacokinetic studies [23,24]. Collectively, such circumstantial evidences point to a vital role for the colonic microflora in the metabolism of I. Despite the benefits of SASP in the treatment of IBD, its efficacy is limited because of its unwanted effects and allergic reactions that are common and inculde [25,26]:

(a) Sulfasalazine cannot be given to more than 20% of patients because of hypersensitivity or less specific forms of intolerance and a better drug is needed for patients with Crohn's disease.

(b) Development of adverse haematological, generalized side effects, agranulocytosis, toxic epidermal necrolysis, parethesis, hepatotoxicity, pancreatitis, pulmonary disease and male infertility.

The toxic symptoms ascribed to SASP have been correlated with high serum concentration of SP (>50 μg/ml) and with decreased ability to acetylate SP or explained by allergic reactions similar to those seen with sulfonamides [27]. The toxic symptoms have not been correlated with SASP or 5-ASA serum concentrations. To explore which component is resposible for the unsolicited effects patients with ulcerative colitis were administered enemas of SASP, SP, and 5-ASA [28]. About 75% of those receiving SASP or 5-ASA improved, compared to only 38% of those receiving SP. Several other studies strongly suggest 5-ASA to be the therapeutic moiety of SASP [29,30]. This means that the toxic SP portion of SASP serves primarily to limit intestinal absorption until 5-ASA is generated by colonic bacterial reduction. Efforts then have been directed towards avoiding or substituting the carrier moiety SP and development of SP free 5-ASA derivatives with a special concern to the therapeutic efficacy and mode of action of 5-ASA [26].

Pharmaceutical and Chemical Approaches for Colonic Targeting of 5-ASA

To facilitate a topical effect of 5-ASA, it must be transported to the lower part of the GIT in a form that protects it from systemic absorption but promotes its release at the site of the lesion. To achieve this either a prodrug or as a special pharmaceutical preparation can be used [11]. Pharmaceutical preparations inculde: (a) rectal formulations (enemas and suppositories, Rowasa°). The short comings of this route may include problems in both patient acceptability and accessing to the proximal colon leading to a non well tolerated maintenance medication [31]; (b) Slow or sustained release oral formulations may deliver 5-ASA to larger areas of the gut. No other molecule being involved and the release of 5-ASA from the various preparations depends on the transit time, the bacterial environment and the local pH. The major oral forms of 5-ASA include [32]; delayed-release forms which release 5-ASA at pH=6-7 (Asacol°, Claversal°, Salofalk° and Rowasa°), a slow release form with gradual release in the small and large intestine (Pentasa°). In Europe and Canada, both oral and topical forms of 5-ASA have been commercially available since 1982 [32]. FDA approved mesalamine in 1987 and topical 5-ASA preparation has been available in the USA since 1988 (Rowasa° enema) [32]. In the USA, the generic name for the oral and rectal forms of 5-ASA is mesalamine, and in Europe they are referred to as mesalazine [32]. The short coming of these oral preparations include; variations in the intestinal pH and transit time may prevent the release of 5-ASA from these preparations, thereby, diminishing the effectivness of the drug [33]. Moreover, the current cost of a course of topical 5-ASA therapy may be prohibitive for some patients [32]. In addition to the efficacy of 5-ASA in treatment of IBD, reported studies have established the safety of both topical and oral forms of aminosalicylates. More than 80% of the patients unable to take SASP will have no adverse effects on 5-ASA therapies. Infertility, noted in male patients on SASP, has not been seen once these individuals are switched to 5-ASA preparations. Unlike SASP, 5-ASA may have a role in the prophylaxis of Crohn's disease. An added benefit of 5-ASA may be an understanding of some elements of the pathogenesis of IBD [34]. Several reviews were reported about the drug delivery to the colon [32,35,36].

Colonic drug-targeting has several therapeutic advantages [37]. Like any other organ, specific targeting to the colon requires a smaller dose of the drug, which subsequently results in fewer adverse drug reactions. Diseases of the colon such as IBD and irritable bowel syndrome are effectively treated when the drug is applied directly to the affected area. Likewise, vermicides and colonic diagonistic agents need only to be applied in smaller doses to the colon.

Recently, colon targeting has attracted much interest due to rapid development of biotechnology and genetic engineering, resulting in availability of peptides and proteins at reasonable costs. Peptides and protein drugs are destroyed by stomach acid and/or enzymes of the pancrease. These drugs are usually administered by the parenteral route, which is inconvenient. With negligible activity of pancreatic enzymes and much less brush border membrane peptidase activity, the colon is more suitable for delivery of such drugs. Thus colonic delivery of analgesic peptides, contraceptive peptides, oral vaccines, insulin, interferons and interleukin has been attempted [38]. In addition, colonic targeting of drugs would prove useful where intentional delayed drug absorption is required such as nocturnal asthma [39].

The approaches utilized in achieving colonic delivery of drugs inculde [40]: (1) coating with pH-sensitive polymer, (2) time controlled formulation and device, (3) coating with polymer which can be degraded by intestinal microflora, (4) pressure controlled devices, (5) prodrugs which are designed to pass intact and unabsorbed from the upper GIT and undergo biotransformation in the colon releasing the active drug molecule. This biotransformation is carried out by a variety of enzymes, mainly of bacterial origin present in the colon (e.g., azo

reductases, glycosidases, esterases, amidases, etc.) [35]. A prodrug is a latent form of an active drug with certain physicochemical properties that allow it to reach the target organ or tissue. Once there, the active drug is formed chemically or enzymatically *in situ* [41].

Colonic microflora as the targetting element of 5-ASA

It has been estimated that the human body is made up of over 10^{14} cells of which only around 10% are mammalian. The remainders are mainly the microorganisms, which comprise the intestinal microflora of the host [42]. Although these microorganisms are distributed throughout the GIT, most are found in the large intestine where they mediate hydrolytic digestive functions using carbohydrates and proteins as substrates. In addition, these microorganisms have a potential to metabolize drugs and other foregin compounds that have been equated with that of the liver [43].

The microbial flora of the GIT constitutes a very complex ecosystem containing more than 400 bacterial species. The bacterial concentration in the colon is 10^{11}-10^{12}/ml making this region the most heavily colonized part of the GIT. The numerically predominant species are non-spore forming anaerobes belonging to the genera Bacteroides, Bifidobacterium, and Eubacterium, these species outnumber faculative anaerobes and aerobes by a factor of 10^3-10^4 [42].

The metabolic effects of the intestinal microorganisms result mainly from the activities of the colonic microflora, which contain a wide spectrum of enzymes that catalyze various metabolic reactions. Several reviews were reported in the literature about the role of bacterial flora in drug metabolism [42-44].

Access of drugs to intestinal microorganisms

Because intestinal microorganisms are mainly restricted to the terminal part of the gut, only drugs reaching this area are susceptible to microbial metabolism. Therefore, drugs that are administered rectally and those which are not absorbed or incompletely absorbed following oral administration may be subjected to biotransformation by the colonic microflora [42].

Drugs which are fully absorbed from the upper parts of the GIT or are given intravenously still be exposed to metabolism by the colonic microflora after diffusion or secretion from the systemic circulation into the lumen of the GIT [42].

Chemistry-Based Colonic Targeting of 5-ASA: Prodrug Approach

It is evident that the colonic bacterial population has a significant impact on colonic drug delivery; hence it produces a wide spectrum of metabolizing enzymes. A major share of the efforts in designing and developing of colonic drug delivery have attempted to utilize the presence of bacteria as a triggering element to initiate or control drug release on arrival of the delivery system to the colon [45]. Specific biotransformation in the large intestine offers two different possibilities of drug targeting: (1) a therapeutic effect can be selectively directed to and exerted at the colonic site and (2) a systemically active drug that would be unstable in the upper intestinal lumen can be released into the colon and absorbed from there. The first of the two concepts has been translated into practice by making use of microbial metabolism of 5-aminosalicylic acid-prodrugs for treatment of IBD.

To be colon specific, a prodrug of 5- ASA should be chemically, biochemically stable and non absorbale in the upper intestine so that it could be delivered to the colon in intact form, where the linkage between the drug and the carrier should be dissociated to liberate the active drug in the colon [46].

Several prodrugs aiming at the delivery of 5-ASA to the colon have been introduced that use polymers or highly hydrophilic small molecules as carriers. Coupling of 5- ASA to the carrier molecules achieved most commonly by means of azo, glycosidic, amide or ester linkages, all these linkages are dissociated in the large intestine by the action of microflora [46]. Prodrugs for colon specific delivery of 5-ASA can be divided according to type of metabolic reaction required to release 5-ASA into:

### Colonic targeting based on azo-reductase activity (Sulfasalazine analogues)

A number of sulfasalazine analogues have been developed by coupling 5-ASA by an azo link to carriers less toxic than **SP**.

Olsalazine (4, Dipentum°): Disodium azo-disalicylate avoids a carrier molecule with potential side effects. Distribution and metabolic studies in healthy volunteers showed that it is poorly absorbed and delivers twice the amount of 5-ASA as compared with sulfasalazine, it is well tolerated and free from most side effects of sulfasalazine [47].
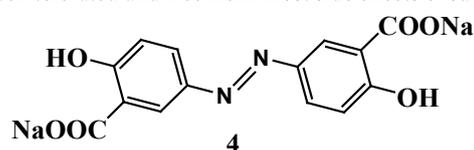

The major side effect of olsalazine is watery diarrhea, which may be attributed to 5-ASA dimer which is a potent secretagogue in the distal ileum [47]. The FDA approved Olsalazine in July 1990.

Salicylazobenzoic acid (5): With a carrier moiety p-aminobenzoic acid was reported to cause fewer side effects than SASP. Furthermore, 5-ASA was liberated by bacteria in the colon lumen faster from (5) than from SASP when both were given to healthy human volunteers orally or as an enema [48].
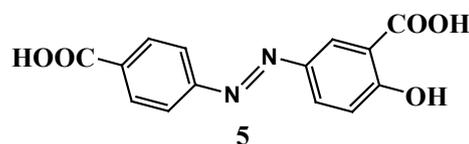
Ipsalazide (7) and Balsalazide (8): Chan [49] have designed analogues of SASP in which the SP has been replaced by certain non heterocyclic organic radicals. Compounds obtained (6) are reported to be useful for the treatment of IBD and have the advantage that their breakdown in the intestinal tract does not give raise undesirable metabolic products. Furthermore, many of them are soluble in water, which is advantageous for ease of administration, and have a very low toxicity.
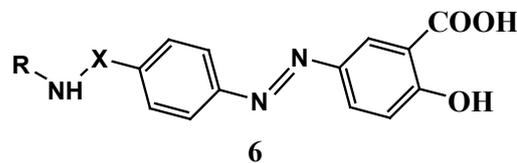

X=SO$_2$ or CO

R=(un)substituted non heterocyclic aromatic ring system and preferably a benzene ring or (CH$_2$)$_n$Y; Y=OH, (un)substituted NH$_2$, COOH, SO$_3$H where n=1-6 in which one or more hydrogen atoms in the alkylene radical can be replaced by (un)substituted amino groups or alkyl radicals. (CH$_2$)$_n$Y is either attached directly to the nitrogen atom or by a benzene ring.

The toxicological and metabolic studies of two of such analogues (Ipsalazide (7) and Balsalazide (8)) have been studied in rats. In these

prodrugs, 5-ASA is linked to either 4-aminobenzoylglycine (Ipsalazide) or 4-aminobenzoyl-β-alanine (Balsalazide). It was found that these prodrugs are not absorbed in the small intestine but reach the colon to liberate to 5-ASA. Faecal recovery of 5-ASA from these prodrugs has been found to be similar to that of SASP [50]. Reports on experiments in rats and mice have not revealed any toxicological effects even in high doses. Unlike ipsalazide, the carrier liberated after splitting balsalazide is poorly absorbed, making it more attractive than sulfasalazine. Balsalazide was approved by the FDA in 1997 and is marketed in UK (Colazide®) and in USA (Colazal®) [51].
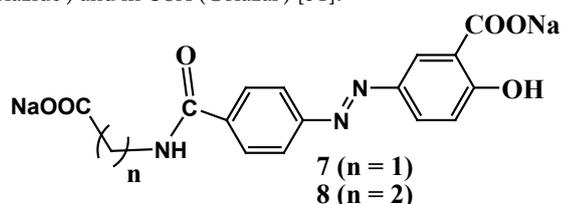
Polysalicylate: Compound (9) is a water-soluble polymer contains sodium salicylate residues linked at the 5-position by an azo-bond to an inert polysulfanilamide backbone. This polymer has been investigated *in vivo* and *in vitro*. In guinea pig poly-5-ASA was significantly more effective in reducing carrageenin-induced colitis than sulfasalazine [25]. Potential therapeutic advantages of this polymer inculde non-absorption, non-metabolism in the small intestine, direct 5-ASA at the disease site, and non-absorption, non-metabolism of the reduction released carrier polymer.
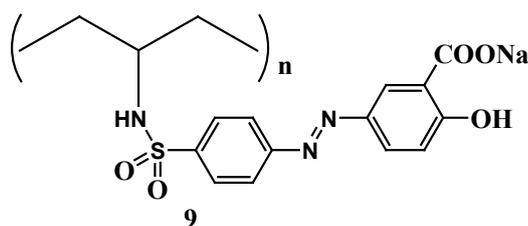
Colonic targeting based on azo-reductase activity using theraputically active carrier

Several scaffolds for colon specific delivery of 5-ASA has been developed taking the advantage of the azo moiety as the colonic drug delivery system but adding a therapeutic activity to the carrier that, if it is not absorbed in the colon, can act topically with 5-ASA to produce a synergistic effect. Two examples are reported in the literature:

The carrier has Platelet Activating Factor (PAF) antagonist activity: PAF is a proinflammatory lipid mediator involved in hypersensitivity and inflammatory reactions such as platelet and neutrophil aggregation, increased vascular permeability, and leukocyte adhesion. PAF also stimulates the release of eicosanoids and cytokines. Such direct and indirect actions contribute to the initiation and amplification of inflammatory process including that of IBD.

The colonic mucosa from patients with IBD has been shown to produce higher levels of PAF than normal mucosa. So inhibition of PAF will suppress inflammation. The authors synthesized aromatic amines with potent PAF antagonist activity and prepared the corresponding azo derivatives of 5-ASA [13].

Pharmacokinetic experiments showed that neither the whole molecule, nor the carrier was absorbed after oral administration in rats and effective cleavage (84%) of the azo-bond was achieved by microflora in the colon. These facts ensure high topical concentrations of 5-ASA and PAF antagonist in the colon and explain the potent anticolitic effect exhibited by these prodrugs (10) in the trinitrobenzenesulfonic acid-induced colitis in rats [13].
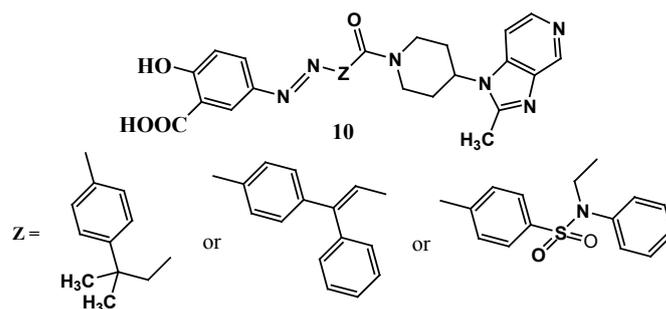
The carrier is a non absorbable antimicrobial agent: Azo derivatives of salicylic acid have been synthesized [52] having the following structures (11):
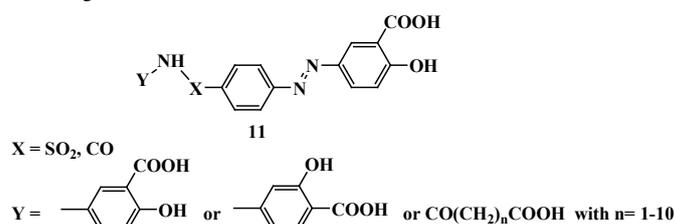
The carrier formed by cleavage of the azo-bond is a non-absorbable antimicrobial agent. It was suggested that these derivatives might be utilized for treatment of IBD or diseases treatable by non-absorbable antimicrobial agent. All the previous prodrugs of 5-ASA contain an azo bond and in turn all of them depend on the azo reductase activity of the colonic flora for activation and release of 5-ASA.

The unknown safety and toxicity of some of the substrates for azo reductase activity [53] and the reported antiandrogenic activity [54] of substituted azo benzenes as well as a possible connection between intestinal tumors and the use of azo dyes have been investigated [55]. So the search to use the more commonly occurring natural substrates may offer important advantages and hence other approaches have been investigated depending on the high hydrolytic activity of the intestinal microflora [53].

Colonic targeting based on the hydrolytic activity of intestinal microflora

5-ASA-O-sulfate ester (12): 5-ASA-O-sulfate ester was investigated [56] *in vivo* and found to be stable in the small intestine while in the large intestine decomposes slowly and uniformly by colonic bacteria to release the desired 5-ASA without the disadvantages of SASP. Compound (12) is extremely sensitive to acids.
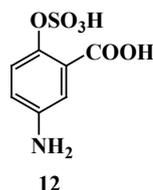
5-ASA-Glucopyranosides (13 and 14): N-and O-β-D-glucopyranosides of 5-ASA (13) and (14) have been shown to have sufficient hydrophilic characters to reach the colon where cleavage occurs by bacterial glycosidases releasing 5-ASA [57]. These compounds have been also investigated as antiulcers and were found to have inhibitory effect on gastric ulceration induced by indomethacin and were four times and one half times less toxic than 5-ASA in mice [58].
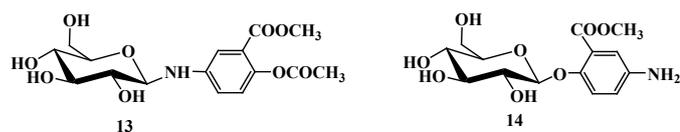
5-ASA-amino acid conjugates (15-17): Amino acids are natural substrates and have hydrophilic characters, so it is expected that 5-ASA-amino acid conjugates to be hydrophilic and membrane permeation might be limited in the upper intestine. Also, the amide bond of the acyl amino acid conjugate is stable in the upper intestine, and so a large fraction of the orally administered derivative might be delivered to the colon in intact form [46]. Once delivered to the colon, it might be degraded by the microbial enzymes to release 5-ASA and the carrier (amino acid). 5-ASA conjugates with aspartic, glutamic, leucine, and proline were prepared and evaluated *in vivo* and *in vitro* [46,59]. The results revealed that a large fraction of 5-ASA-Gly was delivered to the large intestine and activated to liberate 5-ASA. The total recovery of 5-ASA and N-acetyl-5-ASA from feces was greater than that from SASP [46].
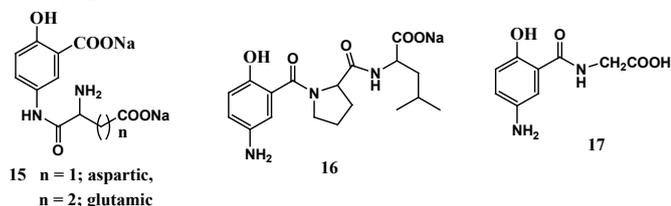

5-ASA-Ursodeoxycholic acid conjugate (18): Ursodeoxycholic acid (UDCA) is the bacterial product of chenodeoxycholic acid and has been applied in gall stone dissolution and in the treatment of cholestatic liver diseases and may be beneficial in colon polyp reduction [60]. The conjugate of 5-ASA with UDCA is considered to be a good way to direct them to the colon without intestinal absorption. Experiments, in rats, showed that 5-ASA-UDCA conjugate is poorly absorbed from the intestine and is targeted to the colon where it is partially hydrolyzed to 5-ASA and UDCA to exhibit their anti-inflammatory and cytoprotective effects in the colon as well as liver [60]. 5-ASA-UDCA monophosphate was also investigated to determine its suitability for the evaluation of enteric bacterial overgrowth. It was efficiently deconjugated by microflora, whereas it was completely resistant to deconjugation by pancreatic and intestinal mucosal enzymes. Urinary excretion of acetyl-ASA was found to increase in rats with intestinal bacterial overgrowth. So this compound offers a simple method for the evaluation of microbial overgrowth without the use of radioisotopes or expensive special techniques.
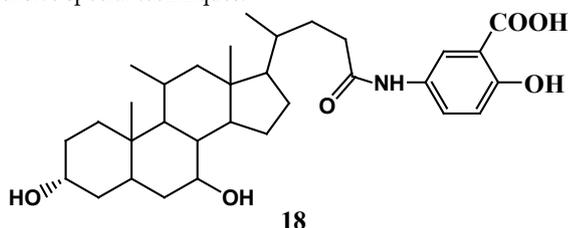

Polymeric prodrugs: 5-ASA has been linked to polymer backbones by ester or amide bonds [35]. Polymers are larger and usually more hydrophilic than the drugs themselves, these properties tend to reduce penetration across biological membranes. The linkage between drug and the polymer is susceptible to enzymatic attack in larage intestine and the drug is released at this site. The advantages of polymeric prodrugs include non-absorption from the small intestine, elimination of SP-produced side effects and non-absorption of polymeric cleavage product.

- **Acrylic type polymeric systems having degradable ester or amide:** bonds linked to 5-ASA have been prepared and evaluated for colon-specific delivery of 5-ASA [61]. The release profiles indicated that the hydrolytic behaviour of the polymer strongly depends on their degree of swelling, type of comonomer, and nature of the hydrolyzable bond.

- **Alginates as 5-ASA carriers (19):** 5-ASA was coupled to linker 6-aminohexanamide-L-phenylalanine and subsequently bound to alginate. *In vitro* release studies showed that 5-ASA was gradually released from this conjugate throughout the whole length of the bowel [62]. This type of release should be suitable for the treatment of both small intestine and colon inflammatory lesions. Alginates are poorly immunogenic and are well tolerated [62].
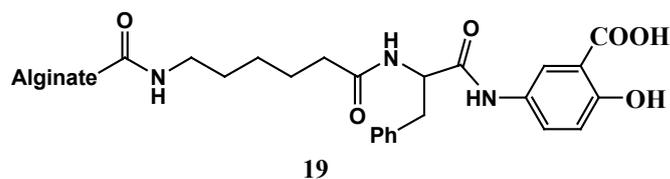

- **Diacetyl anhydride of poly[(5-carboxybutylformamido)-2-acetylsalicylic acid:** 5-ASA incorporated into the polymer backbone (**20**) was synthesized and subjected to degradation. The polymer simultaneously released 5-ASA and its O-acetyl analogue *in vitro* under various conditions. The degradation pattern showed that it is possible to provide high local concentration of 5-ASA in the colon [40].
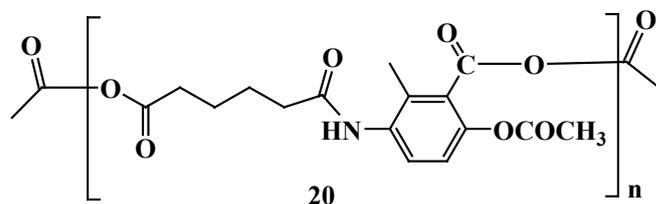
Conjugation with nicotinamide as quaternary less absorbable prodrugs

Several series (21 and 22) of double prodrug-types were synthesized and preliminarily investigatated for their anti-inflammatory activity [63-66]. Some of these derivatives showed better analgesic and anti-inflammatory activities than sulfasalazine (SASP) and 5-ASA. In addition, ulcerogenicity, LD50, *in vivo, in vitro* cleavage and pH stability of some of these derivatives were also investigated for testing their application for colonic targeting of 5-ASA.
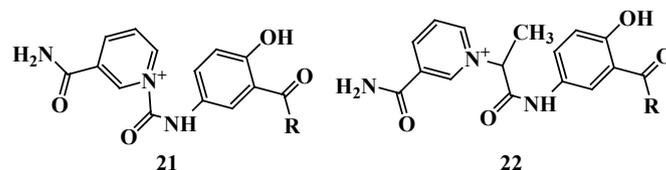
R = OH, OR^1, NHR^2, NR^{2}R^2
    R^1 = C1-6
    R^2 = C1-4
    NR^{2}R^2 = morphine, pyrrolidine and piperidine

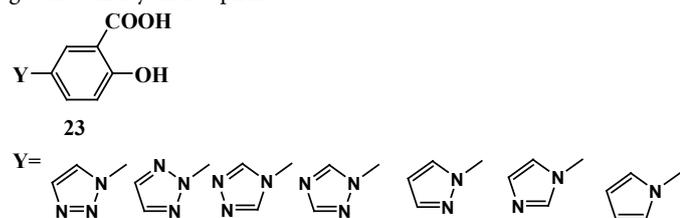## General Anti-inflammatory and Analgesic Activities

Sulfasalazine is clinically effective in the treatment of autoimmune diseases such as rheumatoid arthritis, ulcerative colitis, ankylosis

spondylitis, and psoriasis. It represents a unique salicylate with truely disease modifying effects distinct from symptomatic effects of salicylates and other NSAID and showed penicillamine-like activity [67]. Both sulfasalazine and carbenoxolone find place in prophylactic therapy against ulcerative diseases of the GIT. It has been suggested that the gastroprotective effects of sulfasalazine and carbenoxolone might be due to preservation or potentiation of the cytoprotective effects of prostaglandins consequent to reduced degradation [68].

It has been reported that position 5 of salicylic acid is the most favourable position for substitution by aryl or heteroaryl moities to enhance its anti-inflammatory activity while decreasing toxicity [69,70]. Orlikovs [71] found that prolonged therapy (over 18 month) for rheumatoid arthritis and similar arthitides is possible with the use of 5-ASA.

Clinical efficacy of 5-ASA in the treatment of spondyloarthropathies (SpA) was evaluated [72]. Improvements in clinical, physical, and laboratory measures indicated the efficiency of 5-ASA in treating SpA.

A series of 5-heteroarylsalicylic acid derivatives (23) have been prepared and their anti-inflammatory and analgesic potencies were measured in comparsion to aspirin [70]. The authors observed an improvement of the therapeutic index by 100 times over aspirin. Thus 5-N-pyrrolyl-salicylic acid was the most active in both tests with less gastric toxicity than aspirin.

23

Several substituted 5-ASA derivatives (24) were prepared and showed potent anti-inflammatory, analgesic and antipyretic activities with low side effects [73].
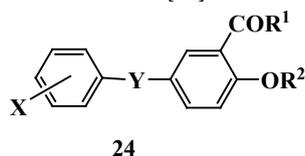
24

R^1=hydroxy, amino, lower alkoxy, lower alkylamino, dialkylamino, (un)substituted phenoxy, phenylamino, hydrazino, morpholino, piperidino, pyrrolidino.

R^2=hydrogen, acyl, alkyl, alkoxycarbonyl.

X=hydrogen, alkyl, hydroxy, alkoxy, acyloxy, halogen, nitro, amino, alkylamino, dialkylamino, mercapto, alkylsulfinyl.

Y=-CH=N-, -CONH-, -CH$_2$NH-

Series of 5-ASA derivatives embodying various moieties of the NSAIDs (25) [74] have been synthesized and tested for their anti-inflammatory activity and their ulcerative effects. Compounds (25) were found to be potent inhibitors of prostagladin release and exhibited measurable anti-lipooxygenase activity *in vitro*. *In vivo* studies, these derivatives showed potent anti-inflammatory activity and the number of gastric lesions observed was negligible in comparison to the controls receiving the respective parent compounds. The beneficial property of these derivatives may be related to combination of prostaglandins synthesis inhibition by NSAIDs moieties with both lipooxygenase inhibition and antioxidant effects of 5-ASA fragment and hence avoid the development of the respiratory distress syndrome. The tested compounds proved to be almost completely non-toxic even at extremely

high doses in both fasting and fed animals and possess typical anti-inflammatory activity *in vitro* as well as *in vivo*.

25
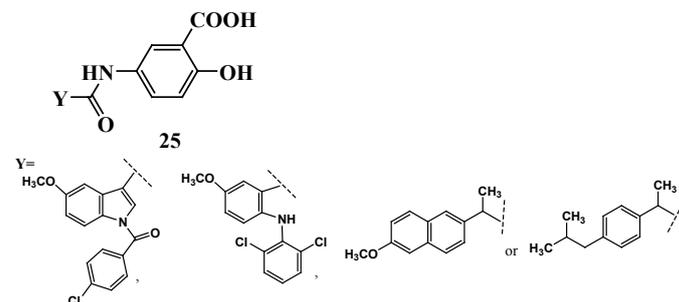
Some Schiff bases of 5-ASA (26) with aryl aldehydes were synthesized and showed promising anti-inflammatory, analgesic, and antipyretic effects [75].


26
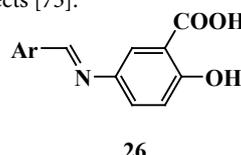
5-Acylaminosalicylic acid and its salts (27) were reported to have anti-inflammatory, antiplatelet aggregation, and antithrombotic properties [76]. The salts usually show higher activity.

27
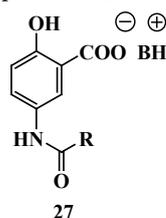
B=H, imidazoline, lysine, or methyl glucosamine

R=H, C_1-C_{25} alkyl, aryl, or heterocylic groups

Another group of 5-acetamidosalicylamides (28) were reported [77] to be of low toxicity and of good effects on the central nervous system. They are useful as analgesics, sedatives, antipyretics, anti-inflammatory, hypnotics, gangloplegics, antimitotics, and bacterostatics.

28
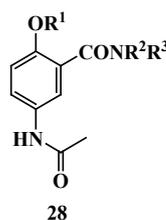
R^1=H, CH$_2$CH$_2$NMe$_2$, CH$_2$CH$_2$NEt$_2$, morpholinoethyl, piperidinoethyl.

NR^{2}R^3=NHMe, NHEt, NHPr, NHPr-iso, NEt$_2$, morpholino, piperidino.

Acylated 5-ASA derivatives (29) were prepared and suggested to have anti-inflammatory activity [78].

29
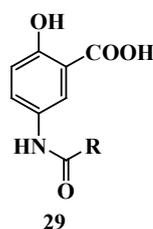
R = OCH$_3$, CH(CH$_3$)$_2$, CH$_2$CH(CH$_3$)$_2$, (CH$_2$)$_{16}$CH$_3$, NHCH$_2$CH$_2$CH$_3$,
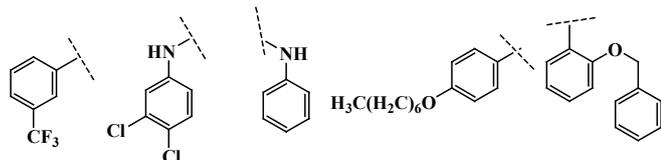
Phenyl 5-aminosalicylate (30) and its acetamido derivative have been investigated as potential analgesic, antipyretic with possible antitubercular activity [79]. (28, R=COCH$_3$) displayed analgesic activity comparable to that of aspirin and phenacetin at doses of 25-50 mg/kg s.c. and was much less toxic than the reference compounds.
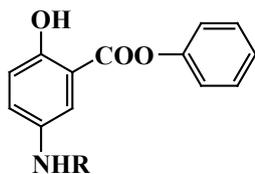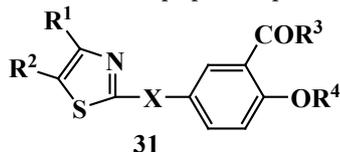

30

R=H or COCH$_3$

Several substituted 5-thiazolylaminosalicylates, 5-thiazolyl-aminosalicylamides and their corresponding 5-thiazolylcarbonylamino derivatives (**31**) were prepared as potential anti-inflammatory agents [80].


31

R^1, R^2=H, halo, (un)substituted phenyl or pyridyl.

R^3=OH, alkoxy, **NR^{5}R^6**

R^4-R^6=H, alkyl.

X=NH, CONH.

A series of 5-substituted amino-N-butylsalicylamides (32-34) were prepared of which parsalamide (32), compound (33), and other related derivatives (34) and were reported to treat arthritic patients. The *in vivo* anti-inflammatroy activity as well the *in vitro* activity on COX-1 and COX-2 have been evaluated for these derivatives and the results showed that (32) and (33) inhibited both COX-1 and COX-2. Both compounds were devoid of gastric effects and prevented indomethacin-induced gastric damage. The authors [81] suggested that these compounds might guide to lead molecules with anti-inflammatory activity.


32   **33**   **34**
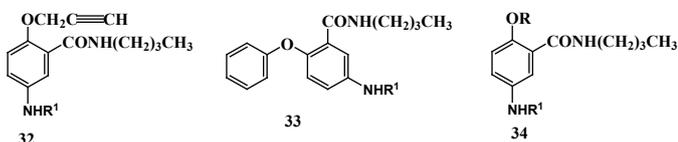
R = CH$_2$C≡CH, CH$_2$CH=C(CH$_3$)$_2$, C$_6$H$_5$, CH$_2$-cyclo-C$_6$H$_{11}$

R^1 = H, COCH$_3$, SO$_2$CH$_3$

5-ASA and its derivatives are recommended in treatment of severe dermatological disorders associated with loss of epithelium including proliferative skin diseases such as psoriasis, various types of dermatitis, mouth and leg ulcers and burns [82-84]. It was reported that 5-ASA and its derivatives are superior to topical corticosteroids because they promote granulation, wound healing and have analgesic activity [84].
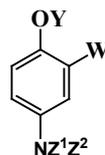
5-ASA esters (35) are active sunscreens for sunburn prevention. They also have local analgesic, anesthetic actions and promote healing of sunburn skin [85].
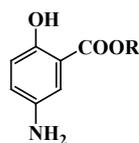
35

R=C1-18 alkyl, vinyl, allyl, undecenyl, oleyl, linolenyl, cyclohexyl, phenyl, menthyl

5-ASA derivatives (36) were prepared for treatment of psoriasis and other inflammatory skin diseases [82].

36

W=CO$_2$X, CO$_2$R, (un)substituted CONH$_2$.

Y=H, alkylcarbonyl, arylalkycarbonyl.

Z^1, Z^2=H, alkyl, alkylcarbonyl or NZ^{1}Z^2=heterocycle.

X=H, (un)substituted N$^+$H$_4$, metals.

Some 5-ASA derivatives (37) which were found to protect cortical neurons from toxicity results by N-methyl-D-aspartate, Zn^{2+}, and reactive oxygen species [86] were prepared. It was suggested that these compounds can be used for treating stroke, traumatic brain and spinal cord injury, epilepsy and other neurodegenerative diseases that are accompained by severe neuronal loss by excitotoxicity, Zn^{2+} neurotoxicity, and free radical toxicity.
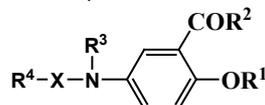
37

R^1=H, alkyl or alkanoyl

R^2=H or alkyl

R^3=H or Ac

R^4=Ph (un)substituted with nitro, halo (alkyl), or alkoxy

X=CO, SO$_2$, or (CH$_2$)n; n=1-5

Mechanism of Anti-inflammatory Activity of 5-ASA

Despite the extensive use of sulfasalazine and 5-ASA in patients with IBD and rheumatoid arthritis, their mode of action has not yet been elucidated in full details. Numerous pharmacological and biochemical effects are described including immunosuppressive, antifolate, and modulatory actions on lymphocyte and leukocyte functions [87]. Interest was focused on actions of 5-ASA on the various enzymes of arachidonic cascade. It is clear that, similarly to indomethacin and acetylsalicylic acid, it inhibits the cyclooxygenase enzyme involved in the biosynthesis of prostaglandins [87]. 5-ASA, unlike most NSAIDs, also inhibits leukotriene synthesis possibly by inhibiting lipooxygenase [81] enzyme that catalyzes the formation of leukotrienes from arachidonic acid and its metabolites. Mucosa of patients with IBD generates excessive amounts of cyclooxygenase products such as prostagladins (PG) as well as 5-lipooxygenase products such as leukotrienes (LTB4 and sulfidopeptide-LT). Both PG and LT exert proinflammatroy effects and are potentially important mediators of mucosal inflammation

[87]. 5-ASA may modulate the expression of the inducible nitric oxide synthase (iNOS), a potential mediator of colitic injury.

An alternative proposal is that 5-ASA acts as a scavenger of superoxide radicals released by neutrophils at the inflammatory sites, hypochlorite formed by this process would react with the amino group of 5-ASA to produce chloramine group (NHCl) and so neutralizes the toxic potential of hypochlorite. It has been suggested that 5-ASA exerts its therapeutic effect through preservation or potentiation of the cytoprotective effects of prostaglandins. Other possible mechanisms include alterations in colonic fluid balance, immunosuppression and alteration of the GI bacterial flora.

Summary

Inflammatory bowel disease (IBD) includes ulcerative colitis and Crohn's disease. The etiology and pathogenesis remains unknown. It is described as auto-immune diseases characterized by inflammation and may affect any part of the small or large bowel. Pharmacologic treatment of IBD involves drugs that belong to different therapeutic classes and have different but nonspecific mechanisms of anti-inflammatory action. Drugs used in inflammatory bowel disease are chosen on the basis of disease severity, responsiveness, and drug toxicity. The aim of therapy is to deliver as mush as possible of the medication at the site of the disease to allow higher doses to be used with least toxicity and to avoid first-pass hepatic metabolism of some drugs. This can be achieved by route of administration (e.g., rectal) or special formulation. 5-aminosalicylic acid used for management of mild to moderate IBD. It is available in different formulations such as mesalamine (5-aminosalicylic acid), Olsalazine (a dimer of 5-ASA) and sulfasalazine (a combination of sulfapyridine and 5-ASA). 5-ASA acts topically (not systemically) within the colon to limit prostaglandin and leukotriene production. Because of specificity of bacterial metabolism (azoreductase) of these drugs, as well as differences in pH of various regions of the lower GI tract, these agents are made more available in the terminal ileum and colon.

Based on our interest and work on 5-ASA and its derivatives, we reported herein 5-aminosalicylates chemistry and unique anti-inflammatory activity, in particular, for treatment of IBD. Different approaches for colonic targeting of 5-ASA are covered with emphasis on chemical methods as well as its proposed mechanisms of action.

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