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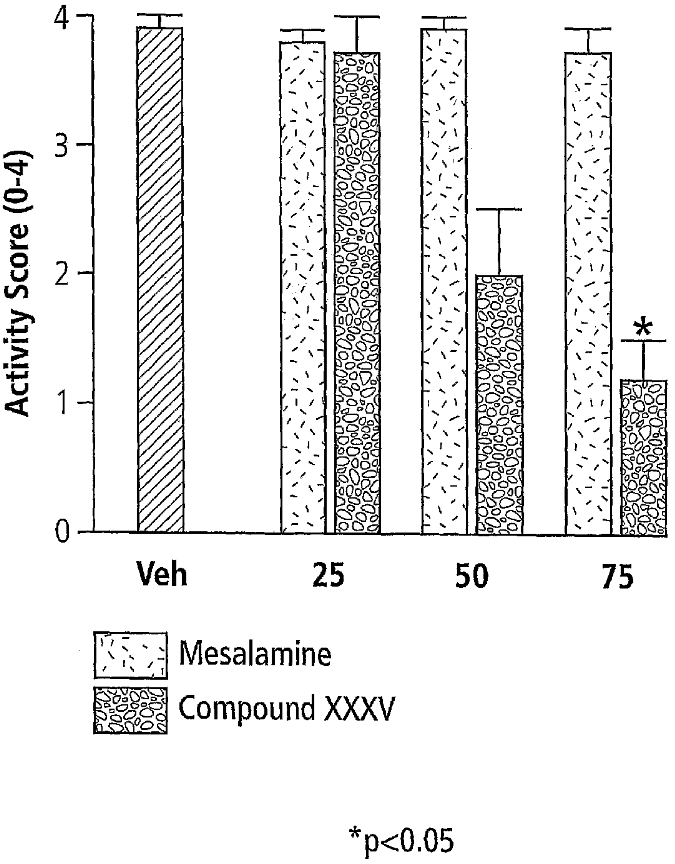
(54) Title: DERIVATIVES OF 4- OR 5-AMINOSALICYLIC ACID



(57) Abstract: The present invention provides new derivatives of 4- or 5-aminosalicylic acid, and a pharmaceutical composition containing these derivatives of 4- or 5-aminosalicylic acid as active ingredients, useful for the treatment of intestinal diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) and for the prevention/treatment of colon cancer. More particularly, these derivatives comprise a hydrogen sulfide releasing carrier linked via an azo, an ester, an anhydride, a thioester or an amide linkage to a molecule of 4- or 5-aminosalicylic acid. Furthermore, the present invention provides a process for preparing these compounds and their use for treating IBD and IBS and the prevention/treatment of colon cancer.

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DERIVATIVES OF 4- or 5-AMINOSALICYLIC ACID

FIELD OF THE INVENTION

The present invention relates to compounds useful in the treatment of an intestinal disease such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) and in colon cancer chemoprevention. In particular, 4- and 5-aminosalicylic acid derivatives have been developed which degrade into useful biologically active compounds. More particularly, these derivatives comprise a hydrogen sulfide releasing carrier linked via an azo, an ester, an anhydride, a thioester or an amide linkage to a molecule of 5- or 4-aminosalicylic acid.

BACKGROUND OF THE INVENTION

Inflammatory bowel disease (IBD) is the general name for diseases that cause inflammation in the small intestine and colon. Ulcerative colitis is the most common inflammatory bowel disease and it affects various portions of the gastrointestinal (GI) tract, particularly the lower GI tract, and more particularly the colon and/or rectum. A second IBD is Crohn's disease, which predominates in the small intestine (ileum) and the large intestine (colon).

Ulcerative colitis can be difficult to diagnose in that its symptoms are similar to other intestinal disorders and to Crohn's disease. Crohn's disease differs from ulcerative colitis because it causes deeper inflammation into the intestinal wall. Also, Crohn's disease usually occurs in the small intestine, although it can also occur in the mouth, esophagus, stomach, duodenum, large intestine, appendix, and anus.

Ulcerative colitis may occur in people of any age, but most often it starts between ages 15 and 30, or less frequently between ages 50 and 70. Children and adolescents sometimes develop this disease. Ulcerative colitis affects men and women equally and appears to run in some families.

It is also important to consider that about 5 percent of people with ulcerative colitis develop colon cancer. The risk of cancer increases with the duration and the extent of involvement of the colon. For example, if only the lower colon and rectum are involved, the risk of cancer is no higher than normal. However, if the entire colon is involved, the risk of cancer may be as much as 32 times the normal rate. Thus, it is possible that drugs useful in the treatment of IBD may also be useful in the prevention of colon cancer.

The pathogenesis of IBD likely involves multifactorial interactions among genetic factors, immunological factors and environmental triggers. Recent evidence suggests that a pathologic activation of the mucosal immune system in response to antigens is a key factor in the pathogenesis of IBD.

The presentation of antigen in the inflammatory process is closely followed by generation of cytokines, small glycoprotein peptide molecules, which provide signals for the communication among different cell populations determining the direction of subsequent immune and inflammatory response. Pro-inflammatory cytokines include interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α). Macrophages are the major source of cytokines, with epithelial cells also being able to produce a number of these peptide factors.

T helper (Th) cells are a further important source of cytokines. Th1 cells, which are associated with a cell-mediated immune response, produce IL-2, interferon gamma (IFN-γ) and TNF-α. A key transcription factor involved in the regulation of inflammation, NFkB, which is specifically implicated in the pathogenesis of IBD, regulates the amount of cytokines produced by the Th1 cells (see Neurath *et al.* (1996) *Nature Med.* **2**: 998-1004). Th2 cells enhance antibody synthesis by B cells and produce IL-4, IL-5, IL-6, and IL-10.

Chemokines are also thought to contribute to the pathogenesis of colitis. Chemokines are pro-inflammatory proteins that participate in immune and inflammatory responses through the chemoattraction and activation of leukocytes. For example, RANTES is a C-C chemokine that promotes the recruitment and activation of inflammatory cells such as monocytes, lymphocytes, mast cells and eosinophils. RANTES has recently been shown to be elevated during the chronic phase of colitis (see Ajuebor *et al.* (2001) *J. Immunol.* **166**: 552-558).

Treatment for ulcerative colitis depends on the seriousness of the illness. Most people are treated with medication. In severe cases, a patient may need surgery to remove the diseased colon.

Irritable bowel syndrome (IBS) is a common but poorly understood disorder that causes a variety of bowel symptoms including abdominal pain, diarrhea and/or constipation, bloating, gassiness and cramping. While these symptoms may be caused by a number of different bowel diseases, IBS is usually diagnosed only after exclusion of a more serious problem. There is increasing evidence suggesting the role of inflammation in the pathogenesis of IBS.

The goal of therapy is to induce and maintain remission, and to improve the quality of life for people with IBD/IBS. Several types of drugs are available.

Aminosalicylates, which are drugs that contain 5-aminosalicylic acid (5-ASA; mesalamine) or 4-aminosalicylic acid (4-ASA), help to control the inflammation. However, both mesalamine and 4-ASA may be absorbed as it passes through the GI tract and may adversely affect the amount of mesalamine that reaches the lower GI tract, particularly the colon and rectum. Thus, various mesalamine formulations have been introduced in an attempt to protect mesalamine as it passes through the gut and upper GI tract.

In addition, several pro-drugs of mesalamine have been introduced which can aid in colon-specific delivery of mesalamine. These pro-drugs are generally less readily absorbed in the gut and upper GI tract and thus can more easily reach the colon.

Sulfasalazine is a combination of sulfapyridine and 5-ASA and is employed to induce and maintain remission. Sulfasalazine is metabolized in the body to form 5-ASA and sulfapyridine. The sulfapyridine component carries the anti-inflammatory 5-ASA to the intestine.

However, sulfapyridine may lead to side effects, such as nausea, vomiting, heartburn, diarrhea, and headache. These adverse side effects are usually attributed to the activity of sulfapyridine in the GI tract, as well as that absorbed into the system.

Other 5-ASA agents such as olsalazine, ipsalazide and balsalazide, each of which have a different carrier, offer fewer side effects, and may be used by people who cannot take sulfasalazine. Unlike sulfasalazine, the breakdown of these 5-ASA compounds in the intestinal tract may not give rise to undesirable metabolic products.

In general, 5-ASA compounds are given orally, through an enema, or in a suppository, depending on the location of the inflammation in the colon. Most people with mild or moderate ulcerative colitis are treated with this group of drugs first. However, in general, this therapy cannot be considered optimal, mainly because of the poor potency of the drug that causes also a poor compliance for the patient.

Other drugs that are used are corticosteroids such as prednisone, hydrocortisone, budesonide etc. and immunomodulators such as azathioprine and 6-mercaptopurine (6-MP). These drugs can cause side effects such as hypertension, increased risk of infections etc.

Sulfasalazine, olsalazide and balsalazide are mesalamine derivatives where the non-mesalamine carrier is linked to mesalamine via a diazo bond. These pro-drugs are not as readily absorbed in the gut and upper GI tract and thus can reach the colon where they are split by azo-reductases of the colonic microflora to release the mesalamine and carrier directly in the colon.

Other derivatives of mesalamine comprise a carrier attached to mesalamine via the carboxylic and hydroxyl functional groups of the molecule. Among these, the preparation of esters or amides with amino acids such as L-serine and L-glycine or the addition of other biological compound such as taurine has been reported. These pro-drugs base their activity on the action of carboxypeptidases and aminopeptidases A for releasing mesalamine. (R. Pellicciari *et al.* in Journal of Medicinal Chemistry, 1993, **36**, pg. 4201-7).

Most of the prior art carrier moieties attached to mesalamine are inert. Thus, it is desirable to link carrier moieties to either 5-ASA or 4-ASA, which are also biologically active and useful in the treatment of IBD/IBS.

SUMMARY OF THE INVENTION

In general, a hydrogen sulfide releasing carrier is linked via an azo, an ester, an anhydride, a thioester or an amide linkage to a molecule of 4- or 5-aminosalicylic acid (4- or 5-ASA) to form 4- or 5-ASA derivatives. These 4- or 5-ASA derivatives undergo hydrolysis or cleavage by various enzymes present in the GI tract to release two active ingredients, namely, 4- or 5-aminosalicylic acid and a hydrogen sulfide releasing carrier.

Surprisingly, the derivatives of the present invention are superior to 5-ASA (mesalamine) in reducing inflammation, as indicated by a reduction in granulocyte infiltration (as measured by a decrease in myeloperoxidase activity), a reduction in mRNA levels for IFN-γ, IL-2 and TNF-α and RANTES, and an overall reduction in bowel edema and weight loss with the hydrogen sulfide releasing derivatives when compared to mesalamine alone. Further, the derivatives of the present invention also reduce mRNA levels of cyclooxygenase (COX)-1, COX-2, constitutive endothelial nitric oxide synthase (eNOS), and inducible NOS (iNOS), all of which are enzymes believed to be involved in inflammation. Without being bound to theory, it is thought that the hydrogen sulfide released from the hydrogen sulfide releasing moiety exerts anti-inflammatory effects via the inhibition of NFkB, the transcription factor that regulates the expression of several of the pro-inflammatory genes.

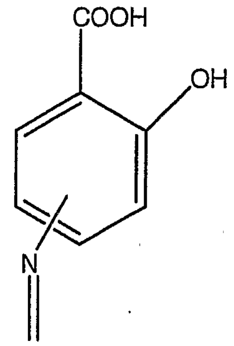
In addition, the 4- or 5-ASA derivatives of the invention are effective in decreasing the viability of HT-29 human colon cancer cells and thus are useful in the prevention and/or treatment of colon cancer.

Broadly stated, compounds of the invention have the following general formula:

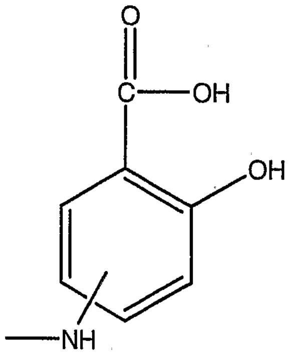
$$A—L—R \qquad (I)$$

where:

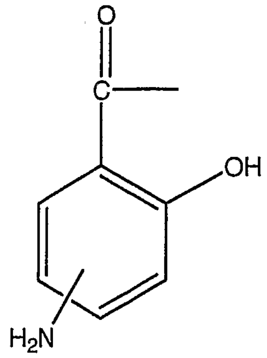
A is



where –N= is either at position 4 or 5,

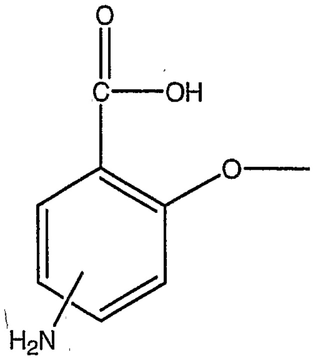


where –NH is either at position 4 or 5,



where $-NH_2$ is either at position 4 or 5,

or

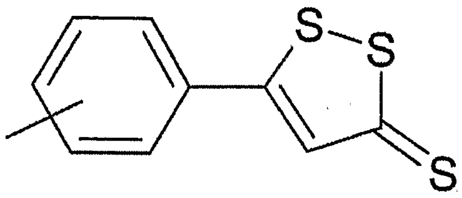


where $-NH_2$ is either at position 4 or 5;

L is either O, O-C=O, S, N or a covalent bond to form an ester linkage, an anhydride linkage, a thioester linkage, an amide linkage or an azo linkage; and

R is a hydrogen sulfide releasing moiety. It is understood that any non-toxic, effective hydrogen sulfide releasing moiety can be used in the present invention.

In a preferred embodiment, R is selected from the group consisting of:



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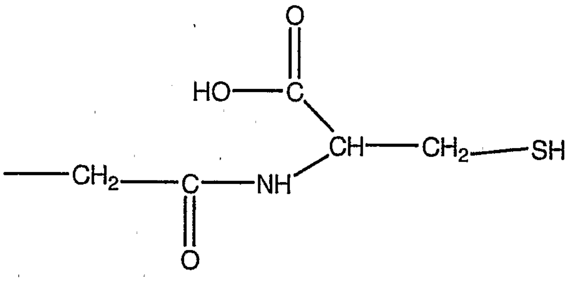

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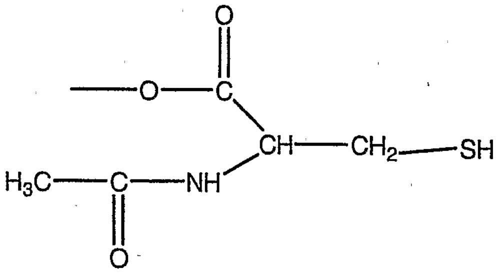

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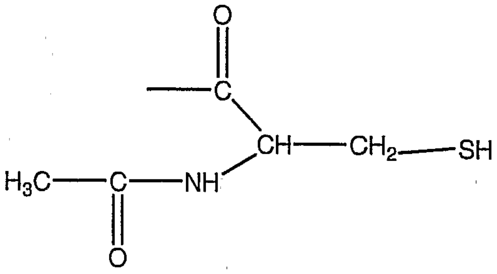

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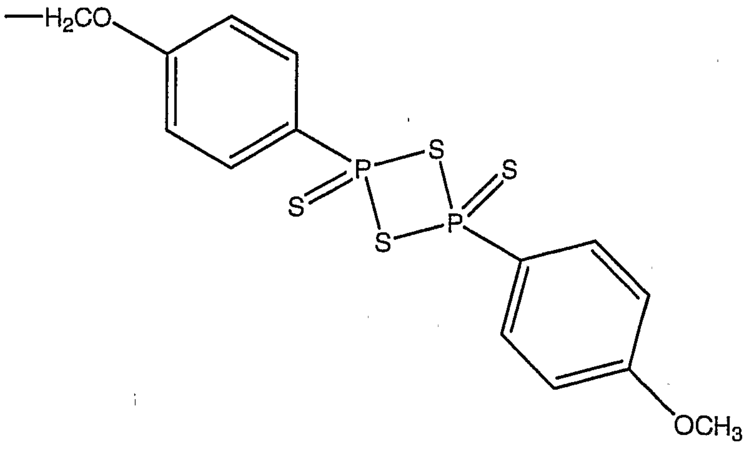
H2CO
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S
P
S
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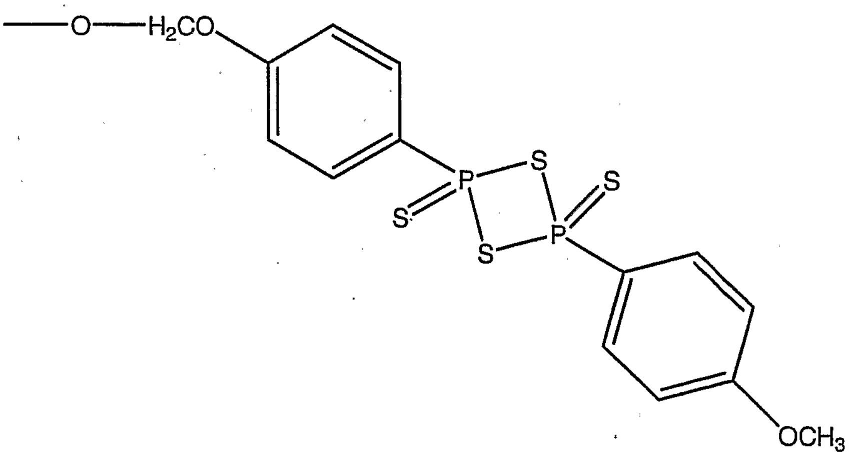
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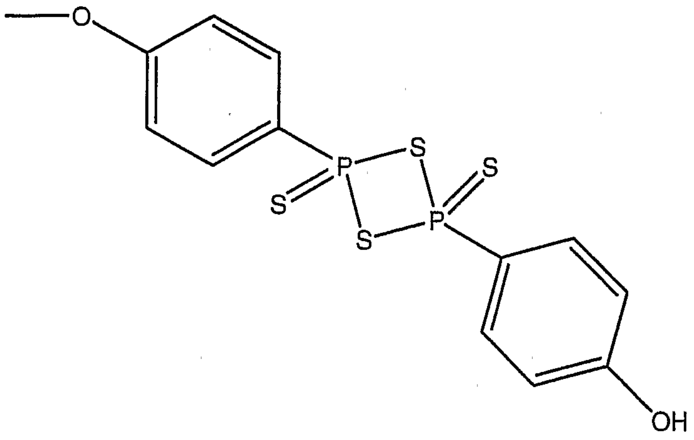

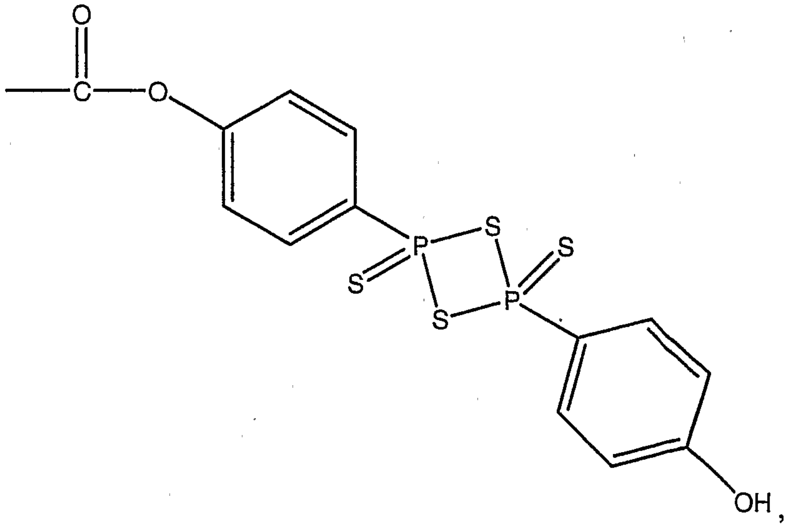

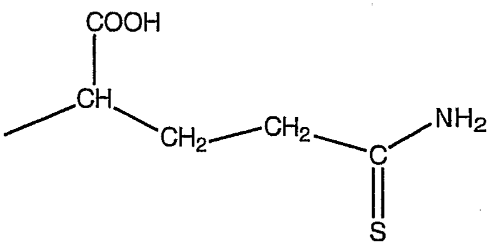

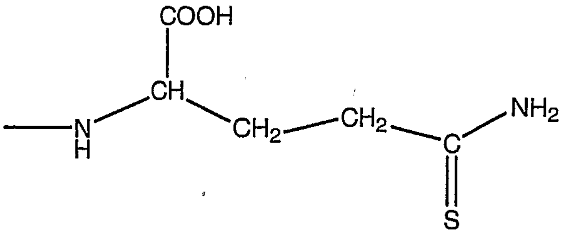
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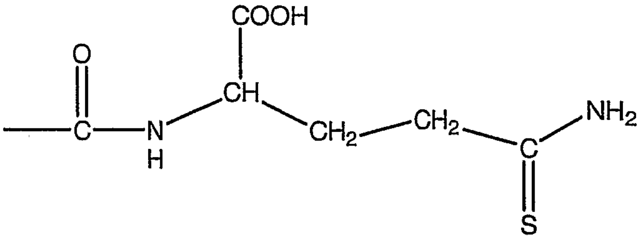
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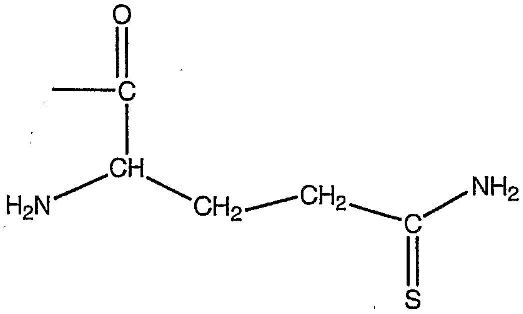
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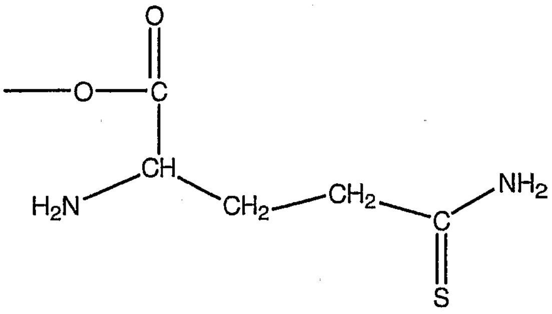
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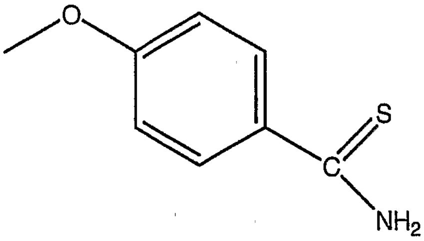
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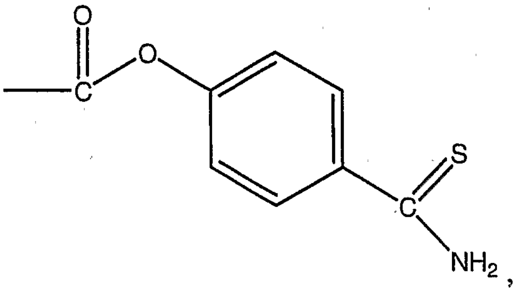
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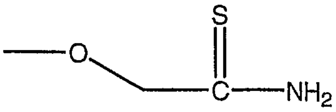
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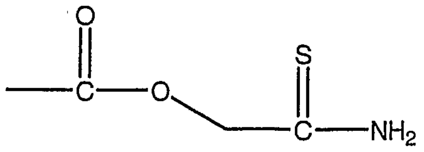
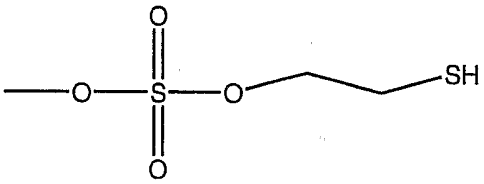


and



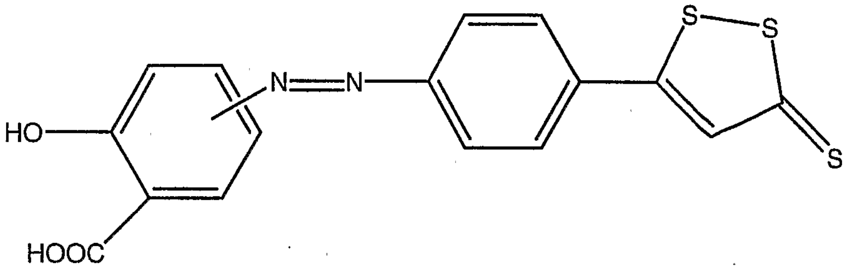
Pharmaceutical acceptable salts such as for example salts with alkaline metals and alkaline earth metals, non-toxic amines and amino acids are also part of the present invention. Preferred salts are the salts with arginine and agmatine. Also included are pharmaceutically acceptable acid addition salts.

In a further aspect the present invention provides a pharmaceutical composition of the compounds of the present invention, and a pharmaceutically acceptable excipient or carrier, particularly one for use in the treatment of an inflammatory condition of the GI tract.

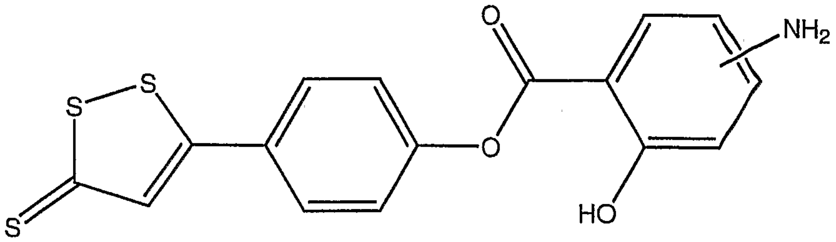
According to other embodiments of the present invention, methods of treating an inflammatory condition of the GI tract, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), in a subject in need of such treatment, include administering to the subject an effective amount of 4- or 5-ASA derivatives and their salts. Further, methods for the treatment or prevention of colon cancer in a subject in need thereof are provided comprising administering to the subject an effective amount of 4- or 5-ASA derivatives and their salts.

In a further embodiment, the present invention provides the use of 4- or 5-ASA derivatives and their salts of the present invention for the manufacture of a medicament for the treatment of an inflammatory condition of the GI tract. The present invention also provides the use of 4- or 5-ASA derivatives and their salts for the treatment of an inflammatory condition of the GI tract.

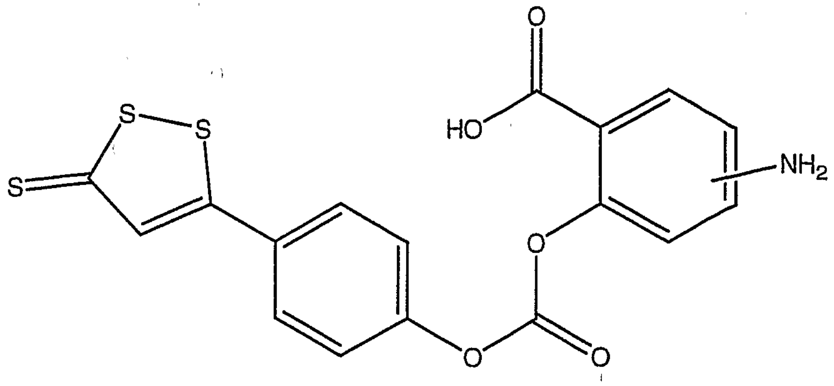
Preferred compounds are those of the following formulae:



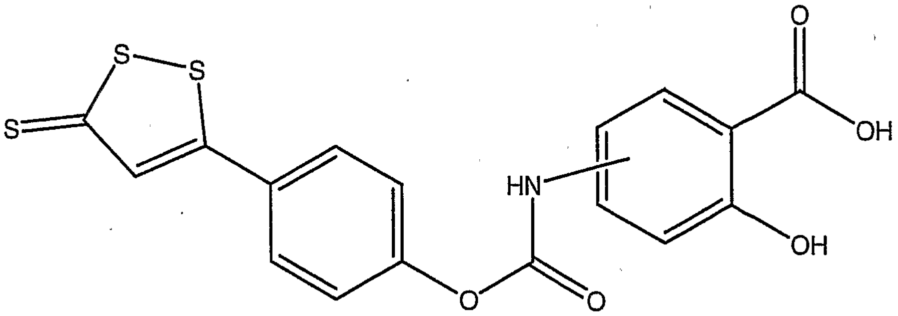
2-Hydroxy-4- or 5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid (**II**),



4-or 5-Amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester (**III**),

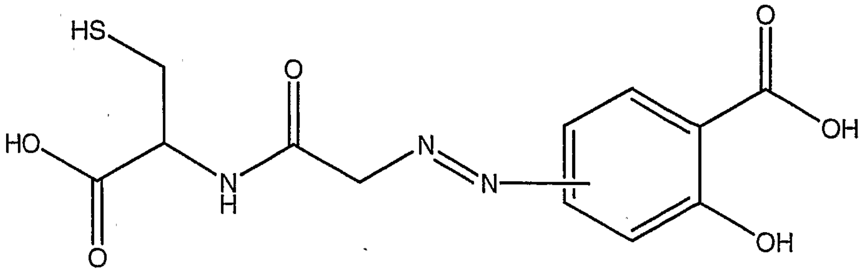


4 or 5-Amino-2-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonyloxy]-benzoic acid (**IV**),



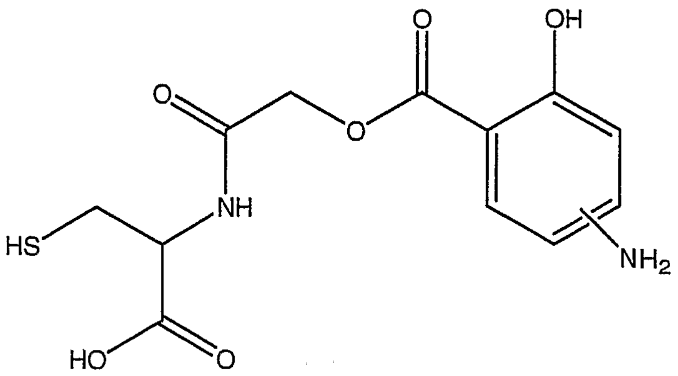
2-Hydroxy-4 or 5[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonylamino]-benzoic acid

(**V**),

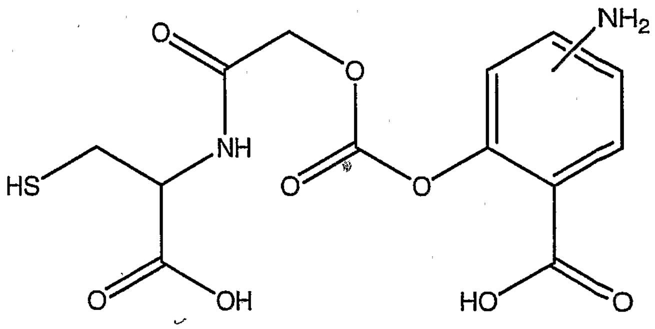


4-or 5-{[(1-Carboxy-2-mercapto-ethylcarbamoyl)-methyl]-azo}-2-hydroxy-benzoic acid

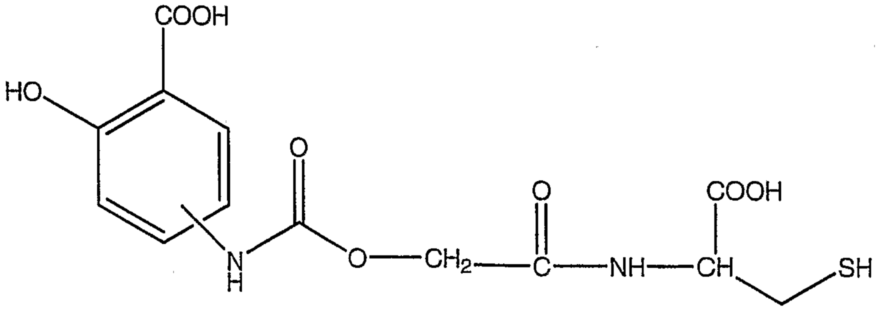
(**VI**),



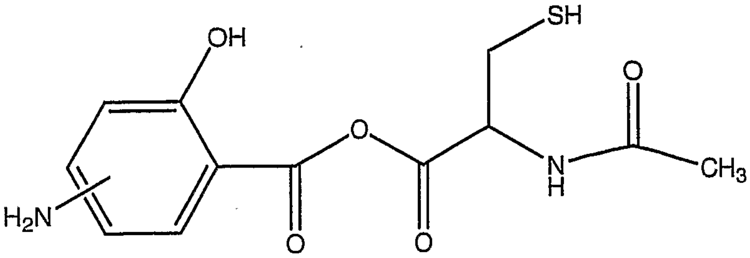
4-or 5-Amino-2-hydroxy-benzoic acid (1-carboxy-2-mercapto-ethylcarbamoyl)-methyl ester (**VII**),



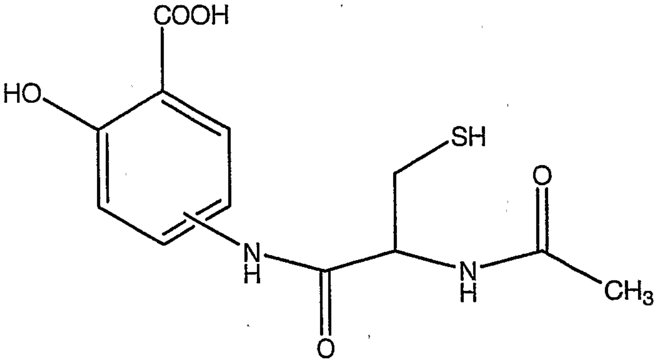
4-or 5-Amino-2-[(1-carboxy-2-mercapto-ethylcarbamoyl)-methoxycarbonyloxy]-benzoic acid (**VIII**),



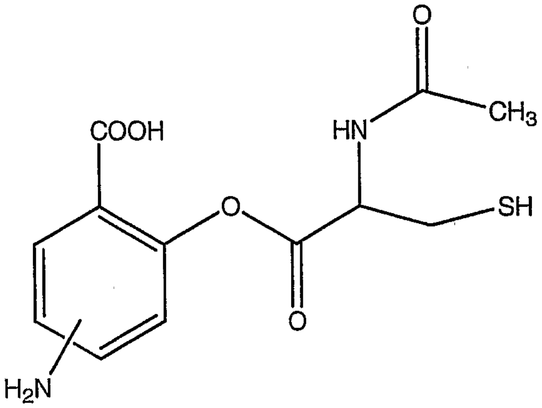
4- or 5-[(1-Carboxy-2-mercapto-ethylcarbamoyl)-methoxycarbonylamino]-2-hydroxy-benzoic acid (**IX**),



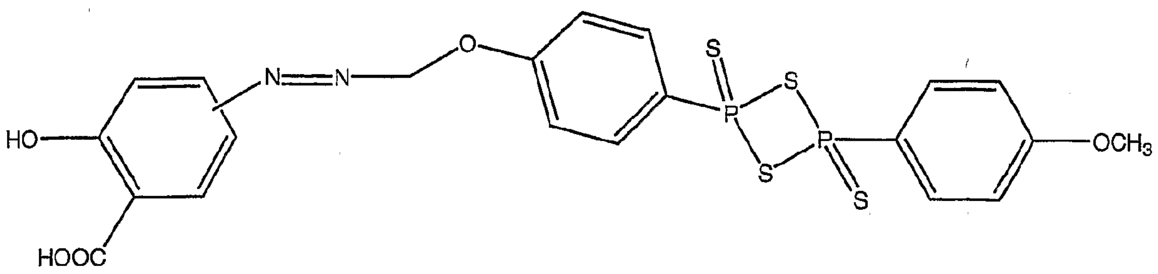
4- or 5- amino-2-hydroxy-benzoic acid anhydride with N-acetyl cysteine (**X**),



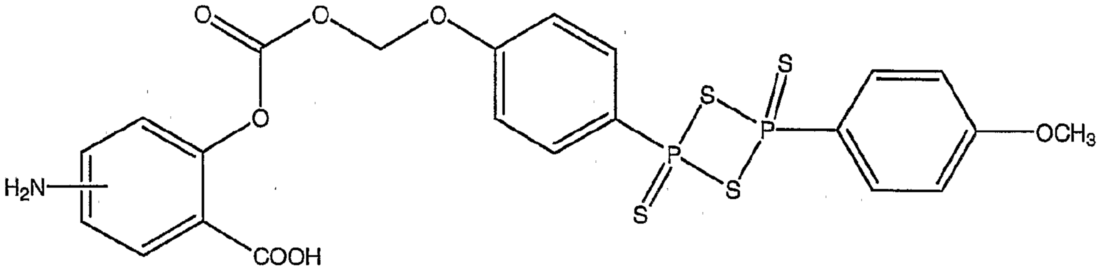
4 or 5-(2-Acetylamino-3-mercapto-propionylamino)-2-hydroxy-benzoic acid (**XI**),



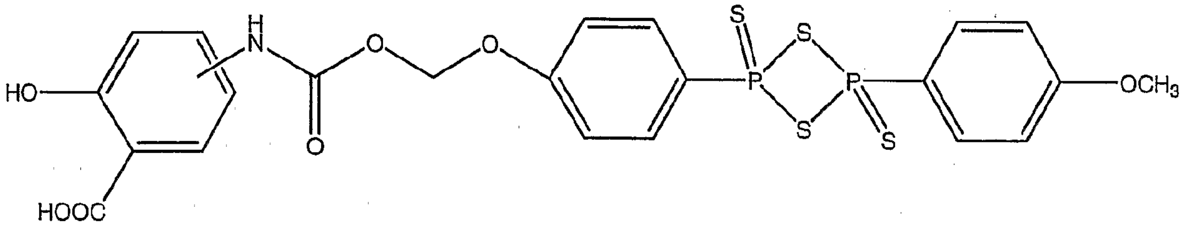
2-(2-Acetylamino-3-mercapto-propionyloxy)-4 or 5-amino-benzoic acid (**XII**),



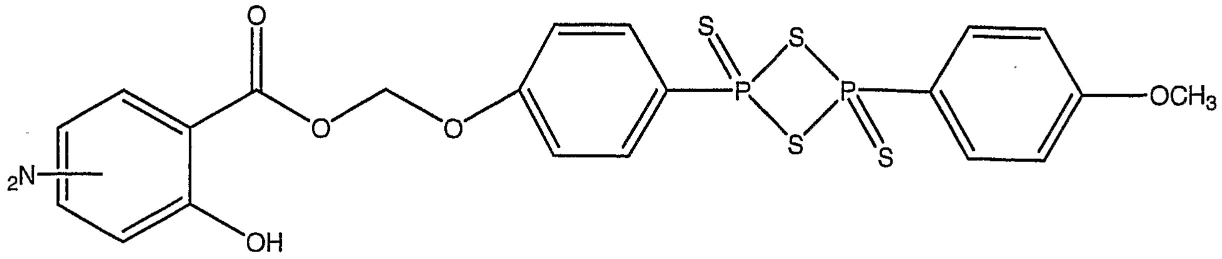
2-Hydroxy-4 or 5-({4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethyl}-azo)-benzoic acid (**XIII**),



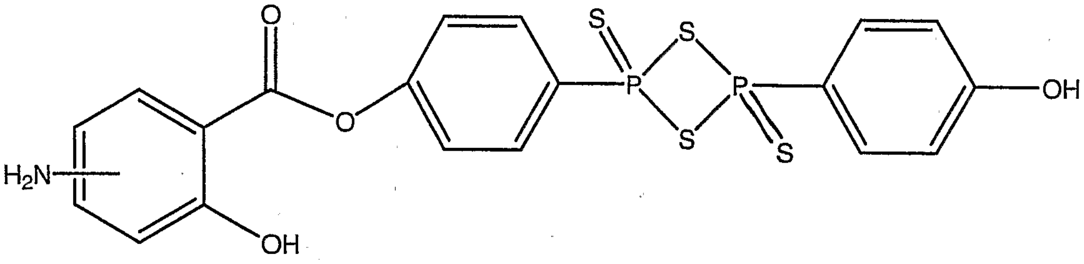
4- or 5-Amino-2-{4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethoxycarbonyloxy}-benzoic acid (**XIV**),



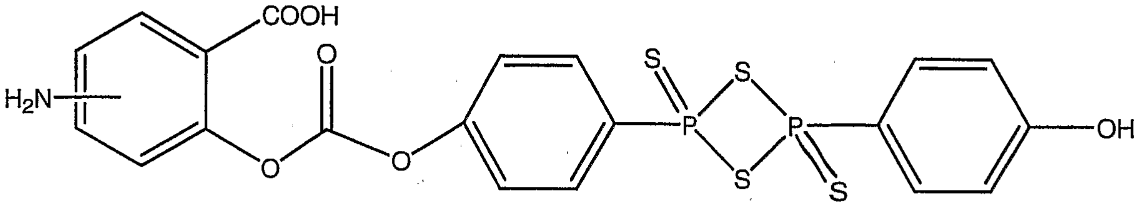
2-Hydroxy- 4- or 5-{4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethoxycarbonylamino}-benzoic acid (**XV**),



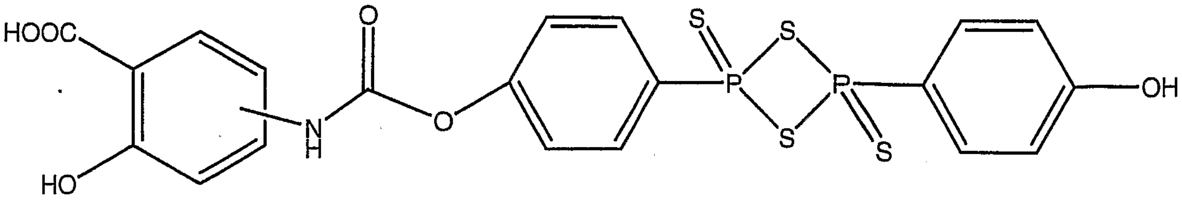
4-or 5-Amino-2-hydroxy-benzoic acid 4-[4-(4-methoxy-phenyl)-2,4-dithioxo-$2\lambda^5,4\lambda^5$-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethyl ester **(XVI)**,



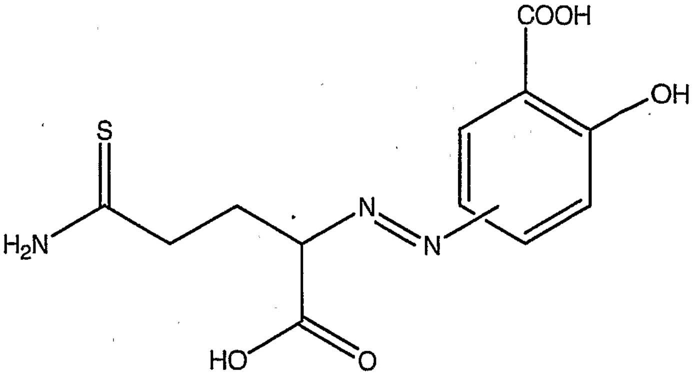
4- or 5-Amino-2-hydroxy-benzoic acid 4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-$2\lambda^5,4\lambda^5$-[1,3,2,4]dithiadiphosphetan-2-yl]-phenyl ester **(XVII)**,



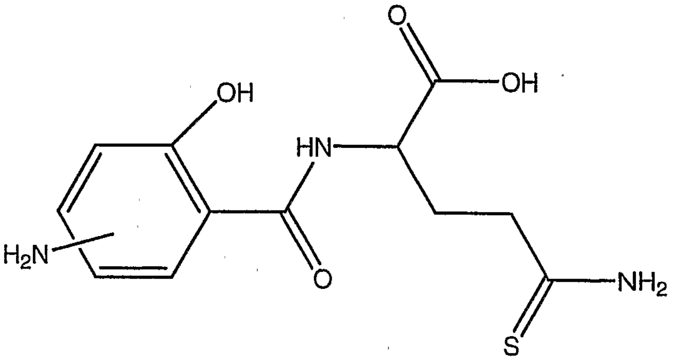
4- or 5-Amino-2-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-$2\lambda^5,4\lambda^5$-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonyloxy}-benzoic acid **(XVIII)**,



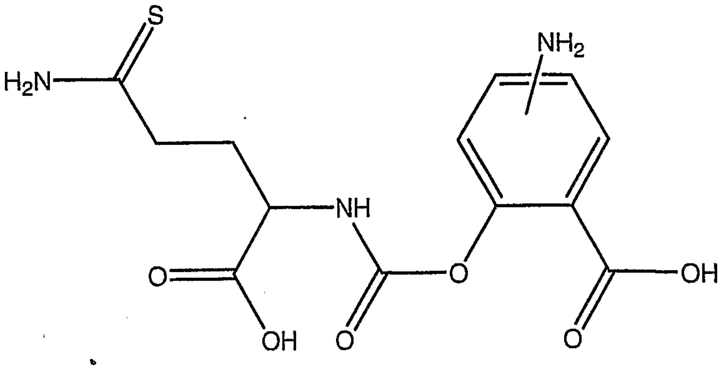
2-Hydroxy-4- or 5-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonylamino}-benzoic acid (**XIX**),



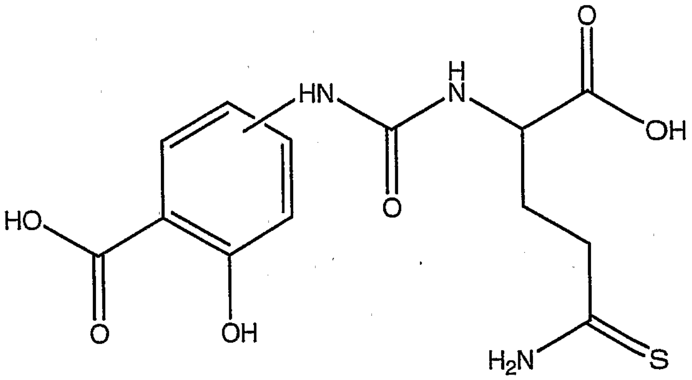
4- or 5-(1-Carboxy-3-thiocarbamoyl-propylazo)-2-hydroxy-benzoic acid (**XX**),



2-(4- or 5-Amino-2-hydroxy-benzoylamino)-4-thiocarbamoyl-butyric acid (**XXI**),

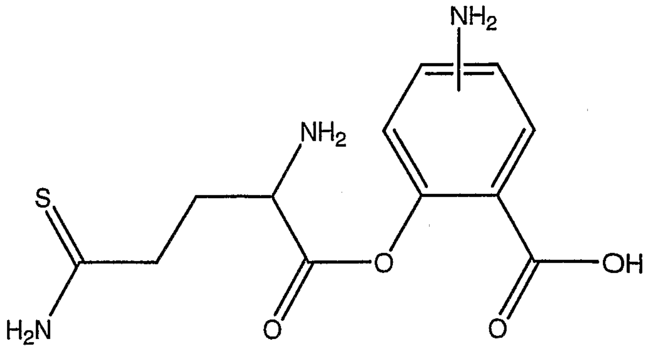


4- or 5-Amino-2-(1-carboxy-3-thiocarbamoyl-propylcarbamoyloxy)-benzoic acid (**XXII**),

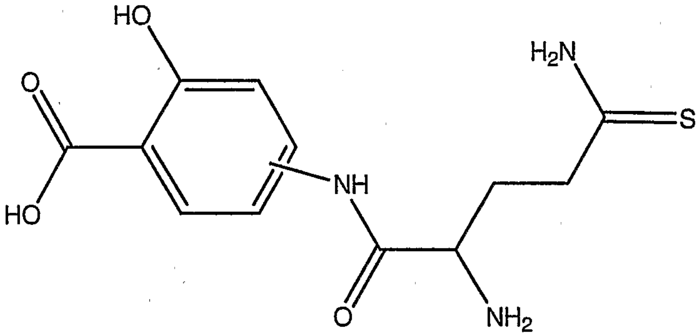


2-Hydroxy-4- or 5-[3-(1-hydroxymethyl-3-thiocarbamoyl-propyl)-ureido]-benzoic acid

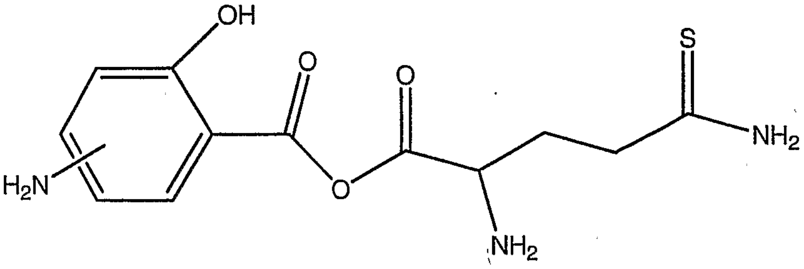
(**XXIII**),



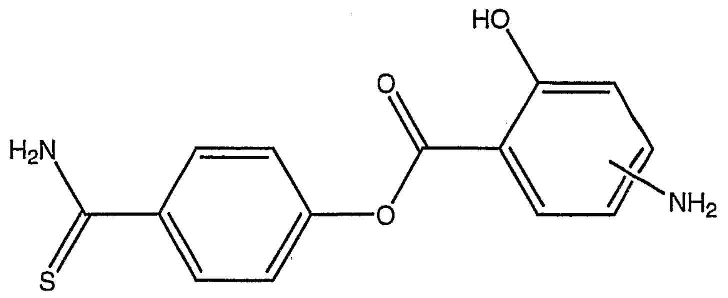
4- or 5-Amino-2-(2-amino-4-thiocarbamoyl-butyryloxy)-benzoic acid **(XXIV),**



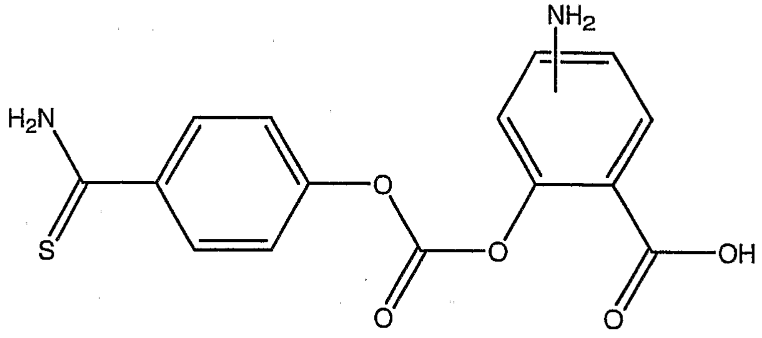
4- or 5-(2-Amino-4-thiocarbamoyl-butyrylamino)-2-hydroxy-benzoic acid (**XXV**),



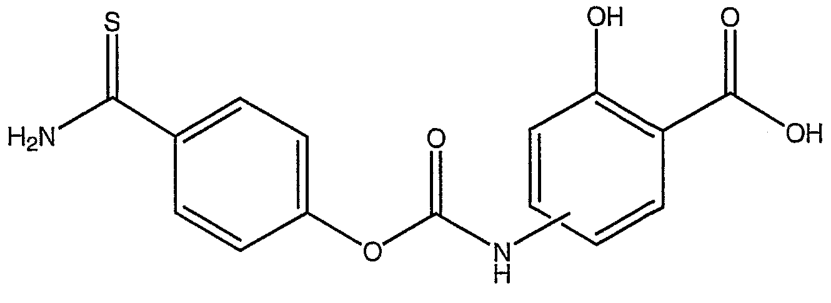
4- or 5- Amino-2-hydroxy-benzoic acid anhydride with 2-amino-4-thiocarbamoyl-butyric acid (**XXVI**),



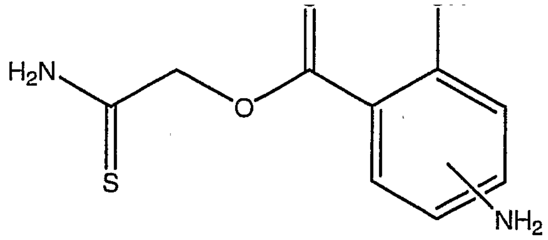
4-thiocarbamoylphenyl 4- or 5-amino-2-hydroxybenzoate (**XXVII**),



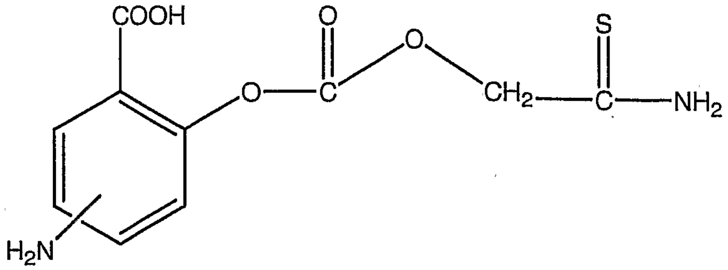
4- or 5-Amino-2-(4-thiocarbamoyl-phenoxycarbonyloxy)-benzoic acid **(XXVIII),**



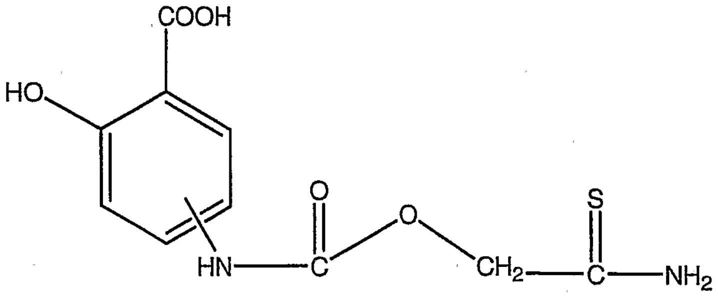
2-Hydroxy-4- or 5-(4-thiocarbamoyl-phenoxycarbonylamino)-benzoic acid **(XXIX),**



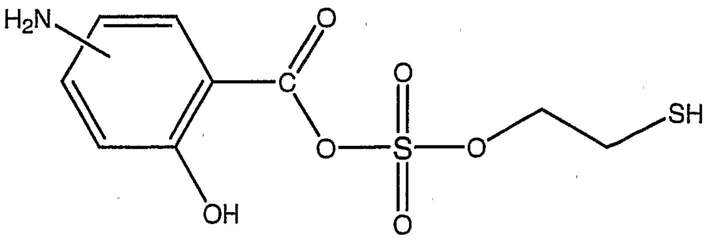
4- or 5-Amino-2-hydroxy-benzoic acid thiocarbamoylmethyl ester **(XXX),**



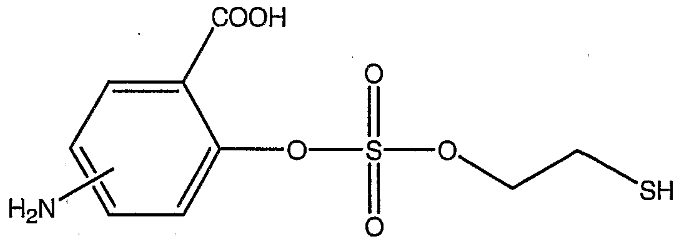
4- or 5-Amino-2-thiocarbamoylmethoxycarbonyloxy-benzoic acid **(XXXI),**



2-Hydroxy-4- or 5-thiocarbamoylmethoxycarbonylamino-benzoic acid **(XXXII),**

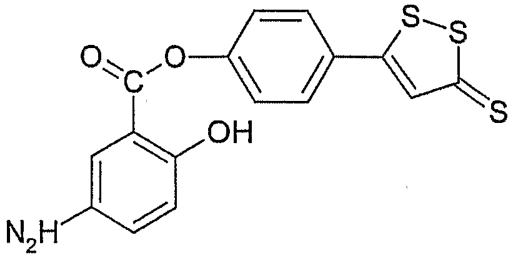


4-or 5- Amino-2-hydroxy-benzoic acid anhydride with sulfuric acid mono- (2-mercapto-ethyl) ester **(XXXIII),** and



4- or 5-Amino-2-(2-mercapto-ethoxysulfonyloxy)-benzoic acid **(XXXIV).**

The most preferred compound is as follows:



5-Amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester **(XXXV).**

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the Disease Activity Score of mice having TNBS-induced colitis after treatment with increasing doses of mesalamine and Compound XXXV of the present invention.

FIG. 2 shows the myeloperoxidase (MPO) activity in mice having TNBS-induced colitis after treatment with increasing doses of mesalamine and Compound XXXV of the present invention.

FIG. 3 shows the myeloperoxidase (MPO) activity in mice having TNBS-induced colitis after treatment with 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 4 shows the Disease Activity Score of mice having TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 5 shows colonic tumour necrosis factor (TNF-α) mRNA expression in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 6 shows interferon gamma (IFN-γ) mRNA expression in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 7 shows various interleukin (IL) mRNA expression, namely, IL-1, -2, 10 and -12 mRNA, in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 8 shows colonic levels of RANTES mRNA in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 9 shows colonic COX-1 and COX-2 mRNA expression in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIG. 10 shows colonic eNOS and iNOS mRNA expression in mice with TNBS-induced colitis after treatment with vehicle (1% CMC), 50 mg/kg mesalamine and equimolar dose of Compound XXXV of the present invention.

FIGS. 11(a) and (b) show the perception score in a rat model of visceral pain perception using mesalamine and Compound XXXV of the present invention.

FIGS. 12(a) and (b) show the intrarectal pressure in a rat model of visceral pain perception using mesalamine and Compound XXXV of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention will now be described with respect to preferred embodiments described herein. It should be appreciated however that these embodiments are for the purpose of illustrating the invention, and are not to be construed as limiting the scope of the invention as defined by the claims.

The compounds of the present invention contain two active moieties, either 4- or 5-ASA and a hydrogen sulfide releasing moiety, linked together by an azo, ester, anhydride, thioester or amide linkage. The presence of azo-reductase enzymes allow for the release of 4- or 5-ASA from the azo bond pro-drugs thus allowing a targeted delivery to the colon and reducing at the same time the systemic absorption. Similarly, the presence of carboxypeptidases and aminopeptidases A also allow for the release of 4- or 5-ASA from the ester and amide bond pro-drugs, respectively. Esterases and thioesterases will also cleave ester and thioester linkages, respectively. Finally, lipases will cleave anhydride linkages. The compounds of the present invention can be made using known starting materials and reagents.

Compounds of the present invention may be utilized for the prophylaxis or treatment of various diseases, particularly inflammatory conditions of the GI tract including, but not limited to, inflammatory conditions of the mouth such as mucositis, infectious diseases (*e.g.*, viral, bacterial and fungal diseases), and Crohn's disease; inflammatory conditions of the esophagus such as esophagitis, conditions resulting from chemical injury (*e.g.*, lye ingestion), gastroesophageal reflux disease, bile acid reflux, Barrett's esophagus, Crohn's disease, and esophageal stricture; inflammatory conditions such as gastritis (*e.g.*, *Helicobacter pylori*, acid-peptic disease and atrophic gastritis), celiac disease, peptic ulcer disease, pre-cancerous lesions of the stomach, non-ulcer dyspepsia, and Crohn's disease; inflammatory conditions of the stomach such as Crohn's disease, bacterial overgrowth, peptic ulcer disease, and fissures of the intestine; inflammatory conditions of the colon such as Crohn's disease, ulcerative colitis, irritable bowel syndrome, infectious colitis (*e.g.*, pseudomembranous colitis such as *Clostridium difficile* colitis, salmonella enteritis, shigella infections, yersiniosis, cryptospiridiosis, microspridial infections, and viral infections), radiation-induced colitis, colitis in the immunocompromised host (*e.g.*, typhlitis), precancerous conditions of the colon (*e.g.*, dysplasia, inflammatory conditions of the bowel, and colonic polyps), proctitis, inflammation associated with hemorrhoids, proctalgia fugax, and rectal fissures; liver gallbladder and/or bilary tract conditions such as cholangitis, sclerosing cholangitis, primary bilary cirrhosis, and cholecystitis; and intestinal abscess.

Depending on the specific condition or disease state to be treated, subjects may be administered compounds of the present invention at any suitable therapeutically effective and safe dosage, as may be readily determined within the skill of the art. These compounds are, most desirably, administered in dosages ranging from about 1 to about 2000 mg per day, in a single or divided doses, although variations will necessarily occur depending upon the weight and condition of the subject being treated and the particular route of administration chosen. However, a dosage level that is in the range of about 0.1 to about 100 mg/kg, preferably between about 5 and 90 mg/kg, and more preferably between about 5 and 50 mg/kg, is most desirable. Variations may nevertheless occur depending upon the weight and conditions of the persons being treated and their individual responses to said medicament, as well as on the type of pharmaceutical formulation chosen and the time period and interval during which such administration is carried out. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effects, provided that such large doses are first divided into several small doses for administration throughout the day.

The compounds of the present invention can be administered in the form of any pharmaceutical formulation, the nature of which will depend upon the route of administration. These pharmaceutical compositions can be prepared by conventional methods, using compatible, pharmaceutically acceptable excipients or vehicles.

Examples of such compositions include capsules, tablets, transdermal patches, lozenges, troches, sprays, syrups, powders, granulates, gels, elixirs, suppositories, and the like, for the preparation of extemporaneous solutions, injectable preparations, rectal, nasal, ocular, vaginal etc. A preferred route of administration is the oral and rectal route.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc can be used for tabletting purposes. Solid compositions of similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar, as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration the active ingredient may be combined with sweetening or flavoring agents, coloring matter and, if so desired, emulsifying and/or suspending agents, together with such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof.

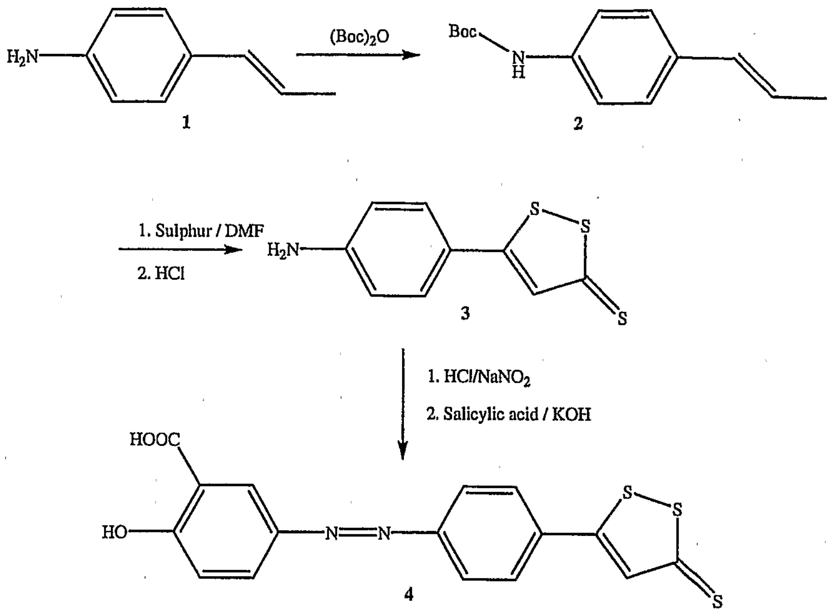
The dosage form can be designed for immediate release, controlled release, extended release, delayed release or targeted delayed release. The definitions of these terms are known to those skilled in the art. Furthermore, the dosage form release profile can be effected by a polymeric mixture composition, a coated matrix composition, a multiparticulate composition, a coated multiparticulate composition, an ion-exchange resin-based composition, an osmosis-based composition, or a biodegradable polymeric composition. Without wishing to be bound by theory, it is believed that the release may be effected through favorable diffusion, dissolution, erosion, ion-exchange, osmosis or combinations thereof.

For parenteral administration, a solution of an active compound in either sesame or peanut oil or in aqueous propylene glycol can be employed. The aqueous solutions should be suitably buffered (preferably pH greater than 8), if necessary, and the liquid diluent first rendered isotonic. The aqueous solutions are suitable for intravenous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The following non-limitative examples further describe and enable a person ordinarily skilled in the art to make and use the invention.

EXAMPLE 1

Synthesis of 2-Hydroxy-5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid (4)



Synthesis of (4-Propenyl-phenyl)-carbamic acid tert-butyl ester (2)

To the solution of 4-propenyl-phenylamine **1** (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine(15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for ½ h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mL), was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/ MeOH (9/1), from which *(4-Propenyl-phenyl)-carbamic acid tert-butyl ester (2)* was obtained (90 % yield).

Synthesis of 5-(4-Amino-phenyl)-[1,2]dithiole-3-thione (3)

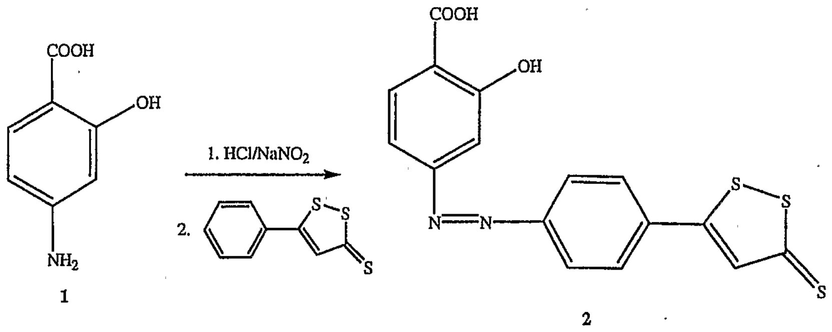
(4-Propenyl-phenyl)-carbamic acid *tert*-butyl ester (**2**, 4.5 mmol) and sulphur (31.5 mmol) were heated in dimethyl formamide (500 ml) for 8 hr; the residue after removal of solvent was almost completely soluble in toluene. An attempt to extract the toluene liquors with 2N aqueous sodium hydroxide, gave a precipitate of an orange solid. This product was dissolved in boiling water, treated with 4N hydrochloric acid for 30 min at room temperature; addition of 4N NaOH furnished the desired product **3** (yield 55%).

Synthesis of 2-Hydroxy-5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid (4)

5-(4-Amino-phenyl)-[1,2]dithiole-3-thione (**3**, 0.56 mmol) was dissolved in a mixture of 5 mL of concentrated HCl and 2.5 mL of water and diazotized with a solution of sodium nitrite (0.56 mmol). In the meantime salicylic acid (0.56 mmol), potassium hydroxide (1.12 mmol) and sodium carbonate are dissolved in water. The diazo suspension is added in portions to the alkaline solution of salicylic acid and the alkalinity maintained at a sufficiently high level during the whole reaction by means of addition of further quantities of potassium hydroxide solution. After 2 days the reaction mixture is heated for 30 min at 50°C. The azo compound **4** was precipitated by means of HCl and filtered off (yield 85%).

EXAMPLE 2

Synthesis of 2-Hydroxy-4-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid (2)

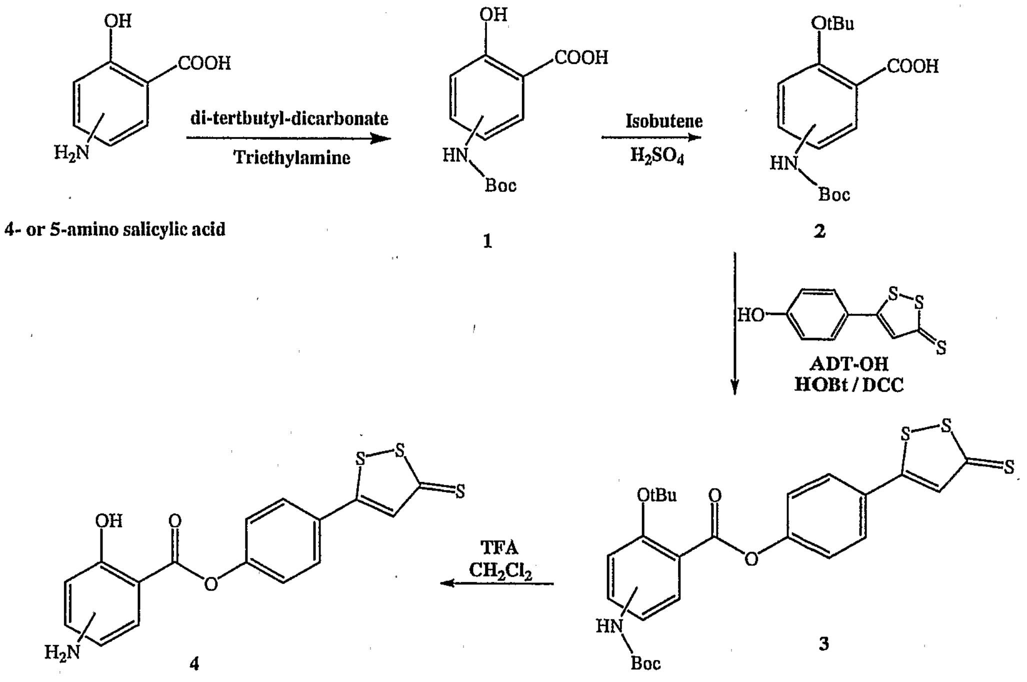


Synthesis of 2-Hydroxy-4-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid (2)

4-Amino-2-hydroxy-benzoic acid (**1**, 1 mmol) was dissolved in a mixture of 10 mL of concentrated HCl and 5 mL of water and diazotized with a solution of sodium nitrite (1 mmol). The diazo suspension is added in portions to a solution of 5-phenyl-[1,2]dithiole-3-thione (1 mmol) in dimethylformamide. After 2 days the reaction mixture is heated for 30 min at 50°C. After cooling the azo compound **2** was precipitated by means of HCl and filtered off (yield 65%).

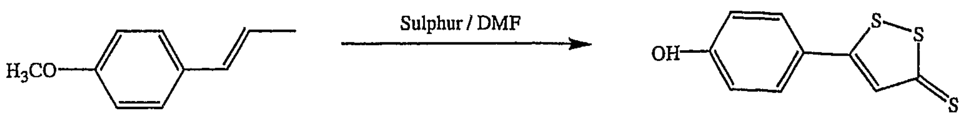
EXAMPLE 3

General synthetic procedure of:
4- or 5-Amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester (4)



Synthesis of 5-p-hydroxyphenyl-1,2-dithione-3-thione (ADT-OH)

Anethole **1** (32.5 g; 0.21 mol) and sulphur (45 g; 1.40 mol) were heated in dimethylformamide (250 ml) for 8 hr; the residue after removal of solvent was almost completely soluble in toluene. An attempt to extract the toluene liquors with 2N-aqueous sodium hydroxide, gave a precipitate of an orange solid (8.5 g). m.p. over 300°C. This product was dissolved in boiling water and gave an orange precipitate **2** after addition of hydrochloric acid (Yield 50%), m.p. 188-189 °C. ^{1}H NMR (DMSO) δ 6.86 (d, 2H), 7.68 (s, 1H), 7.75 (d, 2H), 10.51 (s, -OH); MS (ESI), *m/z* 225(M$^-$).



1

2 (ADT-OH)

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0° C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL), was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (80 % yield).

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2- tert-butoxy-benzoic acid (2)

Compound **1** (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (**2**), which was recrystallized by DCM/ hexane (83 % yield).

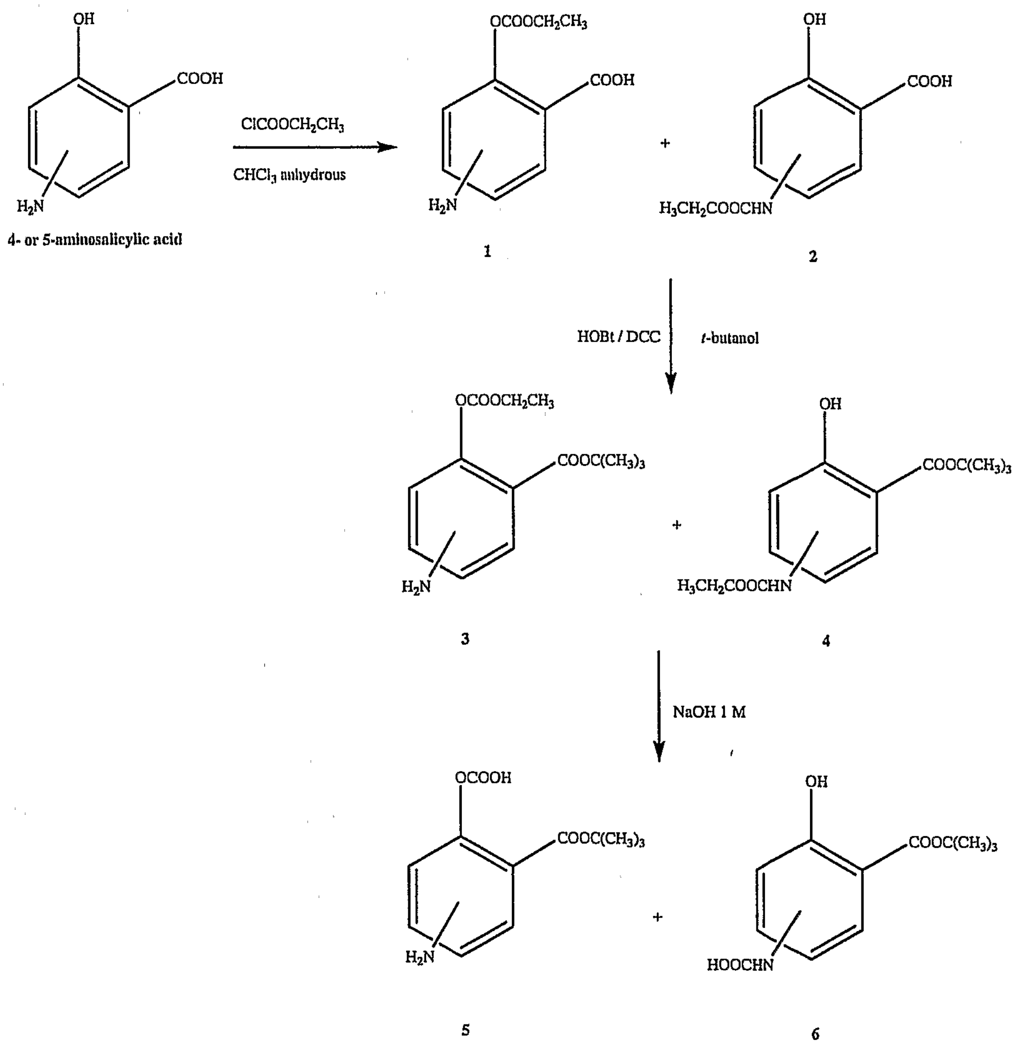
Synthesis of 4-or 5-Amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester (4)

To the solution of 4- or 5-*tert*-butoxycarbonylamino-2-hydroxy-benzoic acid (**2**) (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0° C for 1 h. To the reaction mixture, 5-*p*-hydroxyphenyl-1,2-dithione-3-thione (ADT-OH) (3.0 mmol) was added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **3** was treated with a solution of 40 % TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 3 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4- or 5-amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester* (**4**) was obtained (40% yield).

Compound *5-amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester* (4): ^{1}H NMR (DMSO) δ 7.07 (d, 2H), 7.38 (d, 2H), 7.46 (d, 2H), 7.79 (s, 1H), 7.85 (s, 1H), 8.01 (d, 2H), 10.35 (s, -OH); MS (ESI), *m/z* 362 (M^+).

EXAMPLE 4

General synthetic procedure of:
2-(tert-butoxycarbonyl)-4- or 5-aminophenyl hydrogen carbonate (5)
3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (6)



Synthesis of 4- or 5-Amino-2-ethoxycarbonyloxy-benzoic acid (1) and 4- or 5-ethoxycarbonylamino-2-hydroxy-benzoic acid (2)

4- or 5-amino salicylic acid (3.0 mmol) was dissolved in 40 mL chloroform in round bottomed flask fitted with a drying tube. Ethyl chloroformate (3.0 mmol) was added gradually and the solution refluxed for 2 hours. The chloroform was evaporated in vacuo and the residue then taken up in ether. The ether phase was decolorised using charcoal, filtered and the solvent removed in vacuo. The residue obtained was then dissolved in ethanol and the product recovered by precipitation using n-hexane as a crude oily semisolid. The crude product was purified by Flash-chromatography on silica gel eluting with diethyl ether/hexane (7:3, v/v) to obtain the title compounds: *4-* or *5-Amino-2-ethoxycarbonyloxy-benzoic acid* (**1:** yield: 58%) *and 4- or 5-Ethoxycarbonylamino-2-hydroxy-benzoic acid* (**2:** yield: 34%).

Synthesis of 4- or 5-Amino-2-ethoxycarbonyloxy-benzoic acid tert-butyl ester (3)

To the solution of 1 (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, t-butanol (3.0 mmol) was added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9.5/0.5), from which *4-* or *5-Amino-2-ethoxycarbonyloxy-benzoic acid tert-butyl ester* (3) was obtained (55% yield).

Synthesis of 4- or 5-ethoxycarbonylamino-2-hydroxy-benzoic acid tert-butyl ester (4)

Compound **4** was obtained according the procedure reported to obtain compound **3**. Yield: 74%

Synthesis of 2-(tert-butoxycarbonyl)-4- or 5-aminophenyl hydrogen carbonate (5)

To a solution of the compound 3 (3.5 g; 0.011 mol) in ethanol (80 ml) was added NaOH 1N (40 ml). The reaction mixture was stirred 2 h at room temperature. Then the solution was made neutral with HCl 1N. Ethanol was removed and the extraction performed with ethyl acetate (3 x 150 ml); the organic layers were washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated: *2-(tert-butoxycarbonyl)-4- or 5-aminophenyl hydrogen carbonate* (5) was obtained (3 g; 0.010 mol; yield: 89 %) as a white solid.

Synthesis of 3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (6)

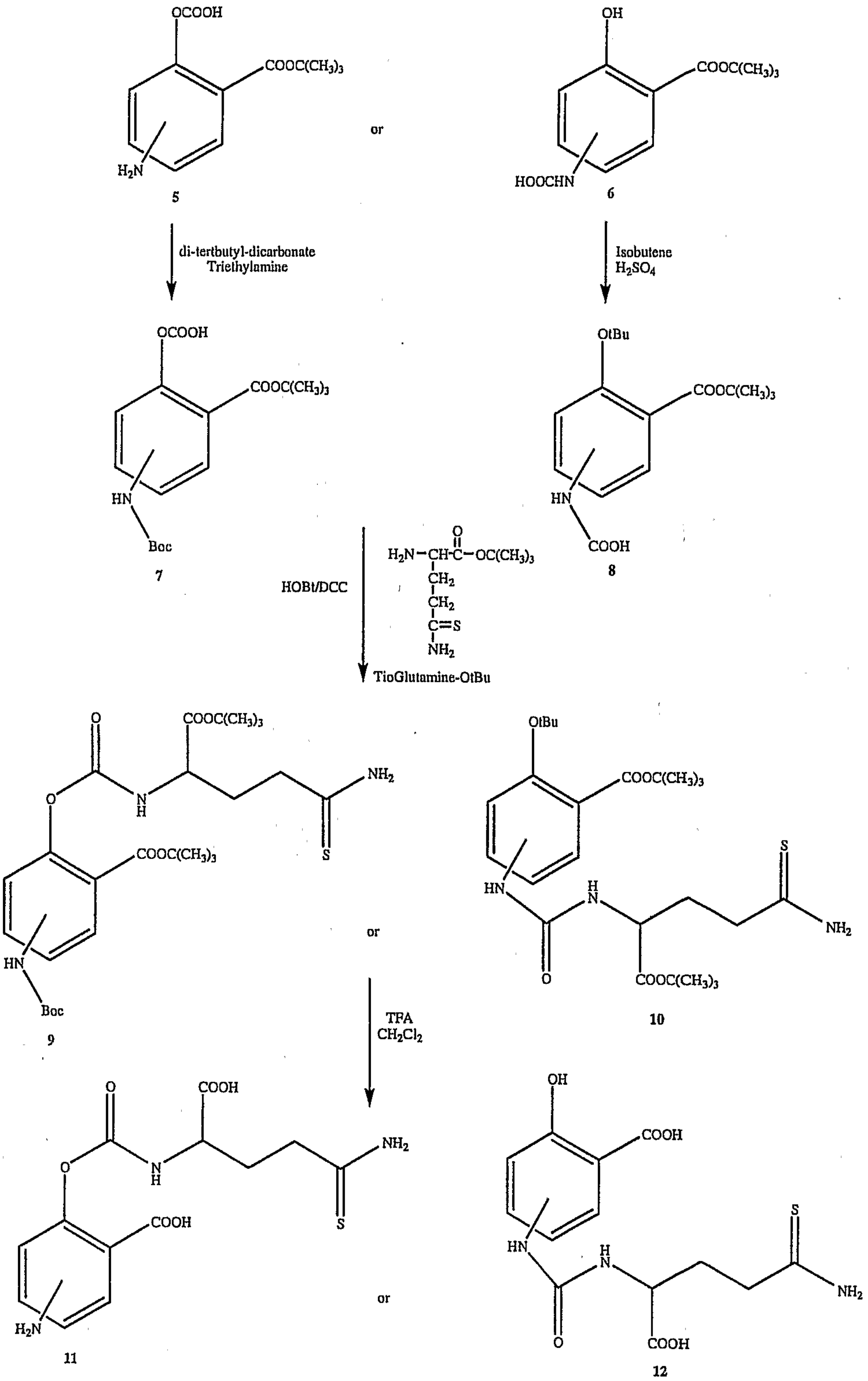
Compound 6 was obtained according the procedure reported to obtain compound 5. Yield: 91%

EXAMPLE 5

General synthetic procedure of:

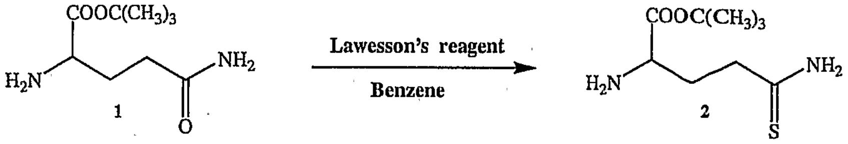
4- or 5-Amino-2-(1-carboxy-3-thiocarbamoyl-propylcarbamoyloxy)-benzoic acid (11)

4- or 5-[3-(1-Carboxy-3-thiocarbamoyl-propyl)-ureido]-2-hydroxy-benzoic acid (12)


OCOOH
COOC(CH3)3
H2N
5
or
OH
COOC(CH3)3
HOOCHN
6
di-tertbutyl-dicarbonate
Triethylamine
Isobutene
H2SO4
OCOOH
COOC(CH3)3
HN
Boc
7
OtBu
COOC(CH3)3
HN
COOH
8
HOBt/DCC
H2N–CH–C(=O)–OC(CH3)3
CH2
CH2
C=S
NH2
TioGlutamine-OtBu
O
COOC(CH3)3
NH2
O
N
H
COOC(CH3)3
S
HN
Boc
9
or
OtBu
COOC(CH3)3
S
HN
H
N
NH2
O
COOC(CH3)3
10
TFA
CH2Cl2
O
COOH
NH2
O
N
H
COOH
S
H2N
11
or
OH
COOH
S
HN
H
N
NH2
O
COOH
12

Synthesis of 5-thio-L-glutamine –OtBu (2)

L-glutamine-OtBu · HCl **1** (1.2 mmol; 0.3 g) and Lawesson's reagent (0.75 mmol; 0.3 g) were added to benzene (20 mL) and the mixture was heated under reflux for 15 min. The reaction was then cooled and evaporated under vacuum. The crude product was chromatographed over 100g of silica gel eluted with mixtures of ethyl acetate and n-hexane. There was obtained 0.2 g (76% yield) of product **2** as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H), 1.8-2.8 (m, 5H), 4.0-4.8 (m, 3H); MS (ESI), *m/z* 219(M^+).



Synthesis of 2-(tert-butoxycarbonyl)-4- or 5-tert-Butoxycarbonylaminophenyl hydrogen carbonate (7)

To the solution of **5** (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for ½ h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/ MeOH (9/1), from which *2-(tert-butoxycarbonyl)-4- or 5-tert-Butoxycarbonylaminophenyl hydrogen carbonate (7)* was obtained (80 % yield).

Synthesis of 3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (8)

Compound 6 (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (8), which was recrystallized by DCM/ hexane (83 % yield).

Synthesis of 4- or 5-Amino-2-(1-carboxy-3-thiocarbamoyl-propylcarbamoyloxy)-benzoic acid (11)

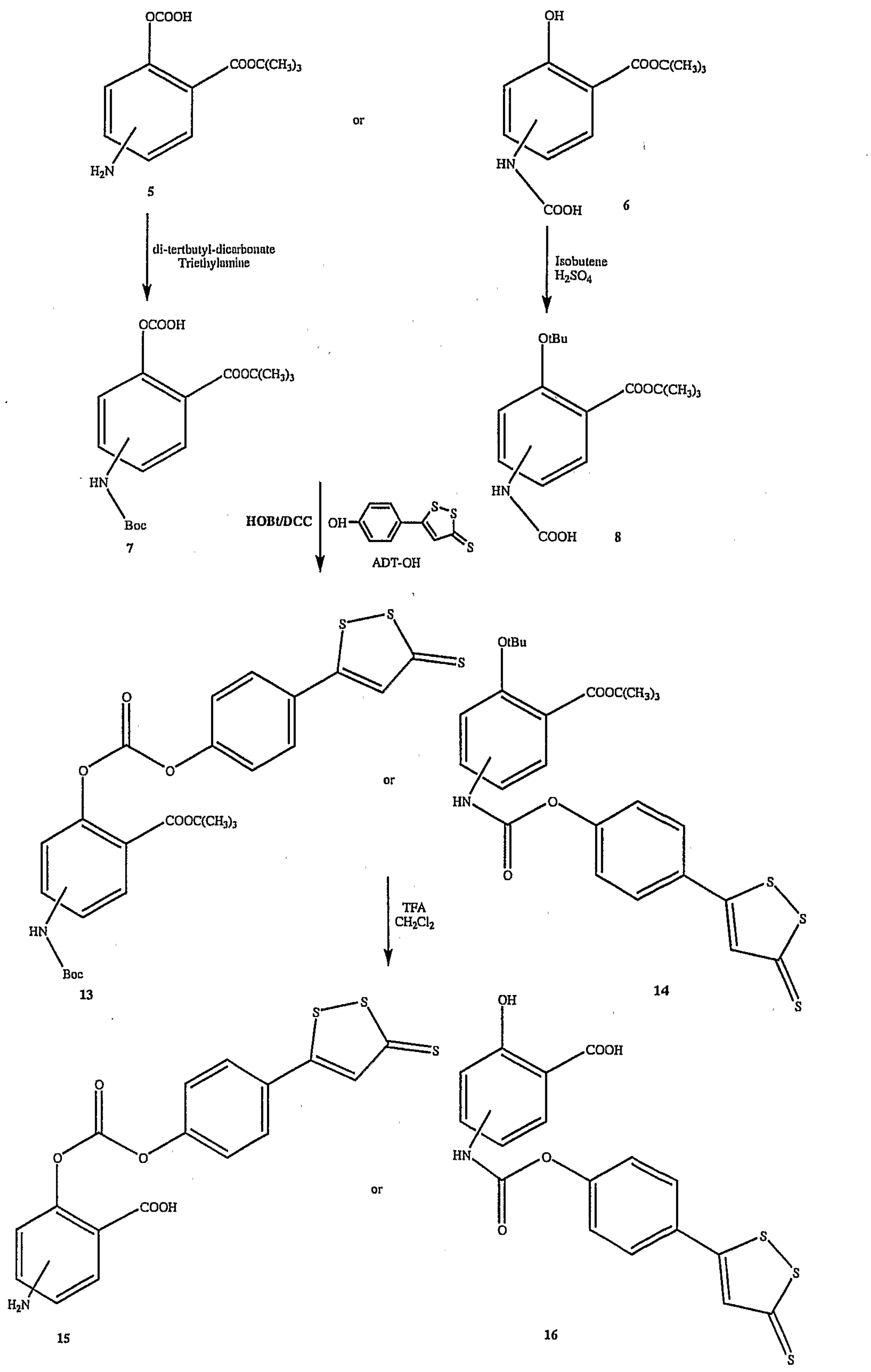
To the solution of **7** (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0° C for 1 h. To the reaction mixture, 2-amino-4-thiocarbamoyl-butyric acid *tert*-butyl ester (3.0 mmol) and triethylamine (3.0 mmol) were added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **9** was treated with a solution of 40% TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound **11** as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (8/2), from which 4- or *5-Amino-2-(1-carboxy-3-thiocarbamoyl-propylcarbamoyloxy)-benzoic acid* (**11**) was obtained (45% yield).

Synthesis of 4- or 5-[3-(1-Carboxy-3-thiocarbamoyl-propyl)-ureido]-2-hydroxy-benzoic acid (12)

Compound 12 was obtained according the procedure reported to obtain compound 11. Yield: 38%

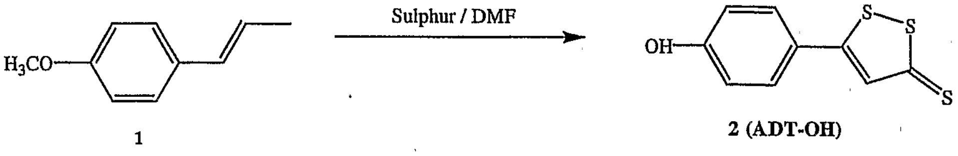
EXAMPLE 6

General synthetic procedure of:
4-or 5-Amino-2-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonyloxy]-benzoic acid (15)
2-Hydroxy-4-or 5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonylamino]-benzoic acid (16)


OCOOH
COOC(CH3)3
H2N
5
or
OH
COOC(CH3)3
HN
COOH
6
di-tertbutyl-dicarbonate
Triethylamine
Isobutene
H2SO4
OCOOH
COOC(CH3)3
HN
Boc
7
HOBt/DCC
OH
S
ADT-OH
OtBu
COOC(CH3)3
HN
COOH
8
S
O
COOC(CH3)3
HN
Boc
13
or
OtBu
HN
O
14
TFA
CH2Cl2
COOH
H2N
15
or
OH
COOH
HN
16

Synthesis of 5-p-hydroxyphenyl-1,2-dithione-3-thione (ADT-OH)

Anethole **1** (32.5 g; 0.21 mol) and sulphur (45 g; 1.40 mol) were heated in dimethylformamide (250 ml) for 8 hr; the residue after removal of solvent was almost completely soluble in toluene. An attempt to extract the toluene liquors with 2 N-aqueous sodium hydroxide, gave a precipitate of an orange solid (8.5 g). m.p. over 300°C. This product was dissolved in boiling water and gave an orange precipitate **2** after addition of hydrochloric acid (Yield 50%), m.p. 188-189 °C. ^{1}H NMR (DMSO) δ 6.86 (d, 2H), 7.68 (s, 1H), 7.75 (d, 2H), 10.51 (s, -OH); MS (ESI), *m/z* 225(M$^-$).



Synthesis of 2-(tert-butoxycarbonyl)-4- or 5-tert-butoxycarbonylaminophenyl hydrogen carbonate (7)

To the solution of **5** (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine(15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for ½ h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *2-(tert-butoxycarbonyl)-4- or 5-tert-Butoxycarbonylaminophenyl hydrogen carbonate (7)* was obtained (80 % yield).

Synthesis of 3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (8)

Compound **6** (12.0 mmol), concentrated H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid **8**, which was recrystallized by DCM/ hexane (83 % yield).

Synthesis of 4- or 5-Amino-2-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonyloxy]-benzoic acid (15)

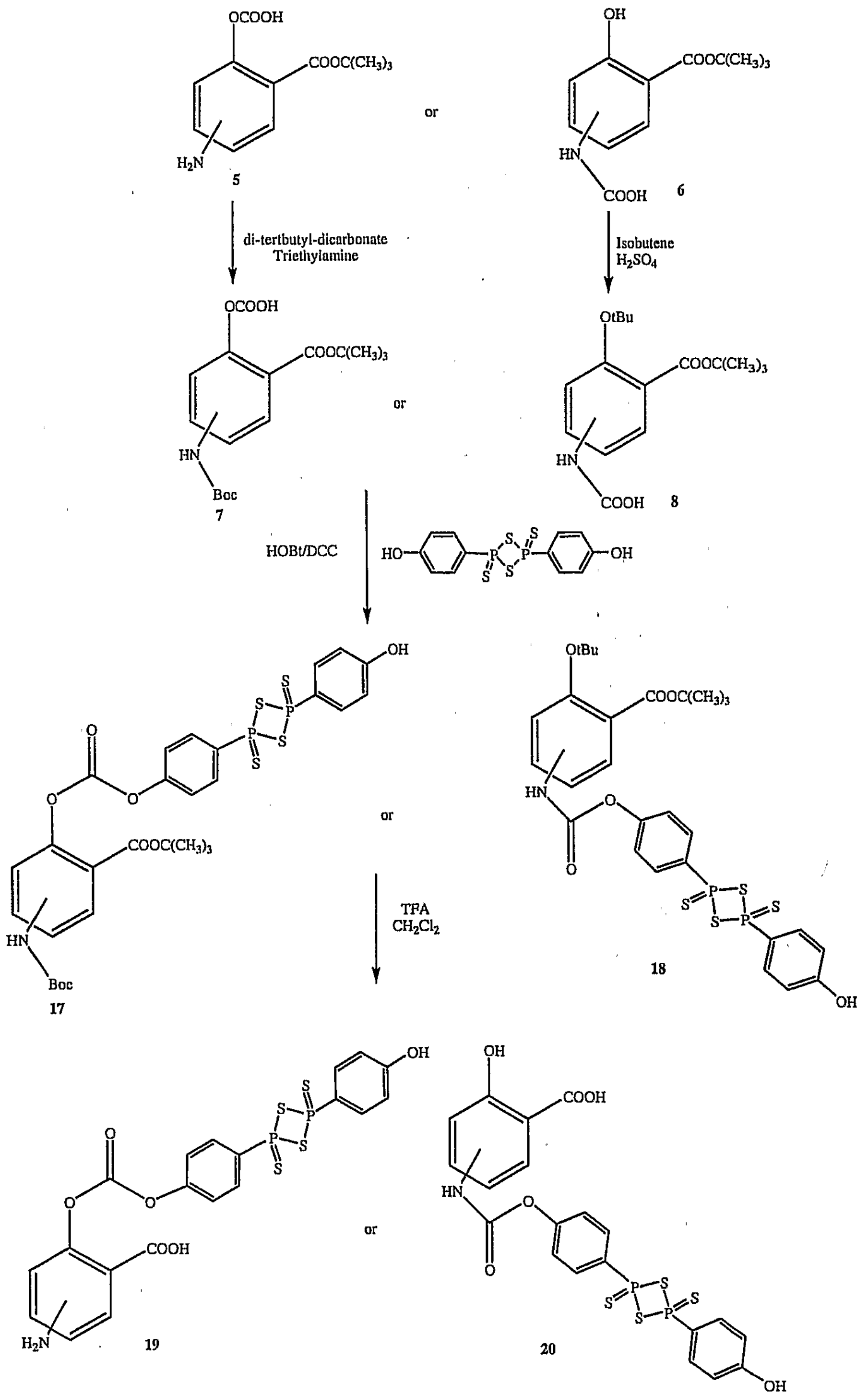
To the solution of **7** (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0° C for 1 h. To the reaction mixture, 5-*p*-hydroxyphenyl-1,2-dithione-3-thione (ADT-OH) (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **13** was treated with a solution of 40% TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound **15** as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4- or 5-Amino-2-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonyloxy]-benzoic acid* (**15**) was obtained (45% yield).

Synthesis of 2-Hydroxy-4- or 5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonylamino]-benzoic acid (16)

Compound 16 was obtained according the procedure reported to obtain compound 15. Yield: 38%

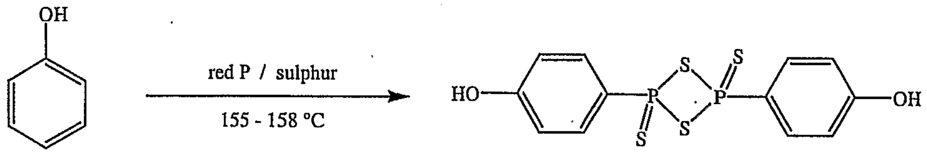
EXAMPLE 7

General synthetic procedure of:
4- or 5-Amino-2-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonyloxy}-benzoic acid (19)
2-Hydroxy-4- or 5-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo--2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonylamino}-benzoic acid (20)


OCOOH
COOC(CH3)3
H2N
5
or
OH
COOC(CH3)3
HN
COOH
6
di-tertbutyl-dicarbonate
Triethylamine
Isobutene
H2SO4
OCOOH
COOC(CH3)3
HN
Boc
7
or
OtBu
COOC(CH3)3
HN
COOH
8
HOBt/DCC
HO
S
S
S
S
P
P
OH
OH
O
O
O
COOC(CH3)3
HN
Boc
17
or
TFA
CH2Cl2
OtBu
COOC(CH3)3
HN
O
O
18
OH
OH
O
O
O
COOH
H2N
19
or
OH
COOH
HN
O
O
20
OH

Synthesis of (p-hydroxyphenyl)dithiophosphonic anhydride

Red P (4 g; 0.129 mol), S (4 g; 0.125 mol) and PhOH (4 g; 0.042 mol) were heated for 5.5 hr at 155-158 °C; the reaction mixture was cooled at room temperature and a precipitate was collected (5.5 g 34% yield). m.p. 224-226 °C. The NMR and MS analysis are consistent with *p*-hydroxyphenyl dithiophosponic anhydride.



Synthesis of 2-(tert-butoxycarbonyl)-4- or 5-tert-butoxycarbonylaminophenyl hydrogen carbonate (7)

To the solution of **5** (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for ½ h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mLl), was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *2-(tert-butoxycarbonyl)-4- or 5-tert-Butoxycarbonylaminophenyl hydrogen carbonate (7)* was obtained (80 % yield).

Synthesis of 3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (8)

Compound **6** (12.0 mmol), concentrated H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutene gas (5 psi) for 6 h at rt. The solution was washed with cold

10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid **8**, which was recrystallized by DCM/ hexane (83 % yield).

Synthesis of 4- or 5-Amino-2-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonyloxy}-benzoic acid (19)

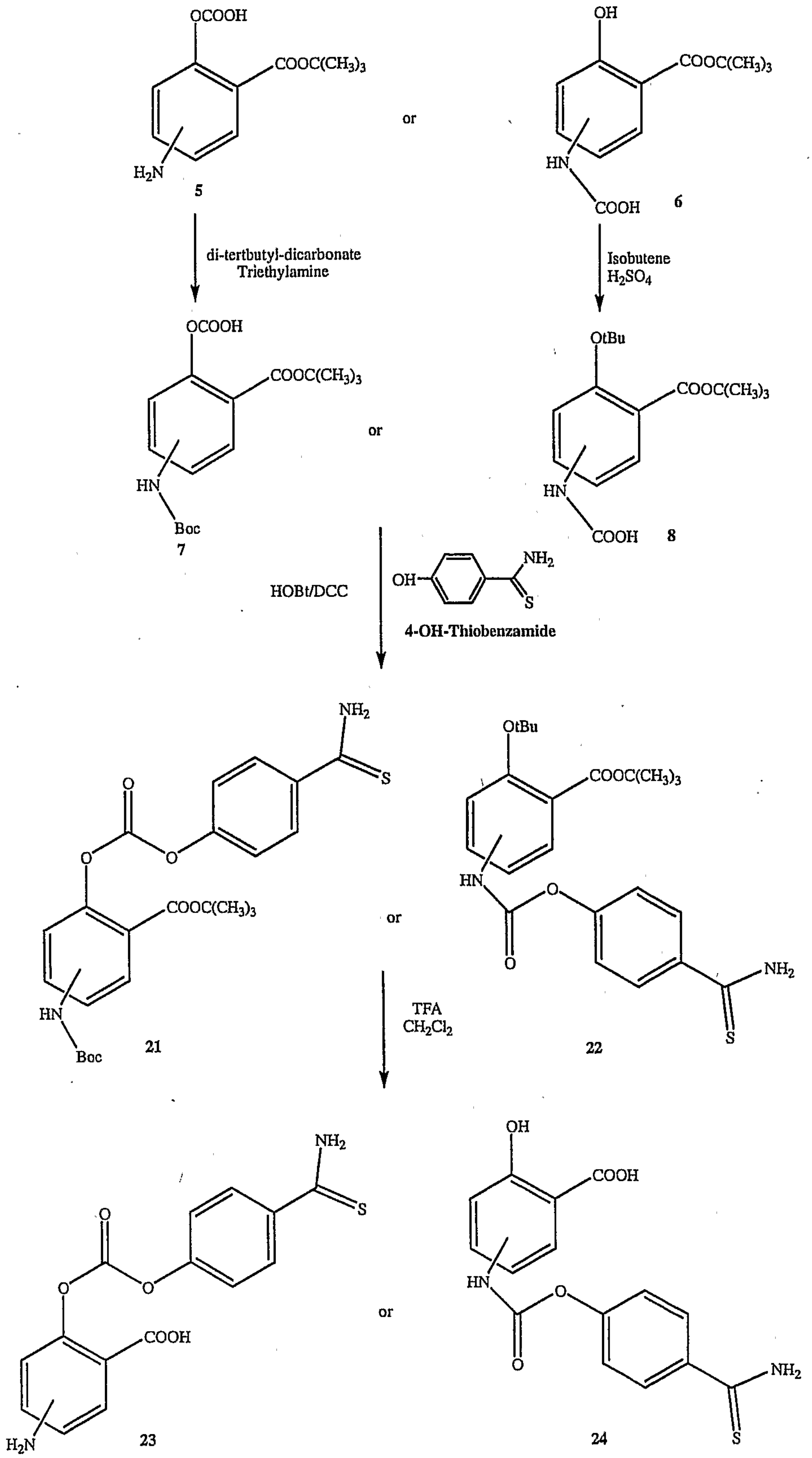
To the solution of 7 (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0° C for 1 h. To the reaction mixture, p-hydroxyphenyl)dithiophosphonic anhydride (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 17 was treated with a solution of 40% TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 19 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4- or 5-Amino-2-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2□^{5},4□^{5}-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarboyloxy}-benzoic acid* (**19**) was obtained (65% yield).

Synthesis of 2-Hydroxy-4- or 5-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo--2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonylamino}-benzoic acid (20)

Compound 20 was obtained according the procedure reported to obtain compound 19. Yield: 48%

EXAMPLE 8

General synthetic procedure of:
4- or 5-Amino-2-(4-thiocarbamoyl-phenoxycarbonyloxy)-benzoic acid (23)
2-Hydroxy-4- or 5-(4-thiocarbamoyl-phenoxycarbonylamino)-benzoic acid (24)


OCOOH
COOC(CH3)3
H2N
5
or
OH
COOC(CH3)3
HN
COOH
6
di-tertbutyl-dicarbonate
Triethylamine
Isobutene
H2SO4
OCOOH
COOC(CH3)3
HN
Boc
7
or
OtBu
COOC(CH3)3
HN
COOH
8
HOBt/DCC
OH
NH2
S
4-OH-Thiobenzamide
NH2
S
O
O
O
COOC(CH3)3
HN
Boc
21
or
OtBu
COOC(CH3)3
HN
O
O
NH2
S
22
TFA
CH2Cl2
NH2
S
O
O
O
COOH
H2N
23
or
OH
COOH
HN
O
O
NH2
S
24

Synthesis of 2-(tert-butoxycarbonyl)-4- or 5-tert-butoxycarbonylaminophenyl hydrogen carbonate (7)

To the solution of **5** (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine(15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for ½ h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/ MeOH (9/1), from which *2-(tert-butoxycarbonyl)-4- or 5-tert-Butoxycarbonylaminophenyl hydrogen carbonate (7)* was obtained (80 % yield).

Synthesis of 3-(tert-butoxycarbonyl)-4- or 5-hydroxyphenyl-carbamic acid (8)

Compound **6** (12.0 mmol), concentrated H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid **8**, which was recrystallized by DCM/ hexane (83 % yield).

Synthesis of 4- or 5-Amino-2-(4-thiocarbamoyl-phenoxycarbonyloxy)-benzoic acid (23)

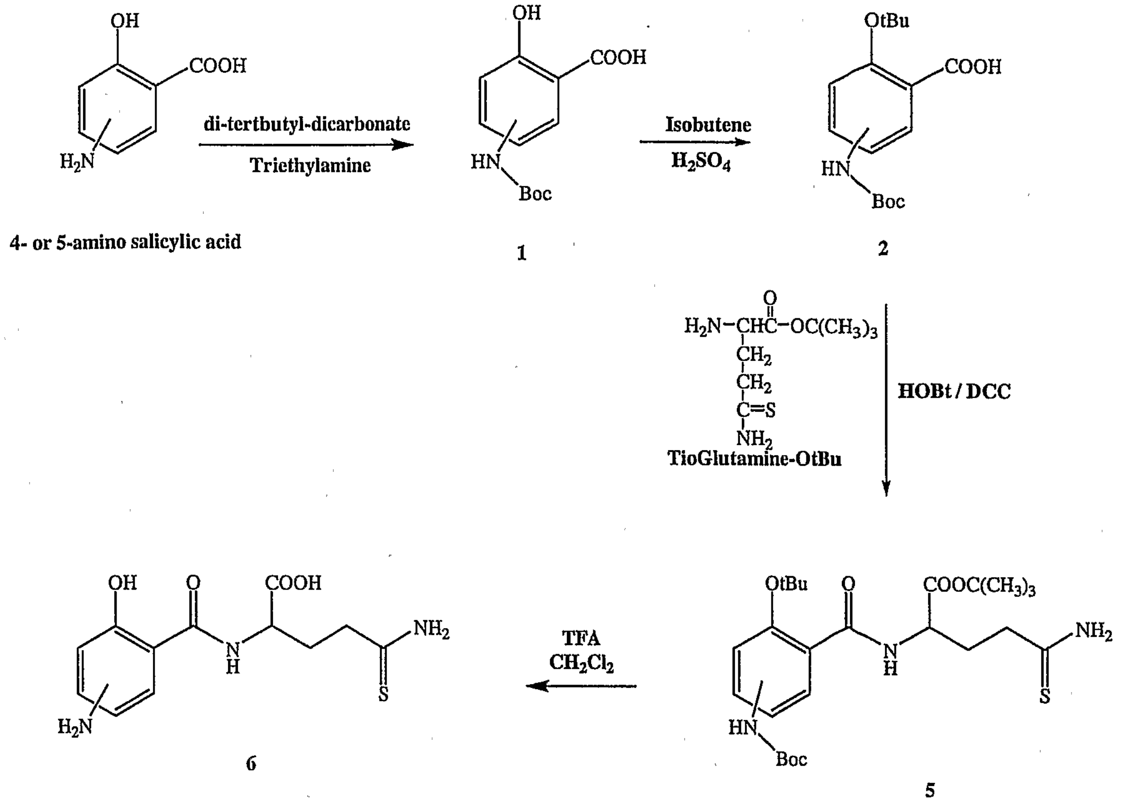
To the solution of **7** (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0° C for 1 h. To the reaction mixture, 4-hydroxy-thiobenzamide (3.0 mmol) was added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **21** was treated with a solution of 40% TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound **23** as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (8/2), from which *4- or 5-Amino-2-(4-thiocarbamoyl-phenoxycarbonyloxy)-benzoic acid* (**23**) was obtained (71% yield).

Synthesis of 2-Hydroxy-4- or 5-(4-thiocarbamoyl-phenoxycarbonylamino)-benzoic acid (24)

Compound 24 was obtained according the procedure reported to obtain compound 23. Yield: 68%

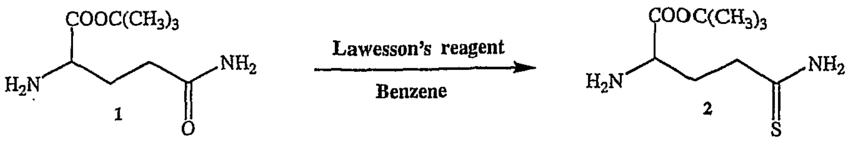
EXAMPLE 8

General synthetic procedure of:
2-(4- or 5-Amino-2-hydroxy-benzoylamino)-4-thiocarbamoyl-butyric acid (6)



Synthesis of 5-thio-L-glutamine –OtBu (2)

L-glutamine-OtBu · HCl **1** (1.2 mmol; 0.3 g) and Lawesson's reagent (0.75 mmol; 0.3g) were added to benzene (20 mL) and the mixture was heated under reflux for 15 min. The reaction was then cooled and evaporated under vacuum. The crude product was chromatographed over 100 g of silica gel eluted with mixtures of ethyl acetate and n-hexane. There was obtained 0.2 g (76% yield) of product **2** as a white solid: ^1H NMR ($CDCl_3$) δ 1.4 (s, 9H), 1.8-2.8 (m, 5H), 4.0-4.8 (m, 3H); MS (ESI), *m/z* 219(M^+).



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-tert-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL), was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (1) was obtained (80 % yield).

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2- tert-butoxy-benzoic acid (2)

Compound 1 (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (2), which was recrystallized by DCM/hexane (83 % yield).

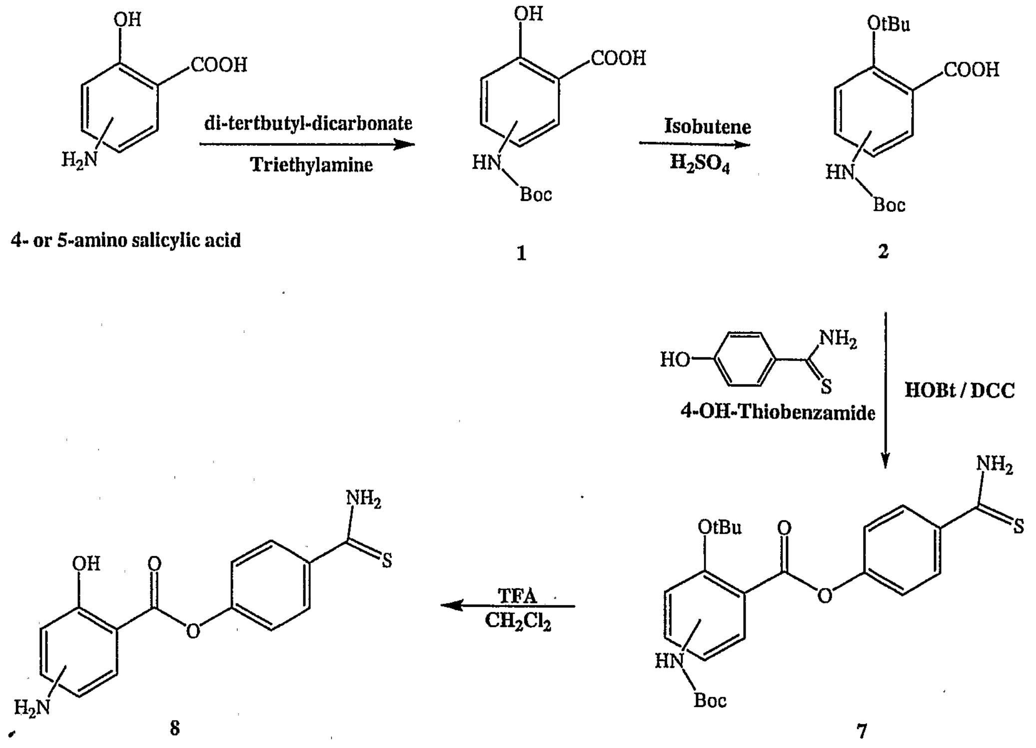
Synthesis of 2-(4- or 5-amino-2-hydroxy-benzoylamino)-4-thiocarbamoyl-butyric acid (6)

To the solution of 4- or 5-tert-butoxycarbonylamino-2-tert-butoxy-benzoic acid (2) (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, 2-amino-4-thiocarbamoyl-butyric acid tert-butyl ester (3.0 mmol) and triethylamine (3.0 mmol) were added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layers were washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 5 was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 6 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *2-(4- or 5-Amino-2-hydroxy-benzoylamino)-4-thiocarbamoyl-butyric acid* (6) was obtained (80% yield). MS (ESI), m/z 298 (M^+).

EXAMPLE 9

General synthetic procedure of:

4- or 5-Amino-2-hydroxy-benzoic acid 4-thiocarbamoyl-phenyl ester (8)



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (80 % yield).

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2- tert-butoxy-benzoic acid (2)

Compound **1** (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (**2**), which was recrystallized by DCM/ hexane (83 % yield).

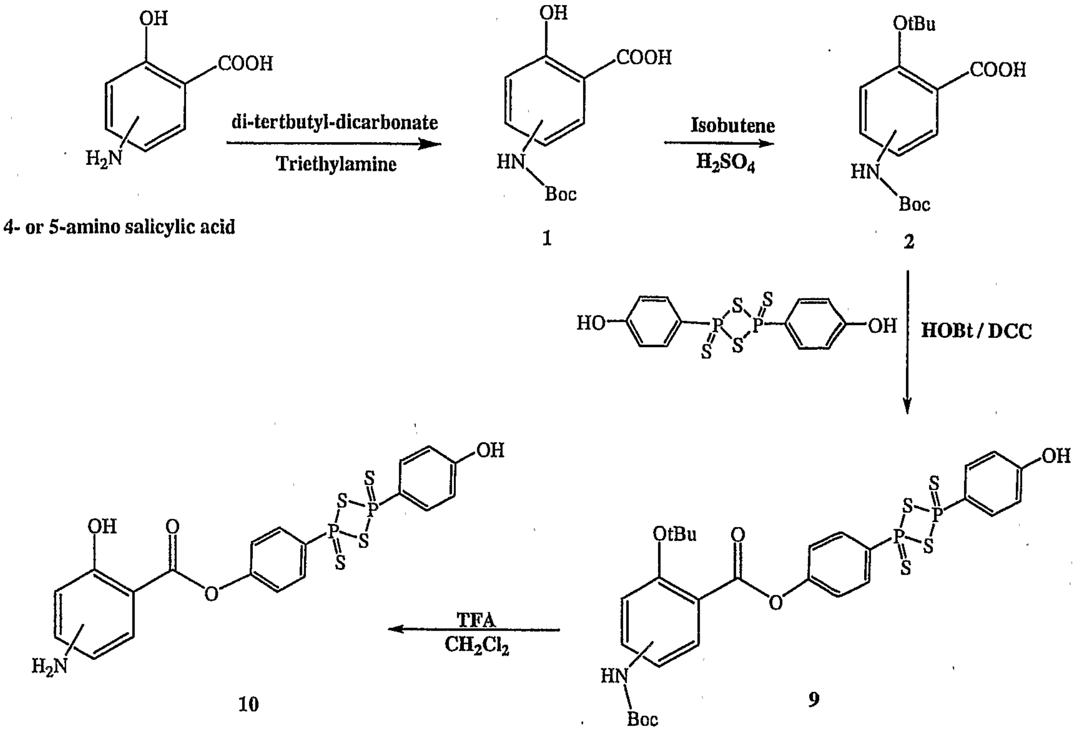
Synthesis of 4- or 5-Amino-2-hydroxy-benzoic acid 4-thiocarbamoyl-phenyl ester (8)

To the solution of 4- or 5-*tert*-butoxycarbonylamino-2-hydroxy-benzoic acid (**2**) (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, 4-hydroxy-thiobenzamide (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **7** was treated with a solution of 40% TFA in CH_2Cl_2. After 2 h the solvent was removed to obtain compound **8** as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4- or 5-Amino-2-hydroxy-benzoic acid 4-thiocarbamoyl-phenyl ester* (**8**) was obtained (48% yield).

EXAMPLE 10

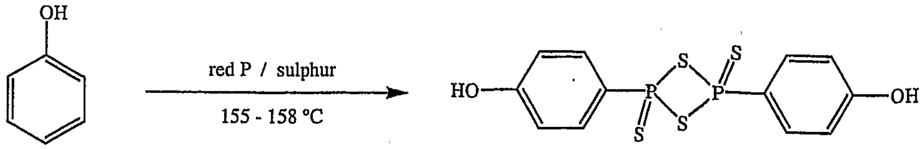
General synthetic procedure of:

4- or 5-Amino-2-hydroxy-benzoic acid 4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenyl ester (10)



Synthesis of (p-hydroxyphenyl)dithiophosphonic anhydride

Red P (4 g; 0.129 mol), S (4 g; 0.125 mol) and PhOH (4 g; 0.042 mol) were heated for 5.5 hr at 155-158°C; the reaction mixture was cooled at room temperature and a precipitate was collected (5.5 g, 34% yield). m.p. 224-226°C. The NMR and MS analysis are consistent with *p*-hydroxyphenyl dithiophosponic anhydride.



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-*tert*-butyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (80 % yield).

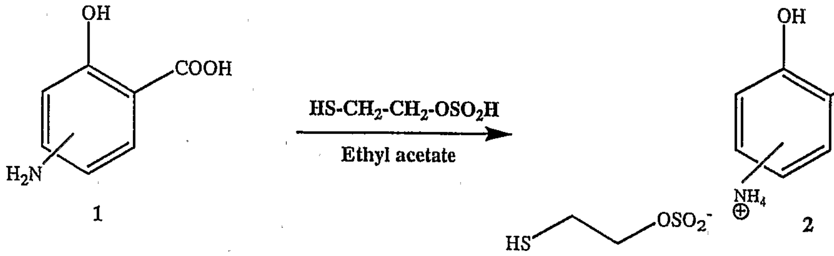
Synthesis of 4- or 5-tert-Butoxycarbonylamino-2- tert-butoxy-benzoic acid (2)

Compound **1** (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (**2**), which was recrystallized by DCM/hexane (83 % yield).

Synthesis of 4- or 5-Amino-2-hydroxy-benzoic acid 4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenyl ester (10)

To the solution of 4- or 5-tert-butoxycarbonylamino-2-tert-butoxy-benzoic acid (**2**) (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, p-hydroxyphenyl)dithiophosphonic anhydride (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 9 was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 10 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (8/2), from which *4- or 5-amino-2-hydroxy-benzoic acid 4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenyl ester* (**10**) was obtained (73% yield).

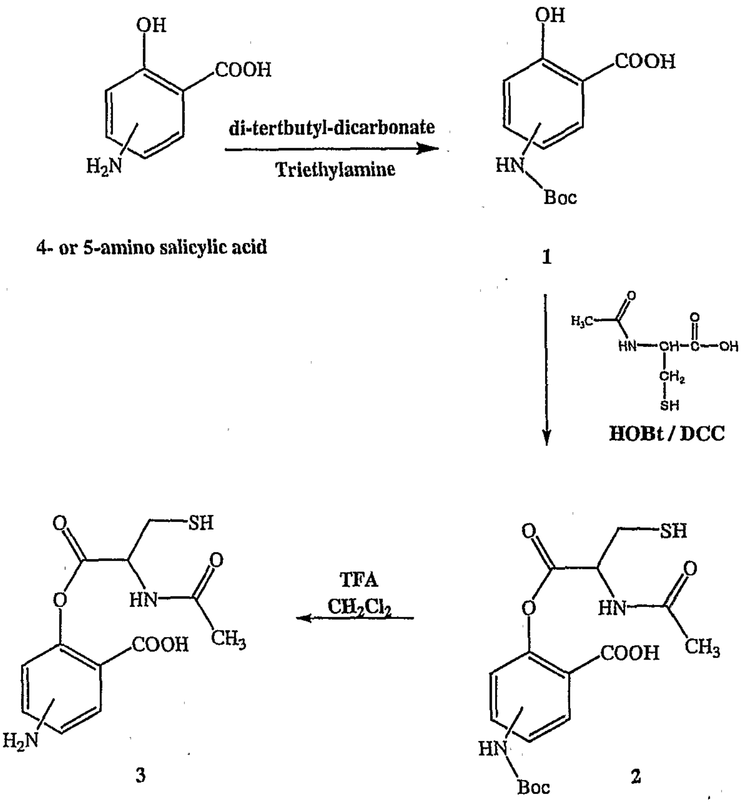
Synthesis of 4- or 5- amino-2-hydroxy-benzoic acid mercaptoethanesulfonate (2)



A mixture of sulfurous acid 2-mercapto-ethyl ester (0.1 mol) in 100ml of ethyl acetate was added to the 4- or 5-aminosalicylic acid **1** (0.1 mole in 100ml of ethyl acetate) solution in 30-45 min at 20-25°C under an inert atmosphere. Then the mixture was stirred at 0-5°C for 1 hour and filtered to give *4- or 5- amino-2-hydroxy-benzoic acid mercaptoethanesulfonate **2*** (yield: 98%).

EXAMPLE 11

Synthesis of 4 or 5-amino -2-(2-Acetylamino-3-mercapto-propionyloxy)-benzoic acid (3)



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

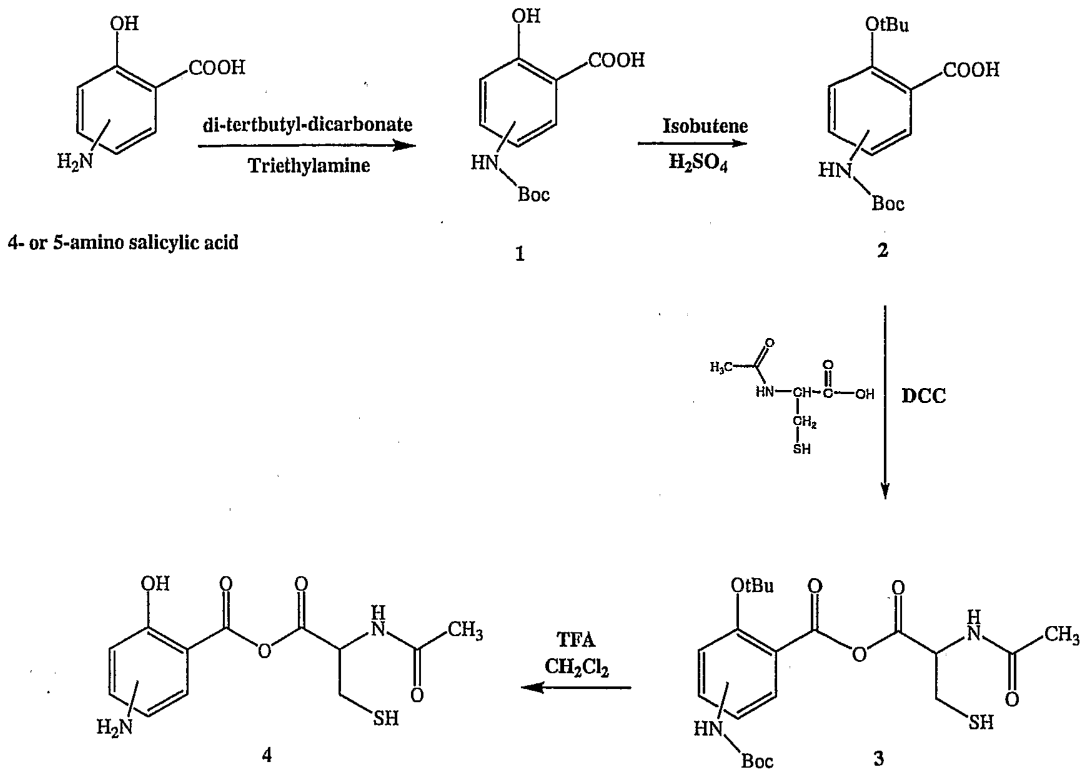
To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-tertbutyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3 M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (80 % yield).

Synthesis of 4 or 5-amino -2-(2-Acetylamino-3-mercapto-propionyloxy)-benzoic acid (3)

To the solution of 2-acetylamino-3-mercapto-propionic acid (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (**2**) (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **2** was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 3 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4 or 5-amino -2-(2-Acetylamino-3-mercapto-propionyloxy)-benzoic acid* (**3**) was obtained (52 % yield).

EXAMPLE 12

Synthesis of *4 or 5-Amino-2-hydroxy-benzoic acid anhydride with 2-Acetylamino-3-mercapto- propionic acid (4)*



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-tertbutyl-dicarbonate (15.0 mmol) were added with stirring at 0° C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL), was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (1) was obtained (80 % yield).

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-tert-butoxy-benzoic acid (2)

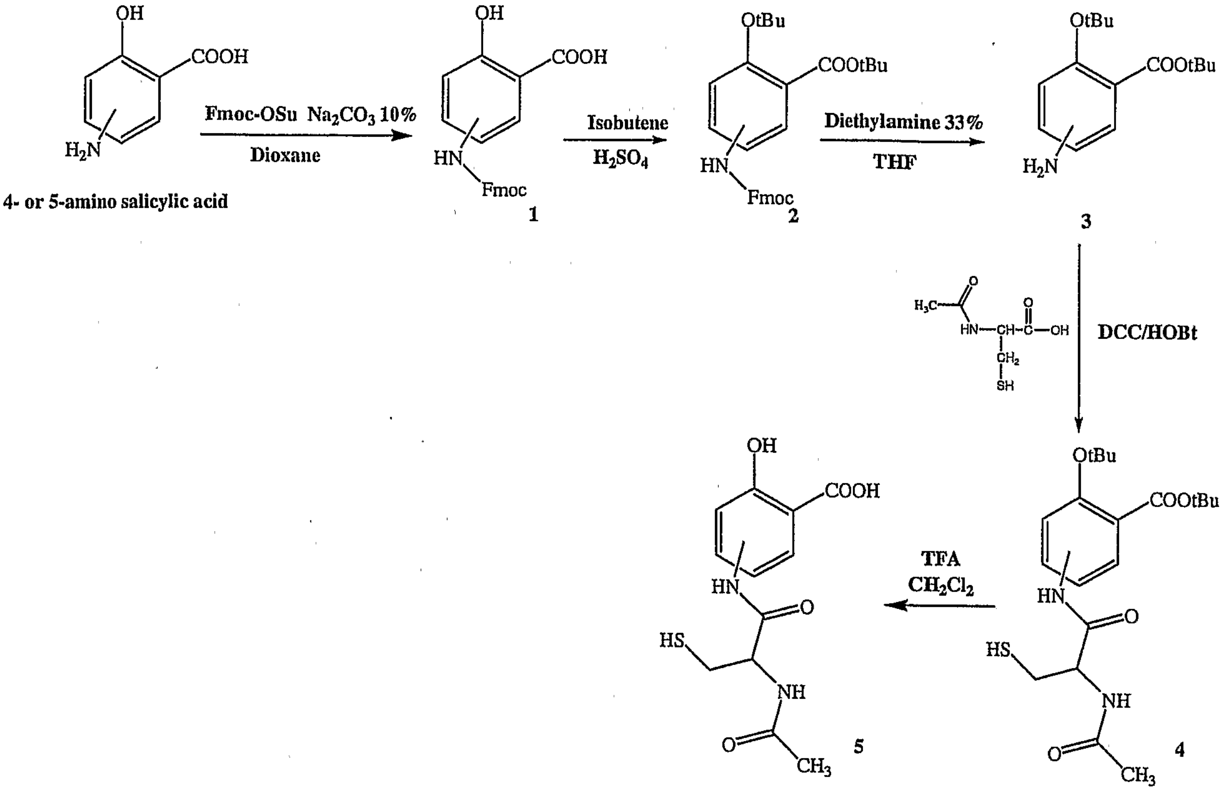
Compound 1 (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (2), which was recrystallized by DCM/hexane (83 % yield).

Synthesis of 4 or 5-Amino-2-hydroxy-benzoic acid anhydride with 2-Acetylamino-3-mercapto-propionic acid (4)

To the solution of 4- or 5-tert-butoxycarbonylamino-2-tert-butoxy-benzoic acid (2) (3.0 mmol) in 50 mL of dimethylformamide, DCC (3.3 mmol) was added with stirring at 0°C for 1 h. To the reaction mixture, 2-acetylamino-3-mercapto-propionic acid (3.0 mmol) was added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layers were washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 3 was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 4 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (8/2), from which *4 or 5-Amino-2-hydroxy-benzoic acid anhydride with 2-Acetylamino-3-mercapto-propionic acid* (4) was obtained (68 % yield).

EXAMPLE 13

Synthesis of 4 or 5-(2-Acetylamino-3-mercapto-propionylamino)-2-hydroxy-benzoic acid (5)



Synthesis of 4 or 5-(9H-Fluoren-9-ylmethoxycarbonylamino)-2-hydroxy-benzoic acid (1)

To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, Na_2CO_3 10 % (15 mL) and Fmoc-OSu (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (90 % yield).

Synthesis of 4 or 5-Amino-2-tert-butoxy-benzoic acid tert-butyl ester (3)

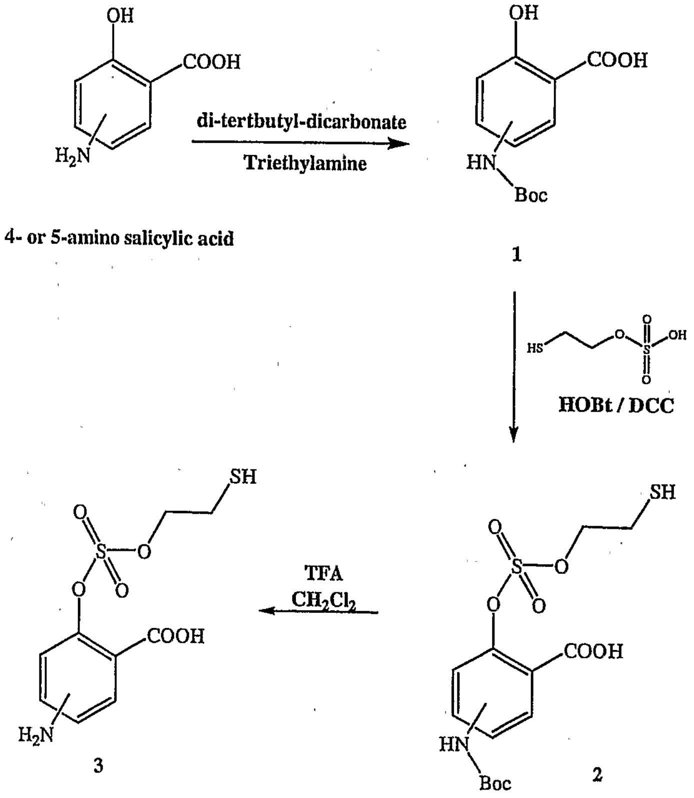
Compound **1** (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (7 psi) for 24 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/ water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (**2**). The crude intermediate **2** was treated with a solution of diethylamine (33%) in THF. After 2 h the solvent was removed to obtain compound **3** as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2 / MeOH (8/2), from which *4 or 5-Amino-2-tert-butoxy-benzoic acid tert-butyl ester **(3)*** was obtained (67 % yield).

Synthesis of 4 or 5-(2-Acetylamino-3-mercapto-propionylamino)-2-hydroxy-benzoic acid (5)

To the solution of 2-acetylamino-3-mercapto-propionic acid (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, 4 or 5-amino-2-tert-butoxy-benzoic acid tert-butyl ester **(3)** (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 4 was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 5 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4 or 5-(2-Acetylamino-3-mercapto-propionylamino)-2-hydroxy-benzoic acid* (5) was obtained (78% yield).

EXAMPLE 14

Synthesis of 4 or 5-Amino-2-(2-mercapto-ethoxysulfonyloxy)-benzoic acid (3)



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

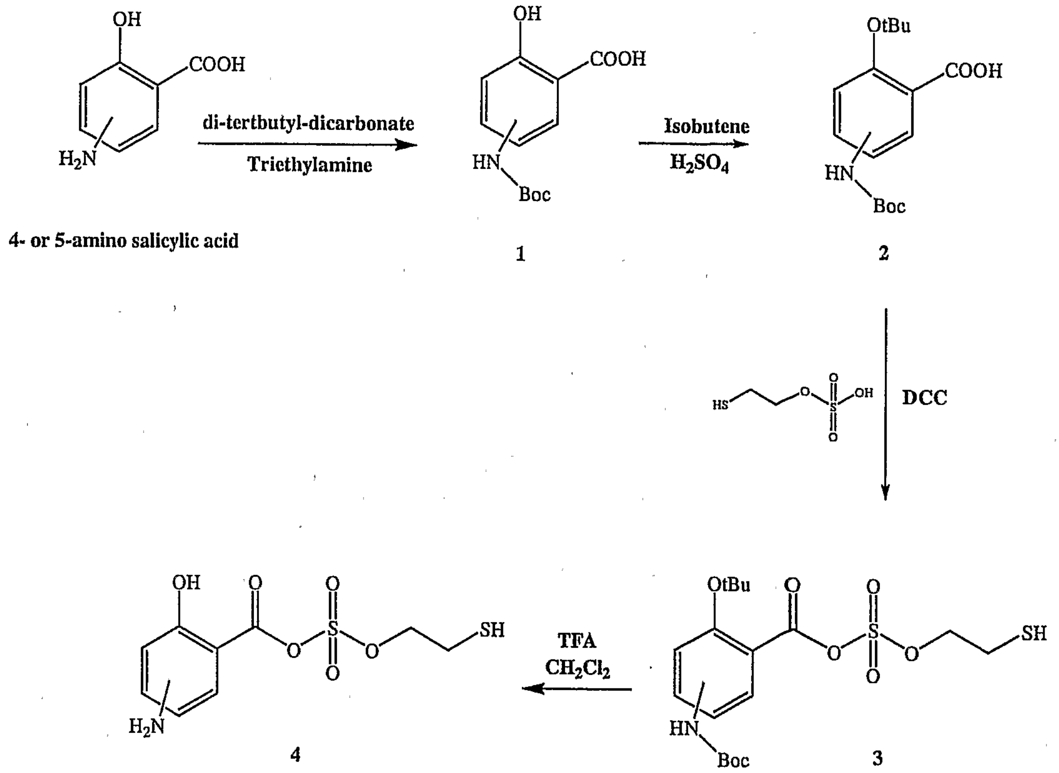
To the solution of 4- or 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-tertbutyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (**1**) was obtained (80 % yield).

Synthesis of: 4 or 5-Amino-2-(2-mercapto-ethoxysulfonyloxy)-benzoic acid (3)

To the solution of sulfuric acid mono-(2-mercapto-ethyl) ester (3.0 mmol) in 50 mL of dimethylformamide, hydroxybenzotriazole (3.3 mmol) and DCC (3.3 mmol) were added with stirring at 0°C for 1 h. To the reaction mixture, 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (**2**) (3.0 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate **2** was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 3 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH (8/2), from which *4 or 5-Amino-2-(2-mercapto-ethoxysulfonyloxy)-benzoic acid* (**3**) was obtained (57 % yield).

EXAMPLE 15

Synthesis of 4 or 5-Amino-2-hydroxy-benzoic acid anhydride with Sulfuric acid mono-(2-mercapto-ethyl) ester (4)



Synthesis of 4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid (1)

To the solution of 5-amino salicylic acid (10.0 mmol) in 25 mL of dioxane and 12.5 mL of water, triethylamine (15.0 mmol) and di-tertbutyl-dicarbonate (15.0 mmol) were added with stirring at 0°C for 1/2 h. The reaction mixture was stirred mechanically for 24 h at room temperature. After evaporation of the solvent, 3M HCl (15 mL) was added dropwise to the residue. The precipitate is filtered, washed with water and dried. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH

(9/1), from which *4- or 5-tert-Butoxycarbonylamino-2-hydroxy-benzoic acid* (1) was obtained (80 % yield).

Synthesis of 4- or 5-tert-Butoxycarbonylamino-2- tert-butoxy-benzoic acid (2)

Compound **1** (12.0 mmol), conc. H_2SO_4 (6.0 mmol), and DCM (100 mL) were stirred under isobutylene gas (5 psi) for 6 h at rt. The solution was washed with cold 10% $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 1:1 MeOH/CCl_4 (400 mL), washed with water (300 mL), and then extracted with 1:1 MeOH/water (2 x 200 mL). The extract was dried (Na_2SO_4) and evaporated to a white solid (**2**), which was recrystallized by DCM/ hexane (83 % yield).

Synthesis of 4 or 5-Amino-2-hydroxy-benzoic acid anhydride with Sulfuric acid mono-(2-mercapto-ethyl) ester (4)

To the solution of 4- or 5-tert-Butoxycarbonylamino-2-tert-butoxy-benzoic acid (2) (3.0 mmol) in 50 mL of dimethylformamide, DCC (3.3 mmol) was added with stirring at 0° C for 1 h. To the reaction mixture, sulfuric acid mono-(2-mercapto-ethyl) ester (3.0 mmol) was added and stirred mechanically for 3 h at 0° C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained was dissolved in ethyl acetate; the organic layers were washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. The crude intermediate 3 was treated with a solution of TFA (40%) in CH_2Cl_2. After 2 h the solvent was removed to obtain compound 4 as a crude residue. The residue was loaded on a silica gel open column and eluted with CH_2Cl_2/MeOH

(8/2), from which *4 or 5-Amino-2-hydroxy-benzoic acid anhydride with sulfuric acid mono-(2-mercapto-ethyl) ester* (4) was obtained (68 % yield).

EXAMPLE 16

Dose-Ranging Study of 2-hydroxy-5-amino-benzoic acid 4-(thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester hydrochloride (Compound XXXV) in TNBS-Induced Colitis in Mice

A standard experimental animal model of colitis induced by intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) to mice is used in the following example. A detailed description of this model has been published (Santucci *et al.* (2003) *Gastroenterology* **124**:1381-94) and is incorporated herein by reference. Briefly, 6-8 week old Balb/c mice were given TNBS intracolonically at a dose of 1.5 mg in 0.1 mL of 30% ethanol. The mice were randomized to the various treatment groups (n=6 per group). Beginning one hour later and continuing every 12 h for 5 days, the mice were treated orally with vehicle (1% carboxymethylcellulose (CMC)), mesalamine (25, 50 or 75 mg/kg) or with equimolar doses of 2-hydroxy-5-amino-benzoic acid 4-(thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester hydrochloride (Compound XXXV). The mice were evaluated (blindly) on the final day of the study for the presence of diarrhea and fecal occult blood, and their body weights were measured. A "disease activity score" was calculated based on these data (0 to 4 scale, as outlined in the paper cited above). After sacrifice, a sample of the colon was excised for measurement of myeloperoxidase (MPO) activity, as a marker of granulocyte infiltration.

The results for the disease activity score and MPO activity are shown in FIG. 1 and FIG. 2, respectively. FIG. 1 shows that Compound XXXV was superior to mesalamine in reducing the activity score at equimolar doses of 50 mg/kg and 75 mg/kg. Further, MPO activity was significantly reduced (almost in half) at the highest doses tested.

EXAMPLE 17

Effects of Mesalamine and Compound XXXV in TNBS-Induced Colitis in Mice

The same model was used as described above. In this example, the effects of mesalamine (50 mg/kg) were compared to those of equimolar doses of Compound XXXV. In addition to measuring the severity of colitis by measuring disease activity score and MPO activity, tissues were processed for measurement of a number of genes for inflammatory cytokines and other mediators.

In particular, mRNA expression in mice of tumour necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), colonic interleukin (IL)-1, IL-2, IL-10, IL-12 p40, RANTES, cyclooxygenase (COX)-1, COX-2, constitutive endothelial nitric oxide synthase (eNOS), and inducible NOS (iNOS) was measured as described in Wallace *et al.* (1999) *Gastroenterology* **117**: 557-566, incorporated herein by reference.

Briefly, reverse transcription-polymerase chain reaction (RT-PCR) was used to detect and quantify mRNA of the particular cytokine/chemokine/enzyme. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the "housekeeping gene" for mRNA expression (*i.e.*, as an internal control). For each sample, the ratio of the amplification of the target gene to the amplification of GAPDH (expression of each is measured by performing densitometry on gels) was obtained. Comparisons were then made between the relative amplification (expression) of the target gene in tissues for the treatment groups in comparison to the expression in tissues from healthy controls. Thus, the data shown in the following FIGS. represent the relative expression of the target gene (normalized to GAPDH expression) as a ratio to the expression in healthy controls.

The results are shown in FIGS. 3 to 10. It is noteworthy that Compound XXXV was superior to mesalamine in every endpoint. It is particularly interesting that Compound XXXV suppressed expression of mRNA for several pro-inflammatory cytokines and chemokines that have been implicated in the pathogenesis of inflammatory bowel disease. However, Compound XXXV did not suppress expression of IL-10 mRNA, which is an anti-inflammatory cytokine.

In addition, Compound XXXV suppressed both COX-1 and COX-2 mRNA. COX-1 and COX-2 are involved in the synthesis of prostaglandins, which are important in inflammation. Further, Compound XXXV also suppressed eNOS and iNOS mRNA. Both eNOS and iNOS have been implicated in diseases of the GI tract

EXAMPLE 18

Comparison of the Effects of Compound XXXV versus Mesalamine in Inhibiting Viability of HT-29 Human Colon Cancer Cells *In Vitro*

HT-29 cells were grown in culture using standard methods. The cells were exposed to vehicle (DMSO), mesalamine or Compound XXXV. Concentrations ranging from 0.1 to 10 μM were tested, with each concentration tested in 6 wells. At the end of 72 h of exposure to the test drugs, cell viability was measured using the MTT [3-(4,5-dimethylthiaxol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Carmichael *et al.* (1978)

Cancer Res. **47,** 936–942), incorporated herein by reference. Cell viability rates were calculated as a percent of the vehicle (DMSO)-treated cells and results are given in Table 1.

Table 1

Concentrations (μM)

Drug	**0.1**	**1**	**10**
Mesalamine	103.8 ± 2.5	101.2 ± 3.5	91.1 ± 3.2
XXXV	88.4 ± 2.9**	87.3 ± 2.3**	79.6 ± 1.9**

**$p<0.01$ versus the mesalamine-treated group (same concentration)

EXAMPLE 19

Comparison of the Effects of Compound XXXV versus Mesalamine in a Rat Model of Visceral Pain Perception

A rat model of visceral pain perception, a pre-clinical model of irritable bowel syndrome, was used in the following example. Rats (male, Wistar, 200-250 g, obtained from Charles River, Monza, Italy), were housed in plastic cages and maintained under controlled conditions with 12-hours light/dark cycle with lights on at 7.00 AM. Tap water and standard laboratory chow were freely available. Before experiments, rats were individually trained by spending 2-3 hours per day in a plexiglass cage for 2-3 days. It allowed them to adjust to a movement-restriction environment. Food was withheld for 12 hours before colorectal distension (CRD) recording were performed. Experiments were performed in awake rats and were conducted in a blind manner in that the observer was not aware of the identity of drug administered to each animal.

In the testing day, rats were sedated with ether inhalation and a 2 cm long latex balloon was inserted intrarectally 2 cm from the anal verge and fixed at the base of the tail. The balloon was connected via a double-barreled cannula to a pressure transducer to continuously monitoring the rectal pressure by a computer (PowerLab PC, A.D. Instruments, Milford, MA, USA) and to a syringe for inflation/deflation of the balloon. The rats were then housed in a small cage (20 x 8 x 8 cm) on an elevated Plexiglas platform and allowed to wake up and adapt for 1 hour. After recovery from sedation, animals underwent the CRD procedure and behavioral responses were tested. The night before the experiments, the balloons were inflated and left overnight so the latex stretched and the balloons became compliant.

CRD of 20 seconds, performed every 5 minutes, was applied in increment of 0.4 ml starting from 0.4 ml up to 1.6 ml water. To achieve an accurate measurement of the colonic parameters and perception, the distensions were repeated twice for each intensity and data for each animal were averaged for analysis. Each animal underwent a double set of CRD. Twenty minutes after the first sequence of CRD (0.4 mL-1.6 ml water), drugs were administered intraperitoneally (i.p.) and a second set of CRD was performed. Behavioral responses during the first and the second set of CRD were assessed and compared.

Behavioral response to CRD was assessed by measuring the abdominal withdrawal reflex (AWR) using a semiquantitative score (1). The AWR is an involuntary motor reflex similar to the visceromotor reflex, but it has the great advantage that, in contrast to the latter, it does not require abdominal surgery to implant recording electrodes and wires in the abdominal muscle wall which may cause additional sensitization (see Ness, T.J. and Gebhart, G.F. (1990) *Pain* **41**:167-234, incorporated herein by reference).

Measurement of the AWR consisted of visual observation of the animal response to graded CRD by blinded observer and assignment of an AWR score according with the behavioral scale as previously described in Al-Chaer, E.D. *et al.* (2000) *Gastroenterology* **19**: 1276-85, incorporated herein by reference, in which grade 0 corresponds to no behavioral response to CRD, grade 1 corresponds to brief head movement at the onset of the stimulus followed by immobility, grade 2 corresponds to a mild contraction of abdominal muscles although the rats does not lift the abdomen off the platform, grade 3 corresponds to a strong contraction of the abdominal muscles with the lifting of the abdomen off the platform, and grade 4 corresponds to a severe contraction of the abdominal muscle manifested by body arching and the lifting of the abdomen and of the pelvic structures and scrotum.

The effects of mesalamine and Compound XXXV on colonic compliance and sensitivity were determined using a total of 8 fasting rats. To investigate whether the administration of mesalamine and Compound XXXV could revert pain induced by CRD, after the first sequence of CRD, 4 rats were treated with mesalamine at the dose of 100 mg/kg i.p. or Compound XXXV at the dose of 100 mg/kg i.p., after which a second set of CRD was repeated. Results from these experiments are shown in FIG. 11.

To determine the effect of mesalamine or Compound XXXV on colonic smooth muscle, the compliance of the colo-rectum during CRD was obtained from intracolo-rectal volume and pressure and expressed as mL/mmHg. These results are shown in FIG. 12.

All data are presented as the mean ± SEM, with sample sizes of 4 rats/group; statistical comparison of paired data was performed by the Wilcoxon signed rank test. An associated probability (p value) of less that 5% was considered significant.

FIG. 11(a) and (b) show that Compound XXXV is more effective than mesalamine (and vehicle) in reducing visceral pain in response to colorectal distension. Further, Compound XXXV successfully reduced intrarectal pressure, as shown in FIG. 12(b).

Thus, Compound XXXV, which has also been shown to have effective anti-inflammatory activity, is useful in treating various inflammatory conditions of the alimentary tract, as well as functional gastrointestinal disorders such as irritable bowel syndrome, dyspepsia, etc., that are characterized by increased visceral nociception (with or without accompanying inflammation).

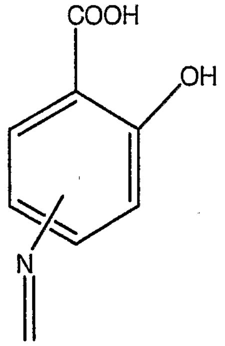
CLAIMS

1. A compound of general formula:

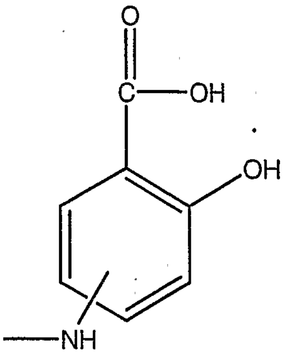
$$A—L—R \qquad (I)$$

where:

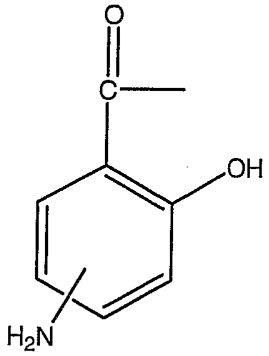
A is



where –N= is either at position 4 or 5,

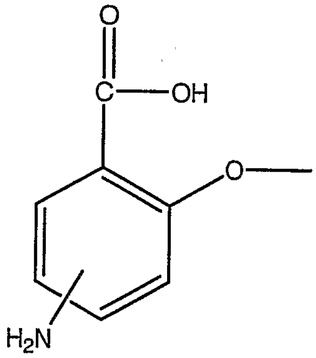


where –NH is either at position 4 or 5,



where $-NH_2$ is either at position 4 or 5,

or

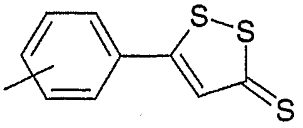


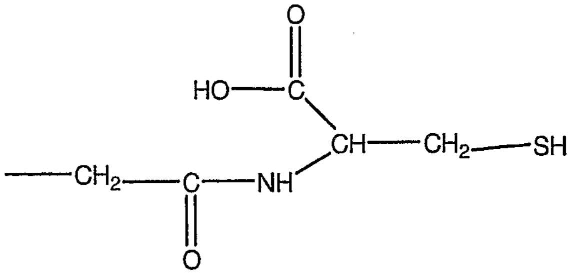
where $-NH_2$ is either at position 4 or 5;

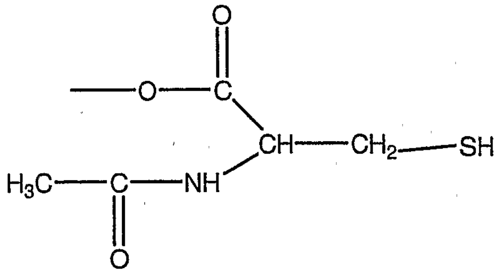
L is either O, O-C=O, S, N or a covalent bond to form an ester linkage, an anhydride linkage, a thioester linkage, an amide linkage or an azo linkage; and

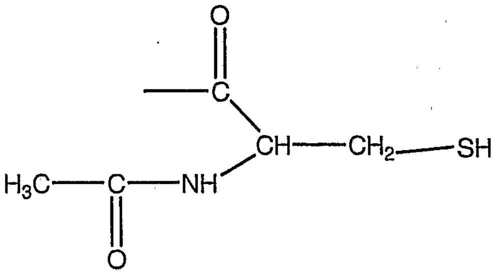
R is a hydrogen sulfide releasing moiety.

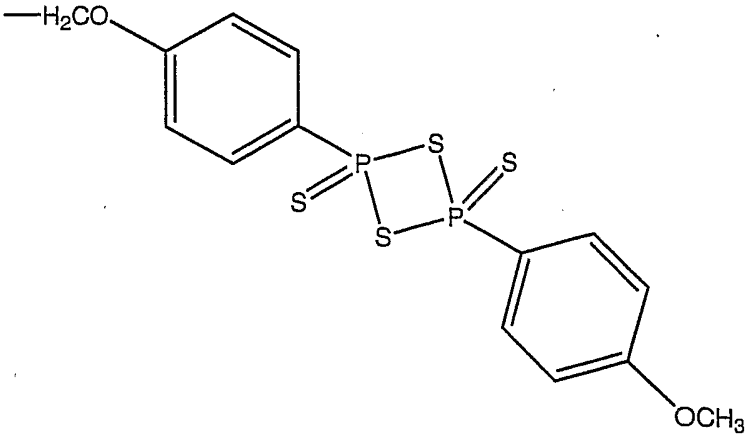
2. The compound according to claim 1, wherein R is selected from the group consisting of:



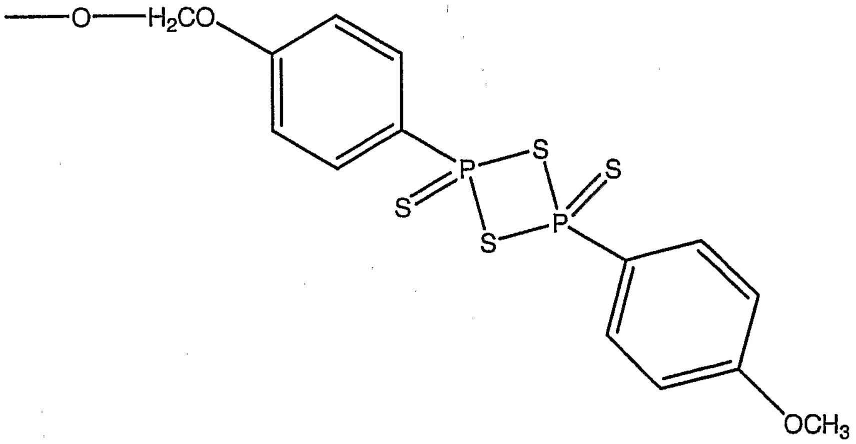




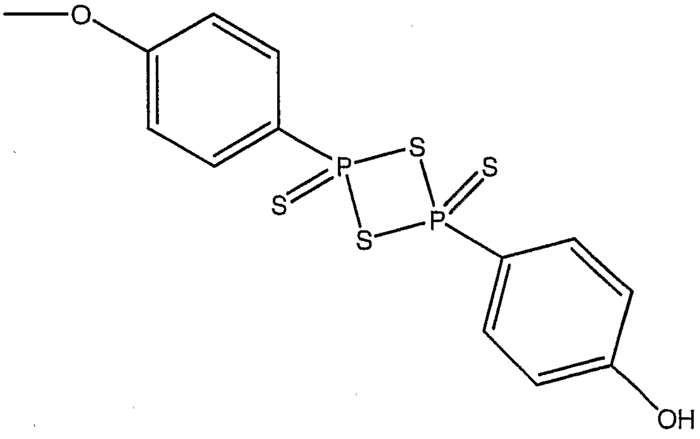




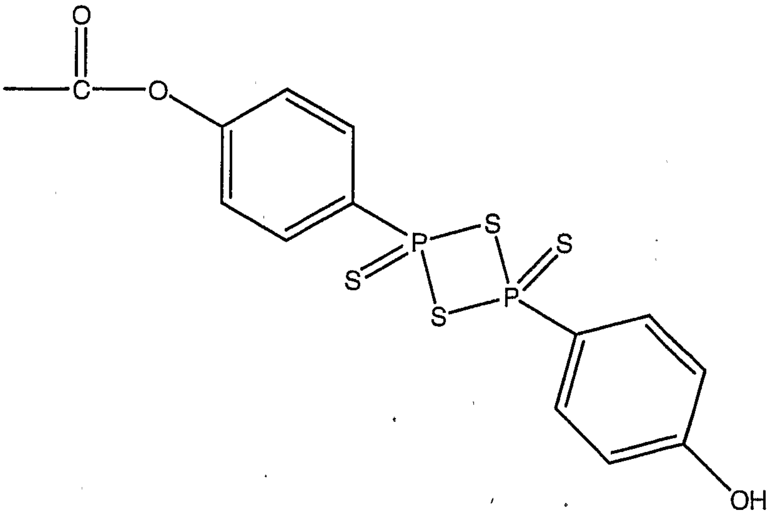
H2CO
S
P
OCH3

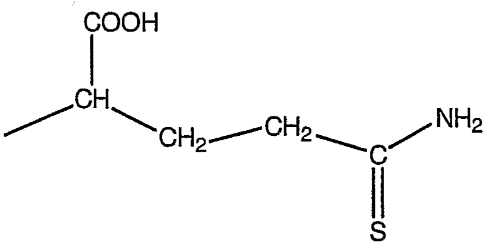


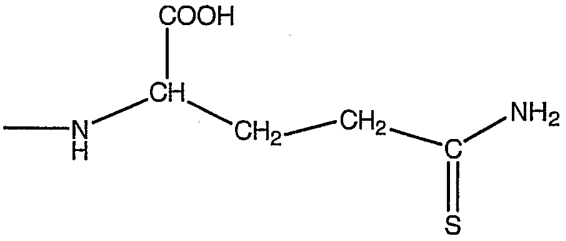
O
H2CO
S
P
OCH3

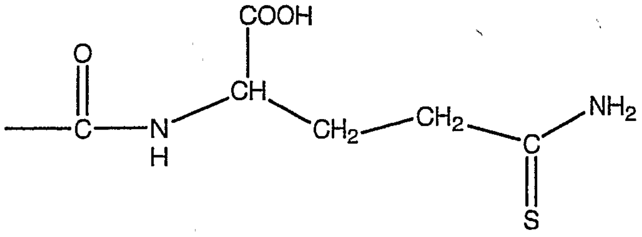


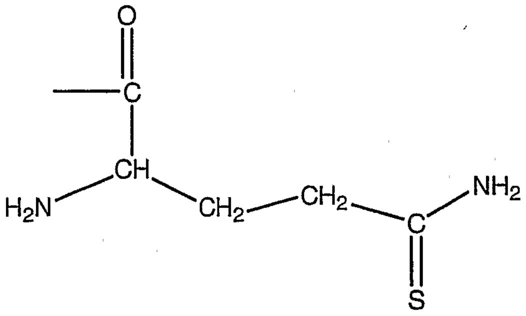
O
S
P
OH

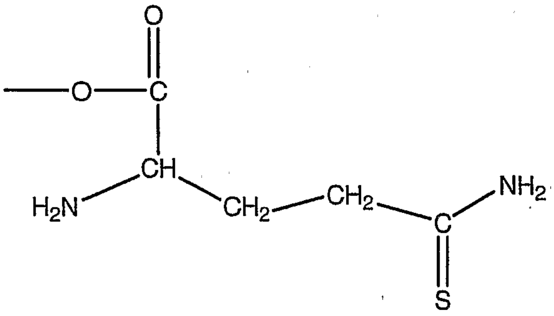


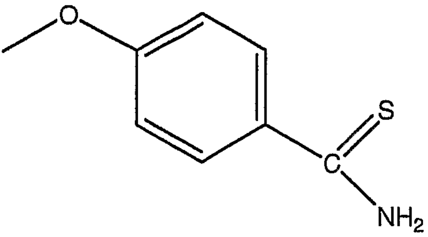


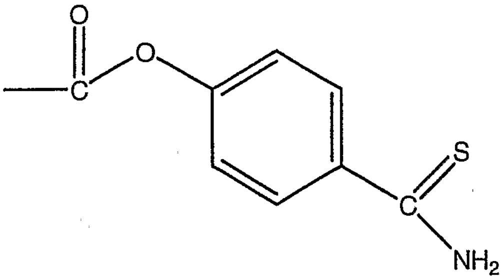


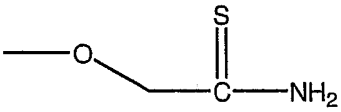


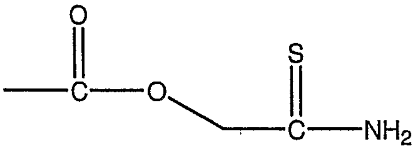




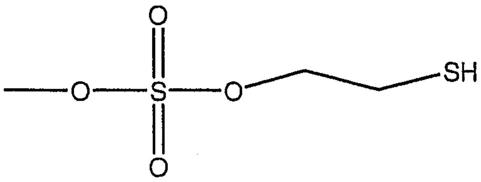








and,



3. The compound according to claim 2, 2-hydroxy-4- or 5-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenylazo]-benzoic acid.

4. The compound according to claim 2, 4-or 5-amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester.

5. The compound according to claim 2, 4 or 5-amino-2-[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonyloxy]-benzoic acid.

6. The compound according to claim 2, 2-hydroxy-4 or 5[4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenoxycarbonylamino]-benzoic acid.

7. The compound according to claim 2, 4-or 5-{[(1-carboxy-2-mercapto-ethylcarbamoyl)-methyl]-azo}-2-hydroxy-benzoic acid.

8. The compound according to claim 2, 4-or 5-amino-2-hydroxy-benzoic acid (1-carboxy-2-mercapto-ethylcarbamoyl)-methyl ester.

9. The compound according to claim 2, 4-or 5-amino-2-[(1-carboxy-2-mercapto-ethylcarbamoyl)-methoxycarbonyloxy]-benzoic acid.

10. The compound according to claim 2, 4- or 5-[(1-carboxy-2-mercapto-

ethylcarbamoyl)-methoxycarbonylamino]-2-hydroxy-benzoic acid.

11. The compound according to claim 2, 4- or 5- amino-2-hydroxy-benzoic acid anhydride with N-acetyl cysteine.

12. The compound according to claim 2, 4 or 5-(2-acetylamino-3-mercapto-propionylamino)-2-hydroxy-benzoic acid.

13. The compound according to claim 2, 2-(2-acetylamino-3-mercapto-propionyloxy)-4 or 5-amino-benzoic acid.

14. The compound according to claim 2, 2-hydroxy-4 or 5-({4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethyl}-azo)-benzoic acid.

15. The compound according to claim 2, 4- or 5-amino-2-{4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxymethoxycarbonyloxy}-benzoic acid.

16. The compound according to claim 2, 2-hydroxy- 4- or 5-{4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithia-diphosphetan-2-yl]-phenoxymethoxycarbonylamino}-benzoic acid.

19. The compound according to claim 2, 4-or 5-amino-2-hydroxy-benzoic acid 4-[4-(4-methoxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-

phenoxymethyl ester.

20. The compound according to claim 2, 4- or 5-amino-2-hydroxy-benzoic acid 4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenyl ester.

21. The compound according to claim 2, 4- or 5-amino-2-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonyloxy}-benzoic acid.

22. The compound according to claim 2, 2-hydroxy-4- or 5-{4-[4-(4-hydroxy-phenyl)-2,4-dithioxo-2λ^5,4λ^5-[1,3,2,4]dithiadiphosphetan-2-yl]-phenoxycarbonylamino}-benzoic acid.

23. The compound according to claim 2, 4- or 5-(1-carboxy-3-thiocarbamoyl-propylazo)-2-hydroxy-benzoic acid.

24. The compound according to claim 2, 2-(4- or 5-amino-2-hydroxy-benzoylamino)-4-thiocarbamoyl-butyric acid.

25. The compound according to claim 2, 4- or 5-amino-2-(1-carboxy-3-thiocarbamoyl-propylcarbamoyloxy)-benzoic acid.

26. The compound according to claim 2, 2-hydroxy-4- or 5-[3-(1-hydroxymethyl-3-thiocarbamoyl-propyl)-ureido]-benzoic acid.

27. The compound according to claim 2, 4- or 5-amino-2-(2-amino-4-thiocarbamoyl-butyryloxy)-benzoic acid.

28. The compound according to claim 2, 4- or 5-(2-amino-4-thiocarbamoyl-butyrylamino)-2-hydroxy-benzoic acid.

29. The compound according to claim 2, 4- or 5- amino-2-hydroxy-benzoic acid anhydride with 2-amino-4-thiocarbamoyl-butyric acid.

30. The compound according to claim 2, 4-thiocarbamoylphenyl 4- or 5-amino-2-hydroxybenzoate.

31. The compound according to claim 2, 4- or 5-amino-2-(4-thiocarbamoyl-phenoxycarbonyloxy)-benzoic acid.

32. The compound according to claim 2, 2-hydroxy-4- or 5-(4-thiocarbamoyl-phenoxycarbonylamino)-benzoic acid.

33. The compound according to claim 2, 4- or 5-amino-2-hydroxy-benzoic acid thiocarbamoylmethyl ester.

34. The compound according to claim 2, 4- or 5-amino-2-thiocarbamoylmethoxycarbonyloxy-benzoic acid.

35. The compound according to claim 2, 2-hydroxy-4- or 5-thiocarbamoylmethoxycarbonylamino-benzoic acid.

36. The compound according to claim 2, 4-or 5- amino-2-hydroxy-benzoic acid anhydride with sulfuric acid mono- (2-mercapto-ethyl) ester.

37. The compound according to claim 2, 4- or 5-amino-2-(2-mercapto-ethoxysulfonyloxy)-benzoic acid.

38. The compound according to claim 2, 5-amino-2-hydroxy-benzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3-yl)-phenyl ester.

39. A pharmaceutical composition comprising a compound according to claim 1, or a pharmacologically acceptable salt thereof, and a pharmaceutically acceptable excipient or carrier.

40. A pharmaceutical composition comprising a compound according to claim 2, or a pharmacologically acceptable salt thereof, and a pharmaceutically acceptable excipient or carrier.

41. A pharmaceutical composition comprising a compound as claimed in any one of claims 3 to 38 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient or carrier.

42. A pharmaceutical composition comprising the compound of claim 38 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient or carrier.

43. A method of treating an inflammatory condition of the gastrointestinal tract in a subject in need of such treatment, said method comprising administering to the subject an amount effective to treat the inflammatory condition of the gastrointestinal tract of a compound according to claim 1.

44. A method of treating an inflammatory condition of the gastrointestinal tract in a subject in need of such treatment, said method comprising administering to the subject an amount effective to treat the inflammatory condition of the gastrointestinal tract of a compound according to claim 2.

45. A method of treating an inflammatory condition of the gastrointestinal tract in a subject in need of such treatment, said method comprising administering to the subject an amount effective to treat the inflammatory condition of the gastrointestinal tract of a compound according to any one of claims 3 to 38.

46. A method of treating an inflammatory condition of the gastrointestinal tract in a subject in need of such treatment, said method comprising administering to the subject an amount effective to treat the inflammatory condition of the gastrointestinal tract of a compound according to claim 38.

47. The method according to any one of claims 43 to 46, wherein the inflammatory condition of the gastrointestinal tract is Crohn's disease.

48. The method according to any one of claims 43 to 46, wherein the inflammatory condition of the gastrointestinal tract is ulcerative colitis.

49. The method according to any one of claims 43 to 46, wherein the inflammatory

condition of the gastrointestinal tract is irritable bowel syndrome.

50. A method for preventing colon cancer in a subject, said method comprising administering to the subject an amount effective of a compound according to claim 1.

51. A method for preventing colon cancer in a subject, said method comprising administering to the subject an amount effective of a compound according to claim 2.

52. A method for preventing colon cancer in a subject, said method comprising administering to the subject an amount effective of a compound according to any of claims 3 to 38.

53. A method for preventing colon cancer in a subject, said method comprising administering to the subject an amount effective of a compound according to claim 38.

54. Use of a compound according to any one of claims 1 to 38 for the preparation of a medicament for the treatment of an inflammatory condition of the gastrointestinal tract in a subject.

55. Use of a compound according to claim 38 for the preparation of a medicament for

the treatment of an inflammatory condition of the gastrointestinal tract in a subject.

56. Use of a compound according to any one of claims 1 to 38 for the preparation of a medicament for the treatment of Crohn's disease in a subject.

57. Use of a compound according to claim 38 for the preparation of a medicament for the treatment of Crohn's disease in a subject.

58. Use of a compound according to any one of claims 1 to 38 for the preparation of a medicament for the treatment of ulcerative colitis in a subject.

59. Use of a compound according to claim 38 for the preparation of a medicament for the treatment of ulcerative colitis in a subject.

60. Use of a compound according to any one of claims 1 to 38 for the preparation of a medicament for the treatment of irritable bowel syndrome in a subject.

61. Use of a compound according to claim 38 for the preparation of a medicament for the treatment of irritable bowel syndrome in a subject.

62. Use of a compound according to any one of claims 1 to 38 for the preparation of a medicament for the prevention of colon cancer.

63. Use of a compound according to claim 38 for the preparation of a medicament for the prevention of colon cancer.

64. Use of a compound according to any one of claims 1 to 38 for the treatment of an inflammatory condition of the gastrointestinal tract in a subject.

65. Use of a compound according to claim 38 for the treatment of an inflammatory condition of the gastrointestinal tract in a subject.

66. Use of a compound according to any one of claims 1 to 38 for the treatment of Crohn's disease in a subject.

67. Use of a compound according to claim 38 for the treatment of Crohn's disease in a subject.

68. Use of a compound according to any one of claims 1 to 38 for the treatment of ulcerative colitis in a subject.

69. Use of a compound according to claim 38 for the treatment of ulcerative colitis in a subject.

70. Use of a compound according to any one claims 1 to 38 for the treatment of

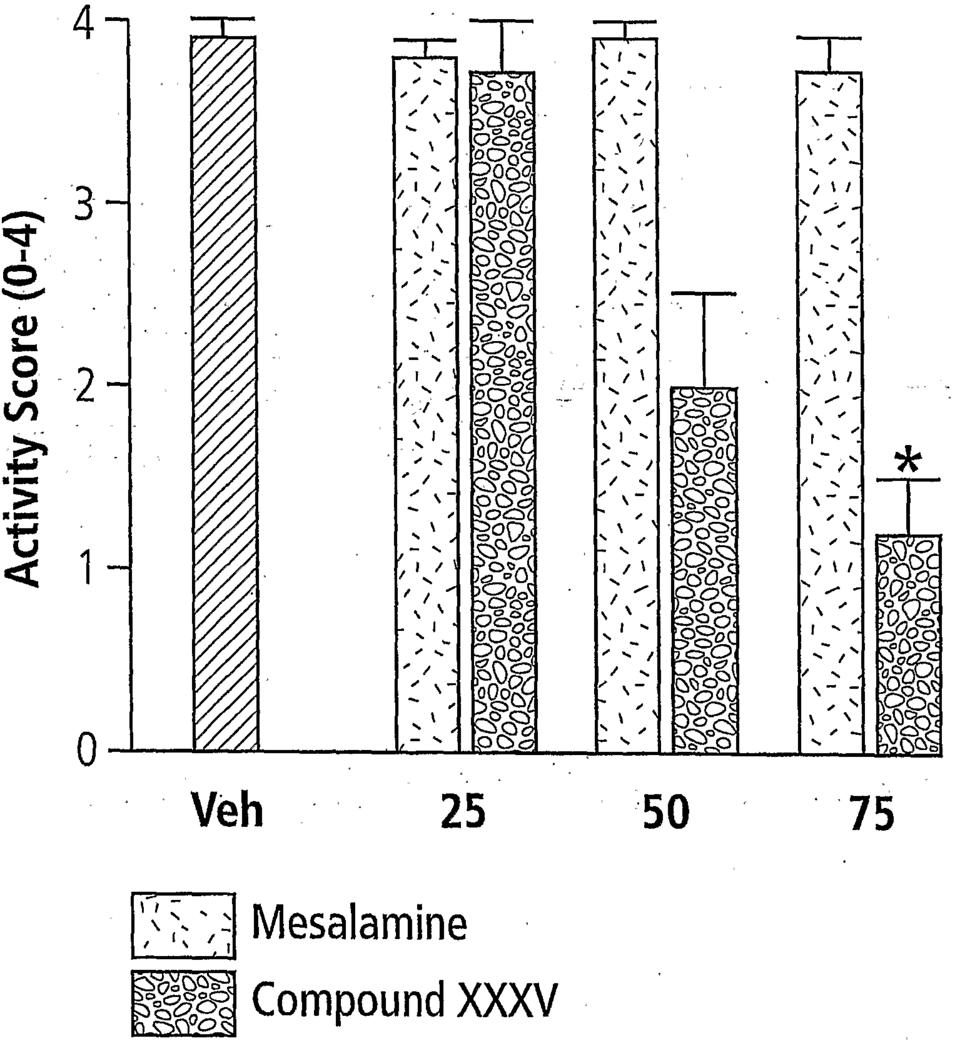
irritable bowel syndrome in a subject.

71. Use of a compound according to claim 38 for the treatment of irritable bowel syndrome in a subject.

72. Use of a compound according to any one of claims 1 to 38 for preventing colon cancer.

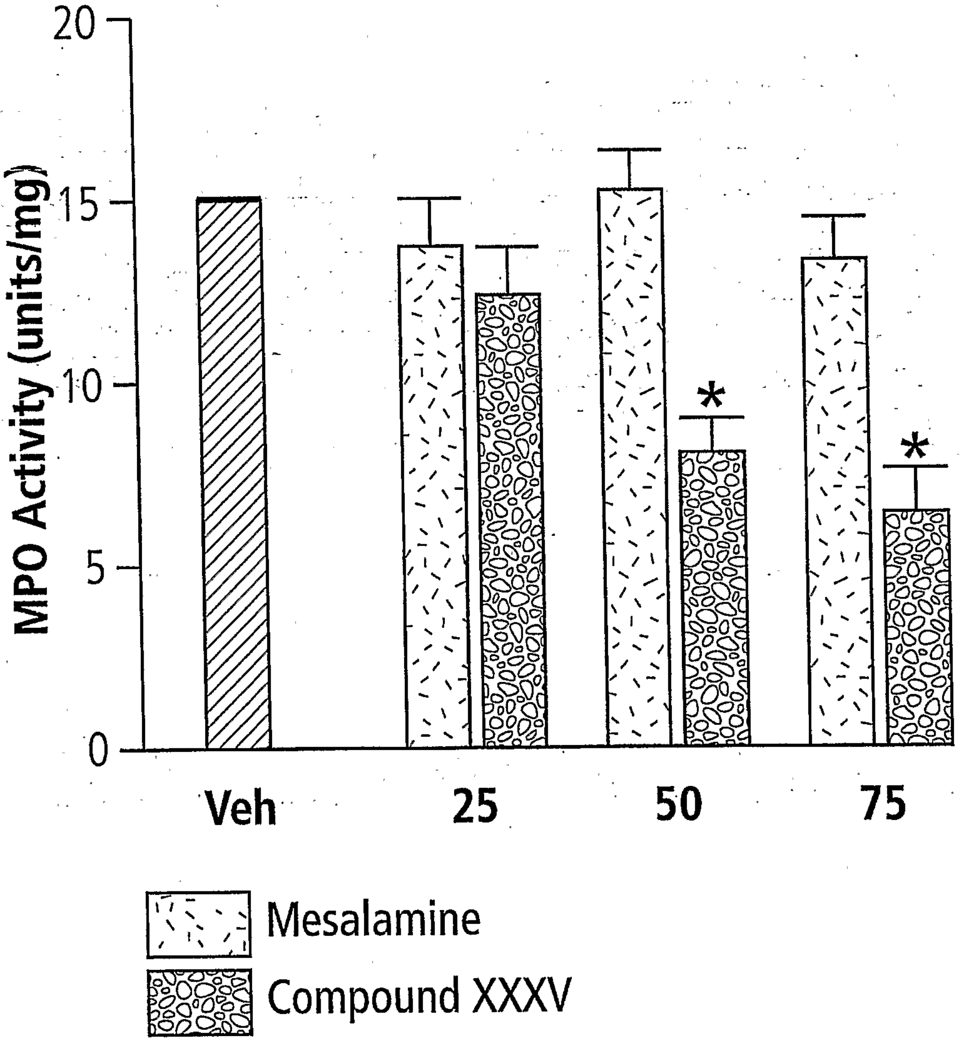
73. Use of a compound according to claim 38 for preventing colon cancer.

Fig. 1


Activity Score (0-4)
4
3
2
1
0
Veh
25
50
75
*
Mesalamine
Compound XXXV

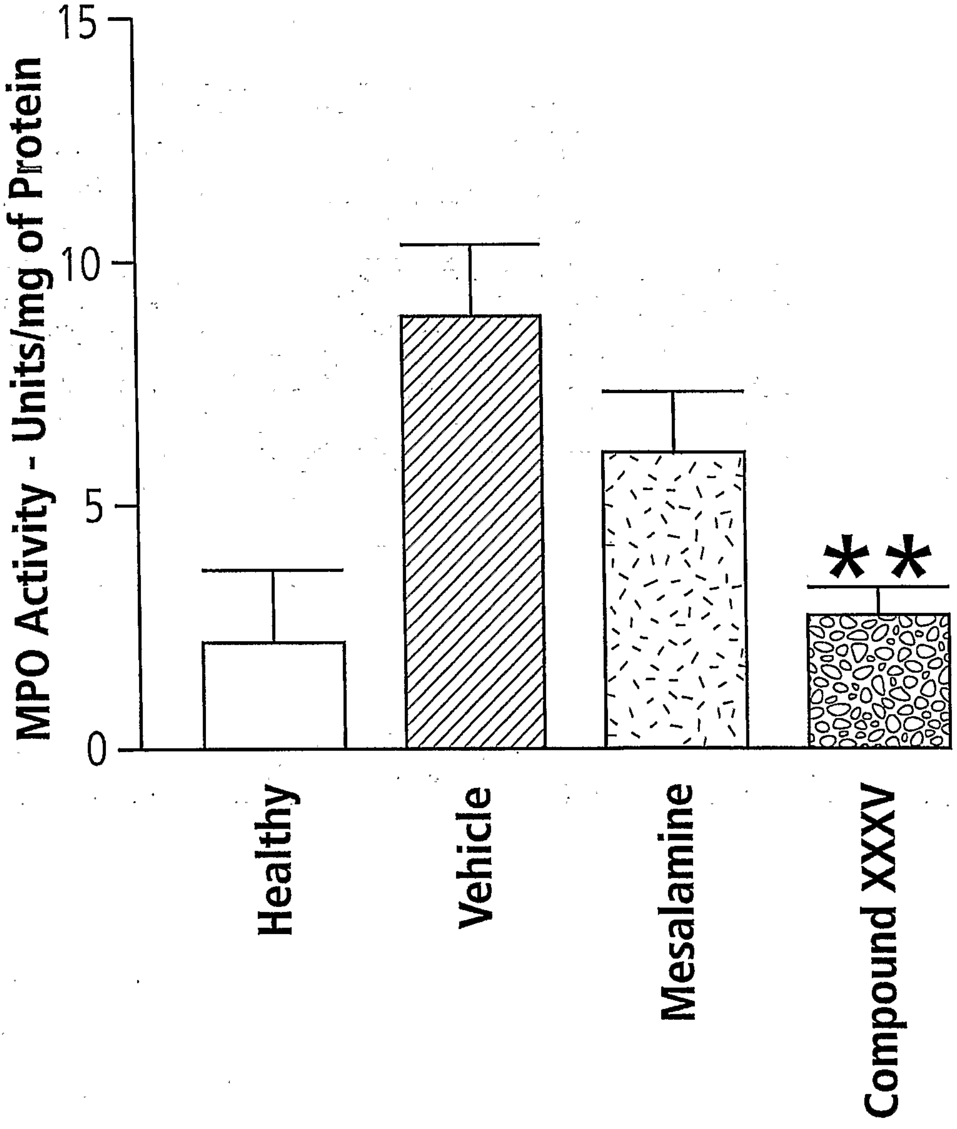
*p<0.05

Fig. 2


MPO Activity (units/mg)
20
15
10
5
0
*
*
Veh
25
50
75
Mesalamine
Compound XXXV

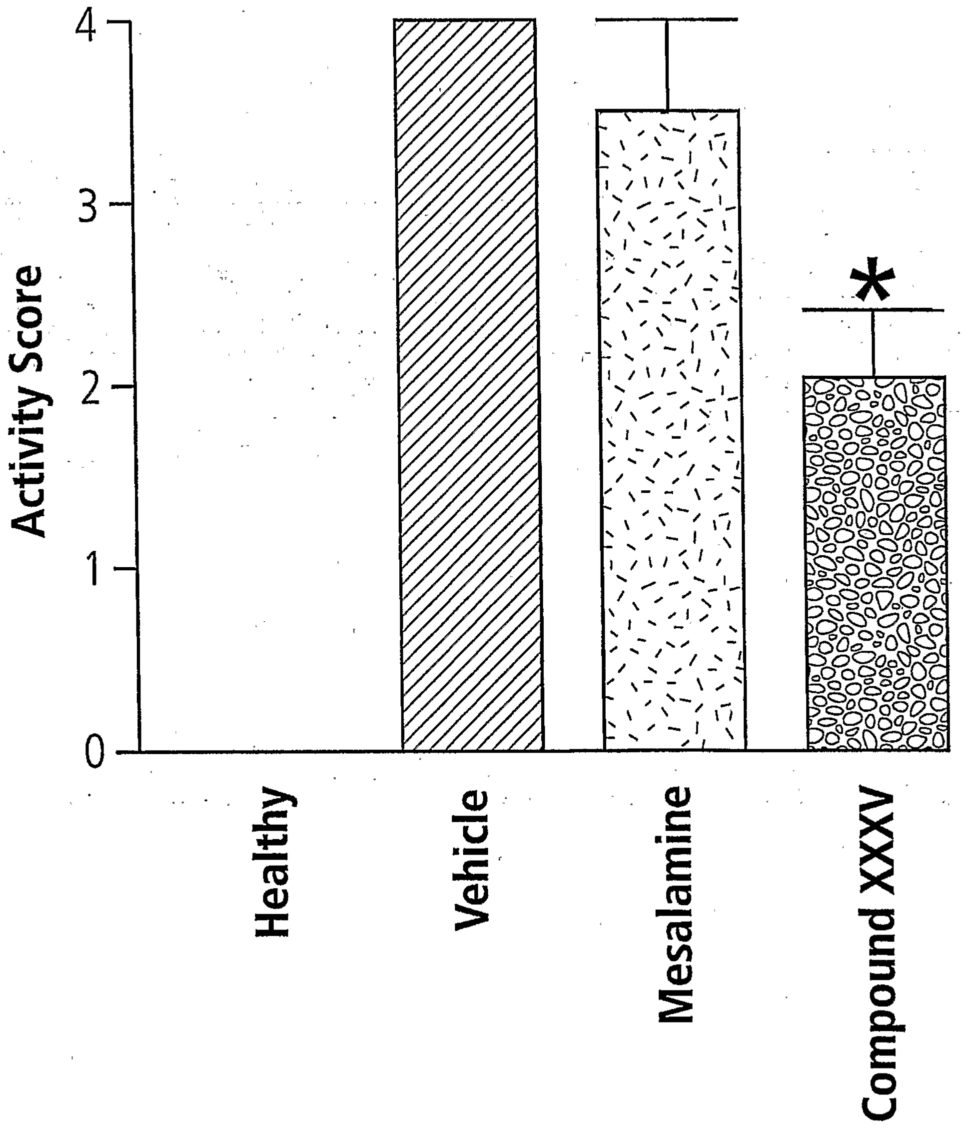
*p<0.05

Fig. 3


MPO Activity - Units/mg of Protein
15
10
5
0
**
Healthy
Vehicle
Mesalamine
Compound XXXV

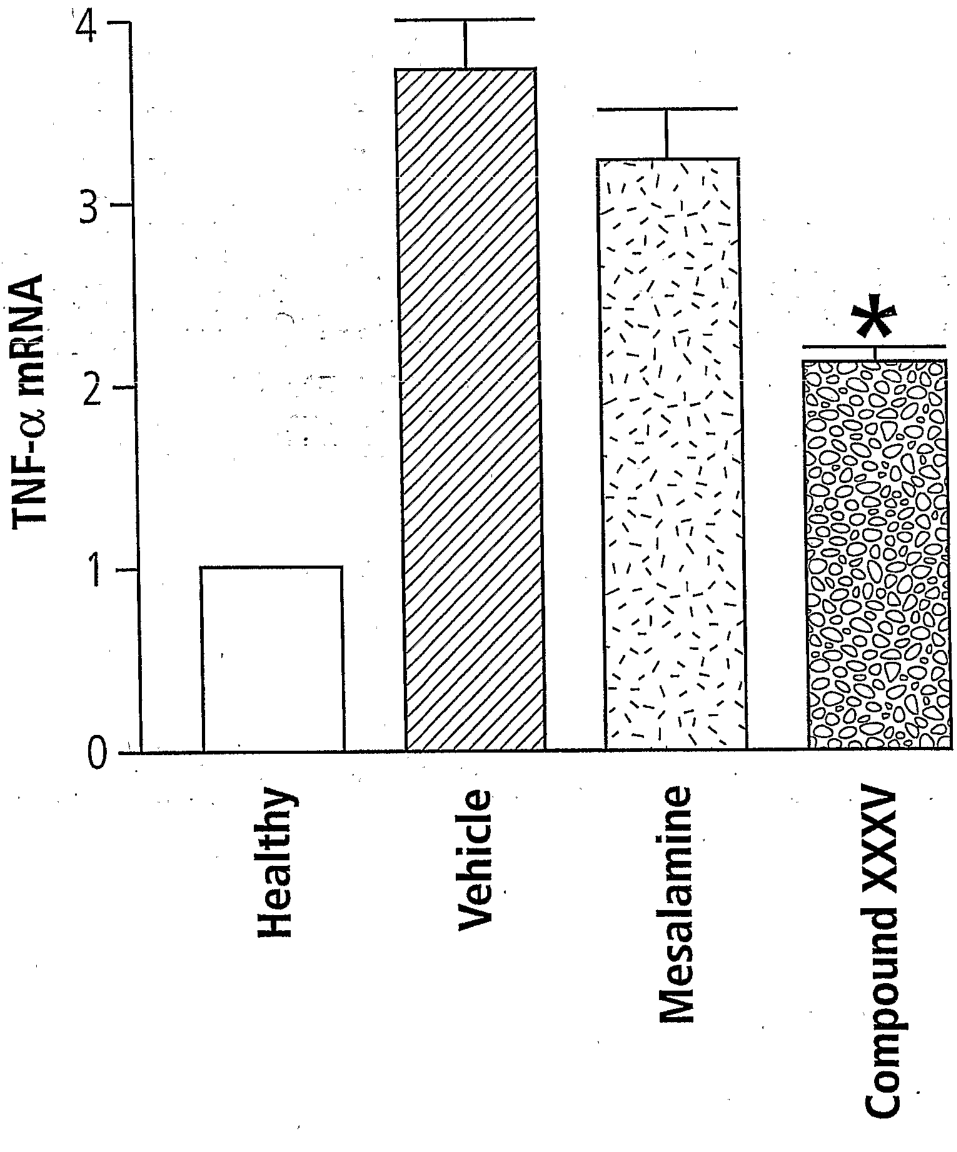
**$p<0.01$

Fig. 4


4
3
2
1
0
Activity Score
*
Healthy
Vehicle
Mesalamine
Compound XXXV

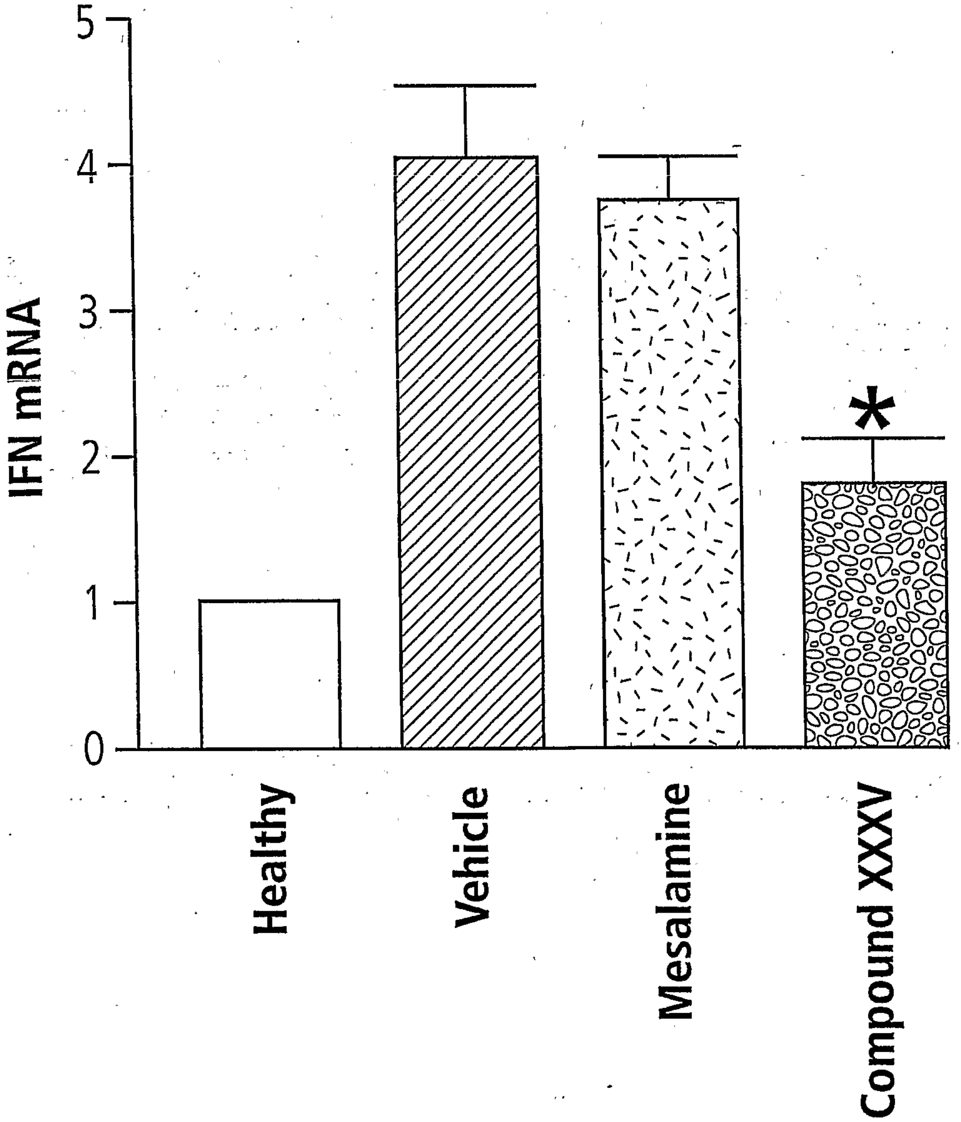
*p<0.05

Fig. 5



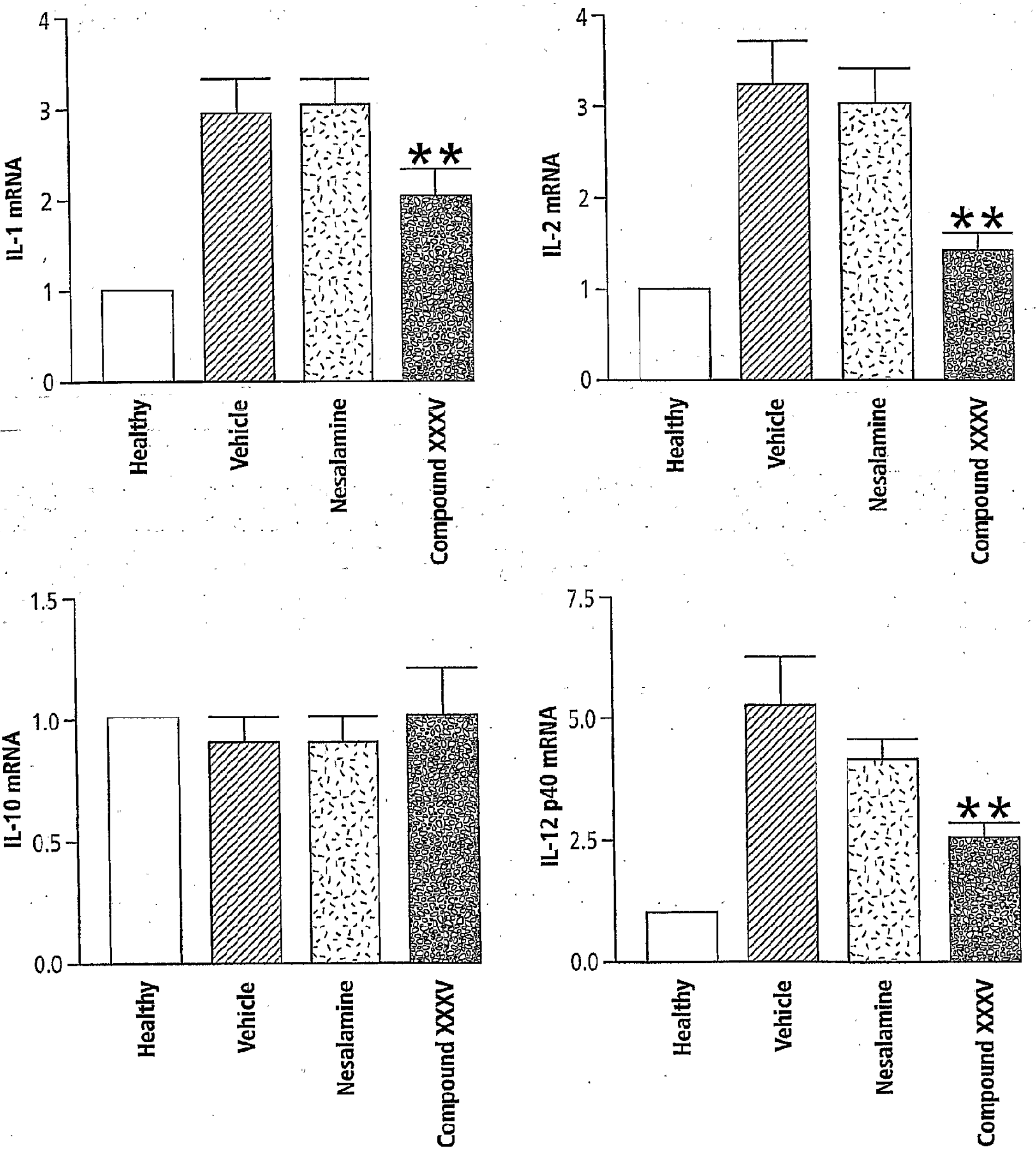
*p<0.05

Fig. 6



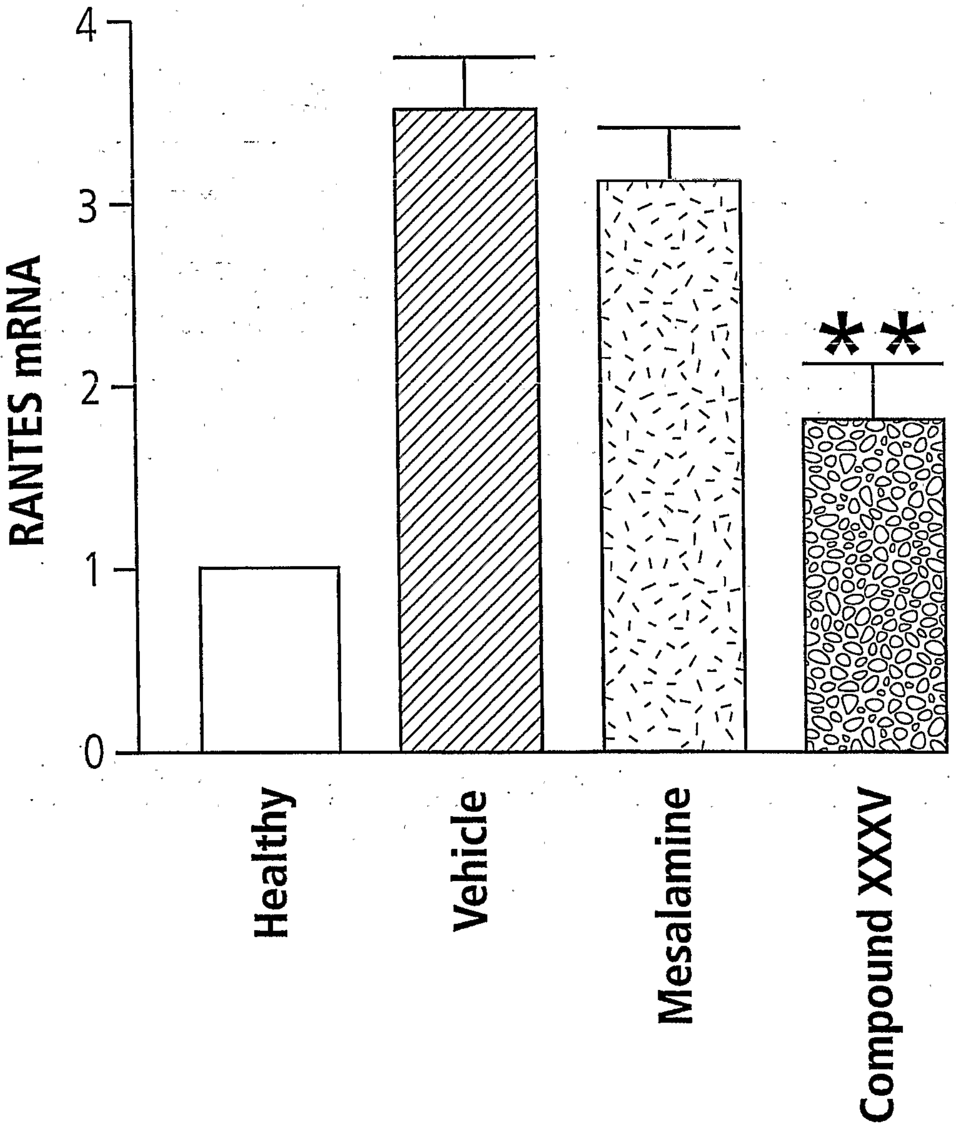
*p<0.05

Fig. 7


IL-1 mRNA
0
1
2
3
4
**
Healthy
Vehicle
Nesalamine
Compound XXXV
IL-2 mRNA
0
1
2
3
4
**
Healthy
Vehicle
Nesalamine
Compound XXXV
IL-10 mRNA
0.0
0.5
1.0
1.5
Healthy
Vehicle
Nesalamine
Compound XXXV
IL-12 p40 mRNA
0.0
2.5
5.0
7.5
**
Healthy
Vehicle
Nesalamine
Compound XXXV

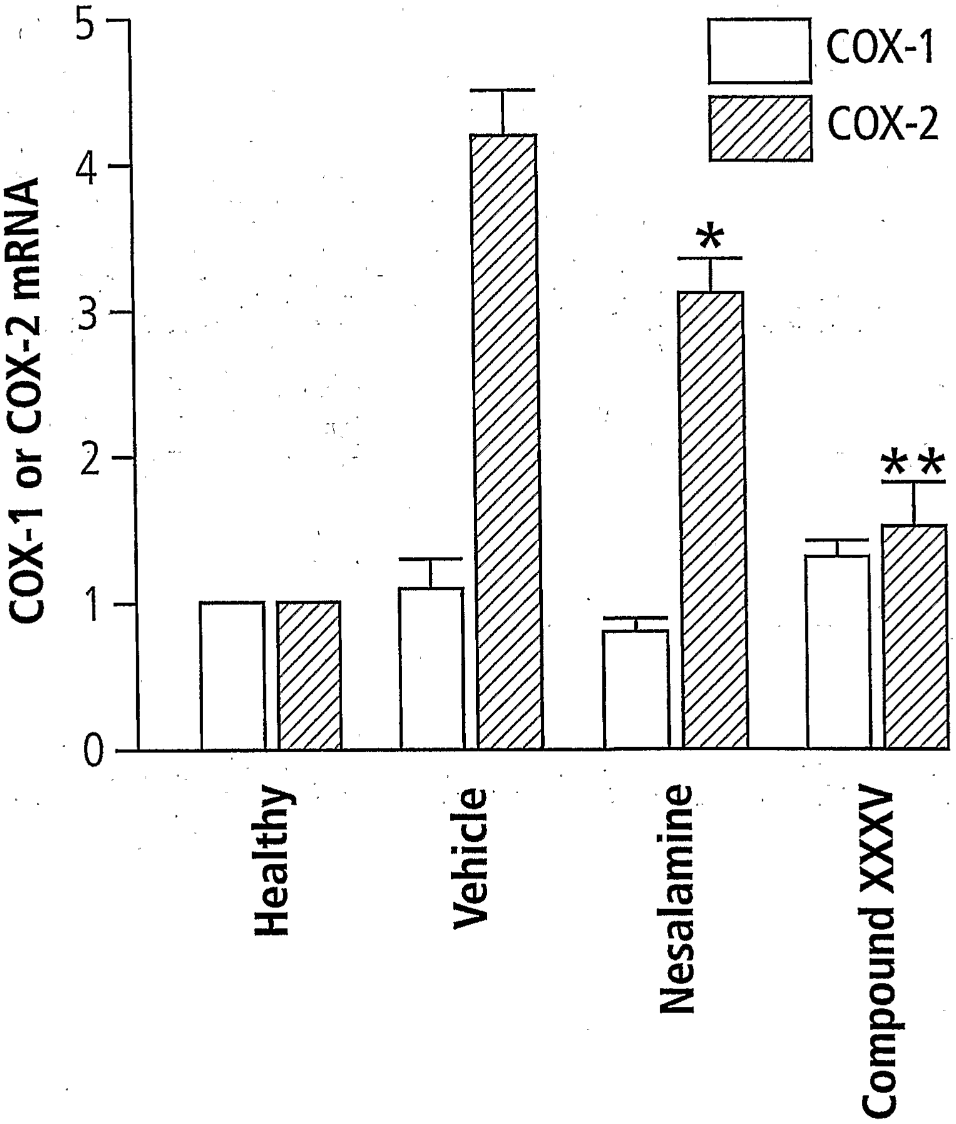
**p<0.01

Fig. 8


RANTES mRNA
0
1
2
3
4
**
Healthy
Vehicle
Mesalamine
Compound XXXV

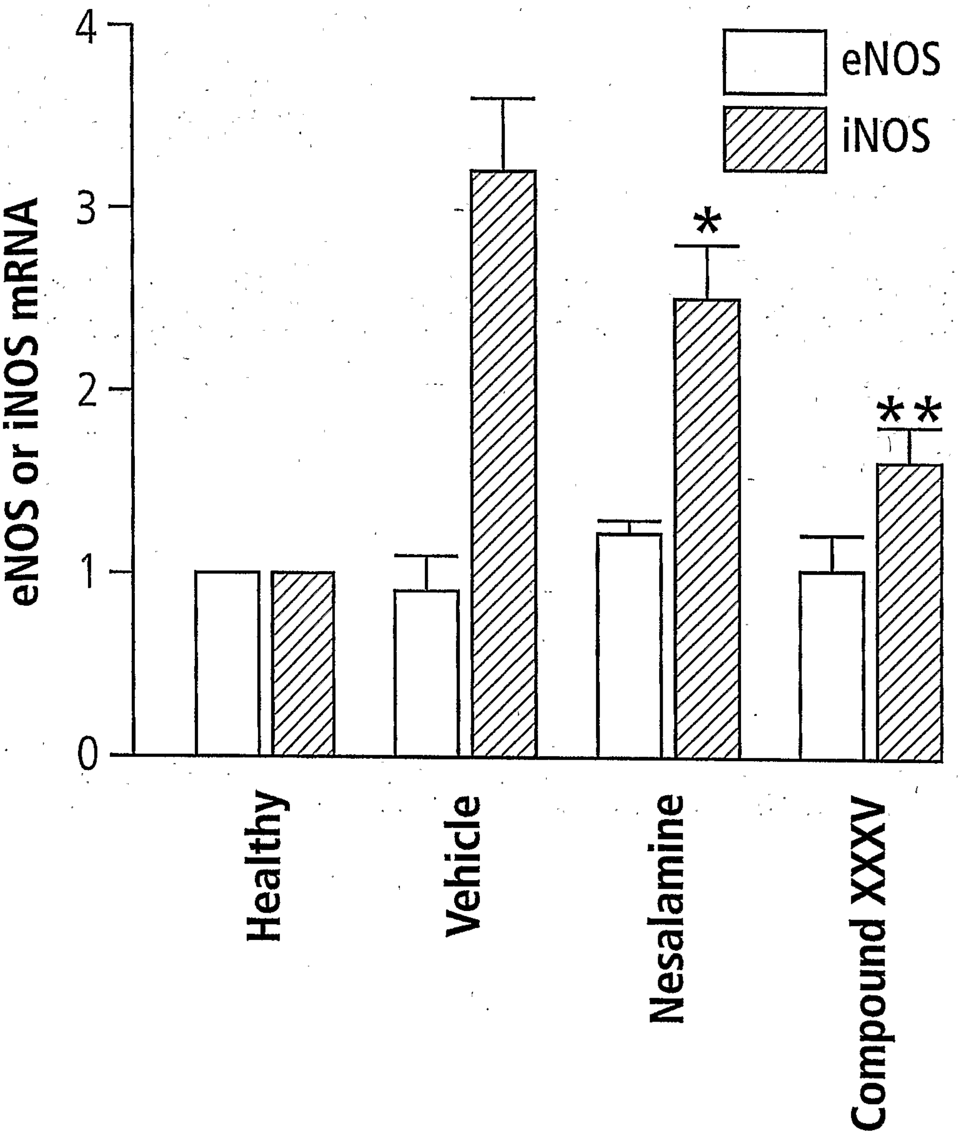
**p<0.01

Fig. 9



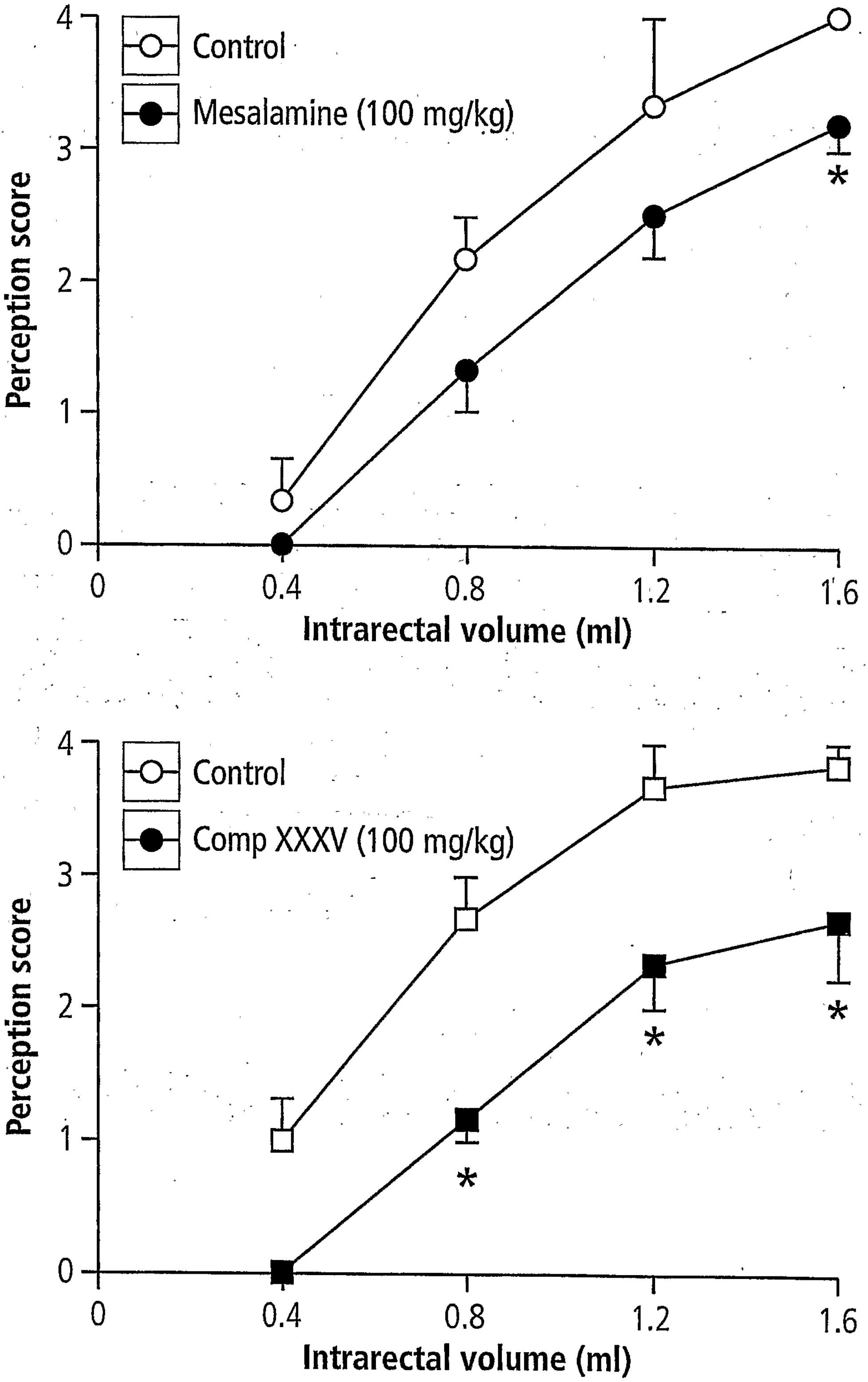
*p<0.05, **p<0.01

Fig. 10



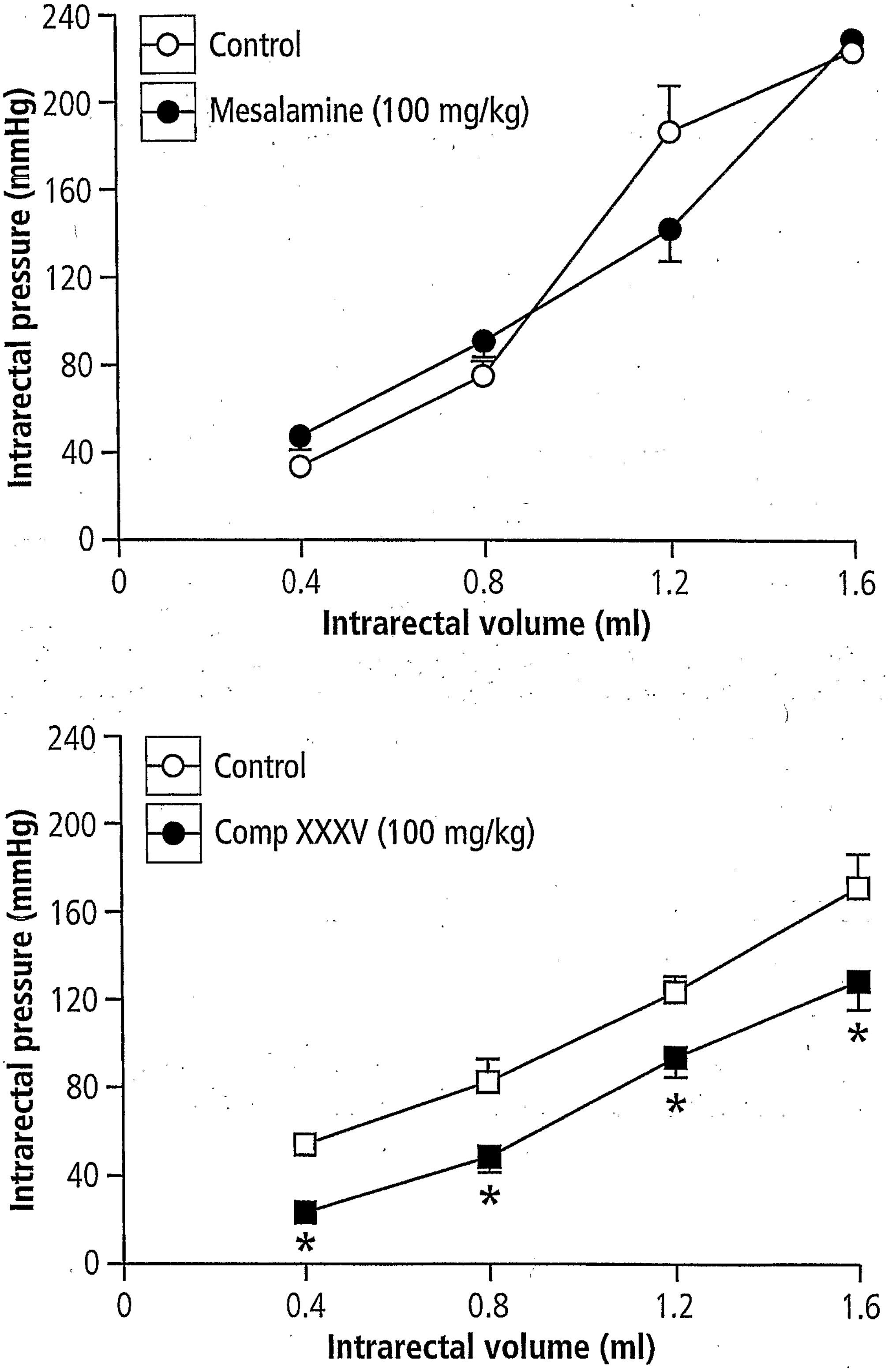
*p<0.05, **p<0.01

Fig. 11


Control
Mesalamine (100 mg/kg)
Perception score
4
3
2
1
0
0
0.4
0.8
1.2
1.6
Intrarectal volume (ml)
*
Control
Comp XXXV (100 mg/kg)
Perception score
4
3
2
1
0
0
0.4
0.8
1.2
1.6
Intrarectal volume (ml)
*
*
*

*$p<0.05$

Fig. 12


240
200
160
120
80
40
0
Intrarectal pressure (mmHg)
Control
Mesalamine (100 mg/kg)
0
0.4
0.8
1.2
1.6
Intrarectal volume (ml)
240
200
160
120
80
40
0
Intrarectal pressure (mmHg)
Control
Comp XXXV (100 mg/kg)
*
*
*
*
0
0.4
0.8
1.2
1.6
Intrarectal volume (ml)

*$p<0.05$

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 45-49, 52 & 53
   because they relate to subject matter not required to be searched by this Authority, namely :

   Although claim 46 and 53, and claims 45, 47-49, and 52, as they depend on claims 4-6 and 38, are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the pharmaceutical compositions.

2. [X] Claim Nos. : 1-3, 7-37, 39, 40, 43-44, 50 & 51
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

   The instant claim 1 is directed to aminosalicylic acid derivatives and pharmaceutical compositions comprising same, wherein the derivative is defined in terms of a desired effect, result or functionality. (**see supplemental sheet for continuation**)

3. [ ] Claim Nos. :
   because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows :

The general formula I contains no fixed structural motif. Further, there is no common, distinct, unifying structural unit linking the subject matter defined in all the claims, and thus, no special technical characterising feature can be ascertained. The International Search Authority concludes that owing to the absence of an identifiable unifying structural motif in all the claims, which represents a technical contribution over the state of the art, unity of invention is lacking for the present invention. **(see supplemental sheet for continuation)**

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] *The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.*

[ ] *The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.*

[ ] *No protest accompanied the payment of additional search fees.*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000819

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7): C07F 9/6578, A61P 1/00, A61P 29/00, A61P 35/00, A61K 31/385, A61K 31/325, A61K 31/27, A61K 31/67, A61K 31/16, A61K 31/255, C07C 327/40, C07C 321/14, C07D 339/04, C07C 333/02

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(7): C07F; A61P; A61K; C07C; C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database; Delphion; STN-CAPLUS; WEST; Science Direct, HighWire; Keywords: irritable + bowel + syndrome + hydrogen + sulfie + releasing + IBS + IBD + inflammatory + disease + 4-ASA + 5-ASA + sulfide + aminosalicylic + azo + anhydride + amide + thioester + ester

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CA 2,204,747 (CARCELLER, ET AL) 13 Mar. 1997 examples 4, 5, 6, 7	1
A	US 4,412,992 (CHAN, ET AL) 01 November 1983 claims, Abstr	1
A	US 6,458,776 (EKWURIBE, ET AL) 01 October 2002 col. 1 line 50 to col. 2 line 64; examples, claims	1
A	US 6,197,341 (FREISS, ET AL) 06 Mar. 2001 see the whole document	1

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search 15 September 2005 (15-09-2005)	Date of mailing of the international search report 20 September 2005 (20-09-2005)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Authorized officer Okemona Oke (819) 956-4108

INTERNATIONAL SEARCH REPORT

[illegible]ernational application No.
PCT/CA2005/000819

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PELLICCIARI, ET AL., "Brush-Border-Enzyme-Mediated Intestine-Specific Drug Delivery. Amino acid Prodrugs of 5-Aminosalicylic Acid" *J. Med. Chem.* 1993, vol. 36, pages 4201-4207, Figure 1	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

[illegible]ernational application No.
PCT/CA2005/000819

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US4412992	01-11-1981	SG0006885 A	14-06-1985
		MY0002986 A	31-12-1986
		JP62025656 B4	04-06-1987
		GB2080796 B2	12-10-1983
		DE3128819 C2	15-02-1990
		FR2493312 B1	21-09-1984
		IT1138450 A	17-09-1986
		HK0031885 A	03-05-1985
US6458766	01-10-2002	CA2421114 A1	07-03-2002
		EP1313688 A1	28-05-2003
		WO0218330 A1	07-03-2002
		AU0185489 A5	13-03-2002
		US20020111334	15-08-2002
		MX3001786 A	04-06-2003
		JP2004507521T2	11-03-2994
		IL0154537 A0	17-09-2003
CA2204747	13-03-1997	AU6987596 A1	27-03-1997
		AT0198751 E	15-02-2001
		BR9606628 A	30-09-1997
		DE69611598 T2	07-06-2001
		DK0790998 T3	05-02-2001
		EP0790998 A1	27-08-1997
		JP11501939 T2	16-02-1999
		WO9709329 A1	13-03-1997
		NO0972113 A0	07-05-1997
		GR3035622 T3	29-06-2001
		US5747477	05-05-1998
US6197341	06-03-2001	AU6430198 A1	12-10-1998
		CA228329 AA	24-09-1998
		EP1003524 A1	31-05-2000
		WO9841212 A1	24-09-1998
		SE9700934 A0	14-03-1997
		PL0335700 A1	08-05-2000
		NO0994431 A0	13-09-1999
		NL10008575 A1	15-09-1999
		JP2001519782 T2	23-10-2001
		HU0000850 AB	29-08-2000
		GR98100096 A	30-11-1998
		FR1003523 A1	18-09-1998

Continuation of Box No. II:

The effect of the lack of clarity in definition of R in formula I, is to provide compounds of no fixed stoichiometry, as such the skilled person is unable to establish the precise scope of the invention. Lack of unity exists between all the possibly infinite combinations and permutations of compounds (arising from the different points of bonding attachments of A, L and R) that can be extracted from formula I. Claim 1 relates to aminosalicylic acid derivatives incorporating the obtuse term “hydrogen sulfide releasing moiety”. Accordingly, the scope of the invention cannot be delineated with any certainty. The claims lack clarity and support and and the application lacks disclosure within the context of Article 5 and Article 6 PCT that a comprehensive search over the entire scope of the claimed subject matter is neither meaningful nor practically feasible. The search has been restricted to the first claimed invention for which there is full support, namely, subject matter encompassing the general formula I, corresponding to Examples 3, 6, Figures 1-7 and claims 4, 5, 6, 38, 42, 46, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73, and claims 41, 45, 47-49, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72, as they depend on claims 4-6 and 38, wherein the fragment A-L is represented by [5-thioxo-5H-(1,2)-dithiol-3-yl]phenyloxycarbonyl and R is [4 or 5] imino-2-hydroxybenzoic acid, [4 or 5] amino-2-oxo-benzoic acid or [4 or 5] amino-2-hydroxyphenyl.

Continuation of Box No. III:

The International Search Authority concludes that owing to the absence of an identifiable unifying structural motif in all the claims, which represents a technical contribution over the state of the art, unity of invention is lacking for the present invention. The application is regarded as non unitary in the absence of appropriate amendments which would make such a characterising feature clearly apparent. Lack of unity exists for each distinct structurally unique entity that can be constructed from general formula I (arising from the different points of bonding attachments of A, L and R). The search has been restricted to the first claimed invention for which there is full support, namely, subject matter encompassing the general formula I, corresponding to Examples 3, 6, Figures 1-7 and claims 4, 5, 6, 38, 42, 46, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73, and claims 41, 45, 47-49, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72, as they depend on claims 4-6 and 38, wherein the fragment A-L is represented by [5-thioxo-5H-(1,2)-dithiol-3-yl]phenyloxycarbonyl and R is [4 or 5] imino-2-hydroxybenzoic acid, [4 or 5] amino-2-oxo-benzoic acid or [4 or 5] amino-2-hydroxyphenyl.