(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2013 (31.01.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/014659 A1

(51) International Patent Classification:
C07C 65/19 (2006.01) *A61P 1/00* (2006.01) *A61K 31/192* (2006.01)

(21) International Application Number:
PCT/IE2012/000037

(22) International Filing Date:
20 July 2012 (20.07.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/510,628 22 July 2011 (22.07.2011) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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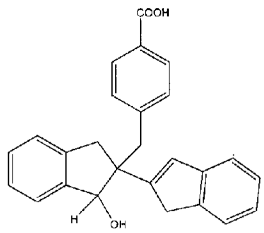
(54) Title: INDENE DERIVATIVES FOR USE IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE



(I)

(57) Abstract: Described are compounds of the structural formula (I): Also provided are pharmacologically acceptable isomers and salts of the compound of (I). The compounds are useful in the treatment of inflammatory bowel disease.

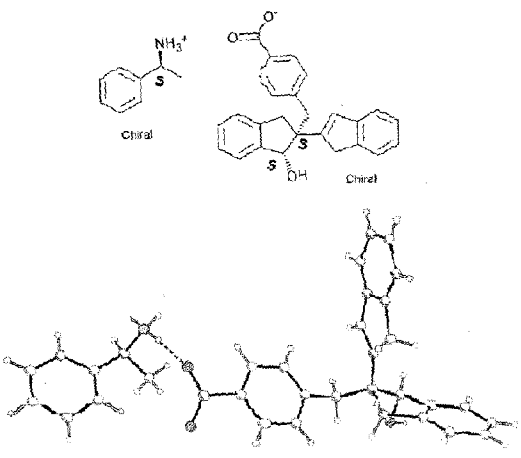


Fig. 2

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

"INDENE DERIVATIVES FOR USE IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE"

This invention relates to new compounds for use in the treatment of inflammatory bowel disease.

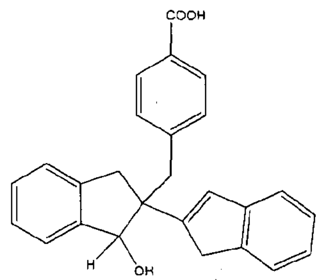
Introduction

Inflammatory bowel disease (IBD) consists of two idiopathic inflammatory diseases, ulcerative colitis (UC) and Crohn's disease (CD). The greatest distinction between UC and CD is the range of inflamed bowel tissue. Inflammation in CD is discontinuously segmented, known as regional enteritis, while UC is superficial inflammation extending proximally and continuously from the rectum. At present, the exact cause of IBD is unknown. The disease seems to be related to an exaggerated mucosal immune response to infection of the intestinal epithelium because of an imbalance of pro-inflammatory and immune-regulatory molecules. The inheritance patterns of IBD suggest a complex genetic component of pathogenesis that may consist of several combined genetic mutations. Currently no specific diagnostic test exists for IBD, but as an understanding of pathogenesis is improved so will our testing methods. Treatment of IBD consists of inducing and maintaining remission. IBD patients may be maintained on remission by use of a 5-aminosalycilate. However, while the use of aminosalycilates in UC provides considerable benefit, both in inducing remission in mild to moderate disease and in preventing relapse, the usefulness of these drugs to maintain remission in CD is questionable and is no longer recommended. The mainstay of treatment of active disease is a corticosteroid, commonly used for limited periods to return both UC and CD patients to remission, though budesonide, designed for topical administration with limited systemic absorption, has no benefit in maintaining remission. Alternatives, such as the immunosuppressive drugs azathioprine and mercaptopurine, together with methotrexate and cyclosporine have limited efficacy and the capability of inducing grave adverse effects. Anti-TNFα antibodies, such as infliximab and adalimubab, may be used in those patients unresponsive to standard immunosuppressive therapy. However, many patients fail to respond to anti-TNFα therapy, either due to their particular phenotype or by the production of autoantibodies.

Statements of invention

In accordance with the present invention there are provided compounds for use in the treatment of inflammatory bowel disease including Crohn's disease and ulcerative colitis.

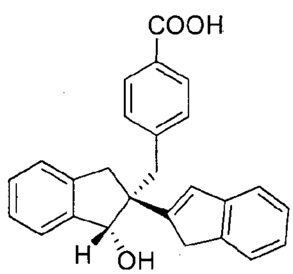
In particular, the present invention provides compounds of the structural formula I:



I

Also provided are pharmacologically acceptable isomers and salts of the compound of formula (I) – compound 1.

In particular, the present invention provides compounds of relative stereochemistry as demonstrated in structural formula II:



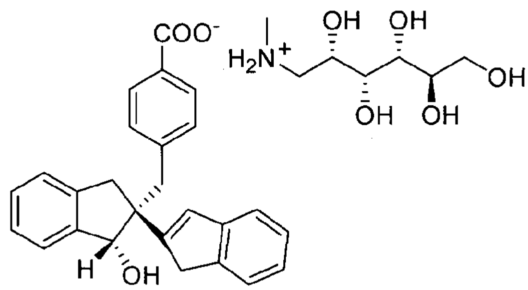
(II)

Also provided are pharmacologically acceptable salts of the compound of formula (II) – compound 2.

The active enantiomers have been characterised, spectroscopically, by their physical and chemical properties and by normal and chiral HPLC retention data.

A specific enantiomeric form has been found to be particularly useful for the treatment of IBD.

The invention also provides the N-Methyl-(D)-Glucamine salt of the compound of formula III:



(III, compound 6)

The compounds of the invention may crystallize in more than one form. This characteristic is referred to as polymorphism, and such polymorphic forms ("polymorphs") are within the scope of the invention. Polymorphism generally can occur as a response to changes in temperature, pressure, or both. Polymorphism can also result from variations in the crystallization process. Polymorphs can be distinguished by various physical characteristics known in the art such as x-ray diffraction patterns, solubility, and melting point.

Certain of the compounds described herein are capable of existing as stereoisomers. The scope of the present invention includes mixtures of stereoisomers as well as purified or enriched mixtures. Also included within the scope of the invention are the individual isomers of the compounds of the invention as well as any wholly or partially equilibrated mixtures thereof. Certain compounds of the invention contain one or more chiral centers. Therefore the present invention includes racemates, purified enantiomers, and enantiomerically enriched mixtures of the compounds of the invention. The compounds of the present invention include racemic and chiral indane dimers.

Salts encompassed within the term pharmaceutically acceptable salts refer to non-toxic salts of the compounds of this invention. Salts of the compounds of the present invention may comprise acid addition salts.

The invention includes a solvate of any of the compounds of the invention. The term solvate refers to a complex of variable stoichiometry formed by a solute (in this invention, a compound of the invention, or a salt or physiologically functional derivative thereof) and a solvent. Such solvents, for the purpose of the invention, should not interfere with the biological activity of the solute. Non-limiting examples of suitable solvents include, but are not limited to water, methanol, ethanol, and acetic acid. Preferably the solvent used is a pharmaceutically acceptable solvent. Non-limiting examples of suitable pharmaceutically acceptable solvents include water, ethanol, and acetic acid. Most preferably the solvent used is water.

The invention includes a prodrug of any of the compounds of the invention. The term prodrug refers to any pharmaceutically acceptable derivative of a compound of the present invention that, upon administration to a mammal, is capable of providing (directly or indirectly) a compound of the present invention or an active metabolite thereof. Such derivatives, for example, esters and amides, will be clear to those skilled in the art.

The invention further provides a pharmaceutical composition comprising any of the compounds described above.

The active compound may be present in the medicament for use in man at a suitable dose to achieve the desired effect. For example, the final dose may be between 0.1 and 10 mg/kg.

It may be possible to administer the compounds of the invention in the form of a bulk active chemical. It is however, preferred that the compounds be administered in the form of a pharmaceutical formulation or composition. Such formulations may comprise one or more pharmaceutically acceptable excipient, carrier or diluent.

The compounds of the invention may be administered in a number of different ways. The compounds may be administered orally. Preferred pharmaceutical formulations for oral administration include tablets, capsules, caplets, solutions, suspensions or syrups.

The pharmaceutical formulations may be provided in a form for modified release such as a time release capsule or tablet.

The medicament may be administered orally, parenterally, intra-nasally, trans-cutaneously or by inhalation.

Pharmaceutical formulations may be adapted for administration by any appropriate route, for example by an oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal, or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such formulations may be prepared by bringing into association the active ingredient with the carrier(s) or excipient(s).

Pharmaceutical formulations adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions, each with aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions. For oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Powders may be prepared by comminuting the compound to a suitable fine size and mixing with an appropriate pharmaceutical carrier such as an edible carbohydrate such as starch or mannitol. Flavorings, preservatives, dispersing agents, and coloring agents and the like may also be included.

Capsules may made by preparing a powder, liquid, or suspension mixture and encapsulating within gelatin or other suitable shell material. Lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate, or solid polyethylene glycol may be added to the mixture. A disintegrating or solubilizing agent such as calcium carbonate or sodium carbonate can also be added to improve the availability of the medicament when the capsule is ingested. Other agents such as binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Examples of suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol and the like. Suitable lubricants for these dosage forms include, for example, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Suitabel disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

Tablets may be formulated by preparing a powder mixture, granulating the mixture, adding a lubricant and disintegrant, and pressing into tablets. A powder mixture may be prepared by mixing the compound, suitably comminuted, with a diluent or base as described above. Optional ingredients include binders such as carboxymethylcellulose, aliginates, gelatins, or polyvinyl pyrrolidone, solution retardants such as paraffin, resorption accelerators such as a quaternary salt, and/or absorption agents such as bentonite, kaolin, or the like. The powder mixture can be wet-granulated with a binder such as syrup, starch paste, or solutions of cellulosic or polymeric materials, and pressing through a screen.

The compounds of the present invention can also be combined with a free flowing inert carrier and compressed into tablets directly without going through other steps such as granulating. A clear or opaque protective coating consisting of a sealing coat of a suitable material such as shellac, sugar or polymeric material, and a polish coating for example of wax can be provided. If appropriate colourants be added to these coatings to distinguish different unit dosages.

Oral fluids such as solutions, syrups, and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of the compound. Syrups can be prepared, for example, by dissolving the compound in a suitably flavored aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound in a non-toxic vehicle. Solubilisers and emulsifiers such as ethoxylated isostearyl alcohols and polyoxy ethylene sorbitol ethers, preservatives; flavor additives such as peppermint oil, or natural sweeteners, saccharin, or other artificial sweeteners; and the like can also be added.

Where appropriate, dosage unit formulations for oral administration can be microencapsulated. The formulation can also be prepared to prolong or sustain the release as for example by coating or embedding particulate material in suitable polymers, wax, or the like.

The compounds described herein and salts, solvates, and physiological functional derivatives thereof, can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The compounds of the invention and salts, solvates, and physiologically functional derivatives thereof may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled.

The compounds may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include, for example, polyvinylpyrrolidone (PVP). The compounds may also be coupled to a biodegradable polymer achieve controlled release of a drug. Such polymers include polylactic acid, polycyanoacrylates, and block copolymers of hydrogels.

Pharmaceutical formulations adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the skin/epidermis of a patient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis.

Pharmaceutical formulations adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols, or oils. For treatments of external tissues the formulations may be applied as a topical ointment or cream.

For topical administration in the mouth the formulation may include lozenges, pastilles, and mouthwashes.

For nasal administration, a powder having a particle size for example in the range 20 to 500 microns may be used. The powder may be administered by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical formulations adapted for administration by inhalation include fine particle dusts or mists, which may be generated by means of of metered dose pressurized aerosols, nebulizers, or insufflators and the like.

For rectal administration the formulation may be presented as suppositories or as enemas.

For vaginal administration the formulation may be in the form of pessaries, tampons, creams, gels, sprays or the like.

For parenteral administration the formulation may be aqueous and non-aqueous sterile injection solutions which may contain various additives such as anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and the like.

The compounds of the present invention and their salts, solvates, and physiologically functional derivatives thereof, may be employed alone or in combination with other therapeutic agents. The compound of the invention and the other pharmaceutically active agent(s) may be administered together or separately. If administered separately, administration may occur simultaneously or sequentially, in any order. The amounts of the compound of the invention and the other pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. The administration in combination of a compound of the invention salts, solvates, or physiologically functional derivatives thereof with other treatment agents may be in combination by administration concomitantly in either a single pharmaceutical composition including both compound or in separate pharmaceutical compositions each including one of the compounds. In some cases the combination of drugs may be administered separately in a sequential manner in which one agent is administered first and a second agent is administered second or the other way around. Such administration may be in a similar time frame or over longer time.

Brief description of the drawings

The invention will be more clearly understood from the following description thereof given by way of example only, in which:-

Fig. 1 is the X-ray crystal structure showing the absolute stereochemistry for the enantiomer compound 4 (R)-(+)-methylbenzylamine salt (compound **9**);

Fig. 2 is the X-ray crystal structure showing the absolute stereochemistry for the enantiomer compound 2 (S)-(-)-methylbenzylamine salt (compound **8**);

Fig. 2A is a view of a molecule of compound **8** from the crystal structure showing the numbering scheme employed. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius. Only the major disorder component is shown;

Fig. 3 is a graph of the effect of compounds **2, 3, 4** and **5** at 30 mg/kg on disease activity index (DAI) over 7 days in 5% DSS colitis;

Fig. 4 is a bar chart of the effect of compounds **2, 3, 4** and **5** at 30 mg/kg on disease activity index (DAI) at day 7 in 5% DSS colitis;

Fig. 5 is a graph of the effect of compounds **5, 7, 2** and **6** at 10 mg/kg on disease activity index (DAI) over 7 days in 5% DSS colitis;

Fig. 6 is a bar chart of the effect of compounds **5, 7, 2** and **6** at 10 mg/kg on disease activity index (DAI) at day 7 in 5% DSS colitis. Asterisks indicate a significant ($P<0.05$) difference (1 way ANOVA) from the vehicle control group.

Fig. 7 Is a graph showing the effect of compound **6** on weight loss in 5% DSS-treated mice. Data are Mean ± SEM from 6-7 mice per group;

Fig. 8 Is a graph showing the effect of compound **6** on DAI in 5% DSS-treated mice. Data are Mean ± SEM from 6-7 mice per group;

Fig. 9 Is a bar chart showing the effect of compound **6** on DAI in 5% DSS-treated mice on day 7. Data are Mean ± SEM. Asterisks indicate a significant (P<0.05) difference (1 way ANOVA) from the vehicle control group;

Fig.10 Is a bar chart showing the effect of compound **6** on Colon length of 5 % DSS-treated mice on day 7. Asterisks indicate a significant (P<0.05) difference (1 way ANOVA) from the vehicle control group;

Fig. 11 Shows representative haematoxylin and eosin-stained sections from distal colons of mice. Higher magnifications (X10) are shown;

Fig. 12 Is a bar chart showing the effect of compound **6** on histology scores of colons from DSS-treated mice. Data are Mean ± SEM from 5-6 mice. Asterisks indicate a significant (P<0.05) difference (1 way ANOVA) from the vehicle control group.. Note, maximum score 10;

Fig. 13 Is a bar chart showing myeloperoxidase (MPO) activity in the colons of untreated or vehicle, prednisolone and compound **6** treated mice exposed to 5% DSS. Data are Mean ± SEM from 5-6 mice. Asterisks indicate a significant (P<0.05) difference (1 way ANOVA) from the vehicle control group;

Fig. 14(A) to (C) are bar charts showing the effect of compound **6** on levels of cytokines (IL1β, TNFα and IL6) in mice treated with DSS. Data are Mean ± SEM from 5-6 mice. Asterisks indicate a significant (P<0.05) difference (1 way ANOVA) from the vehicle control group;

Fig. 15 Is a grph showing weight loss in IL10$^{--/--}$ mice treated with vehicle or compound **6**. Mice were administered compound **6** (300 mg/kg/week) or vehicle orally on a Monday/Wednesday/Friday (MWF) dosing schedule. Mice were ~4 weeks of age at start of experiment and were treated for 9 weeks. Mice were weighed weekly and data are presented as Mean ± SEM from 9-12 mice per group. Mice were monitored for overt disease, rectal prolapse, and moribund animals were humanely killed;

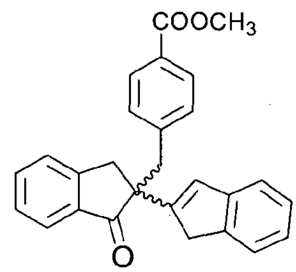
Fig. 16 Is a scatter graph representing Serum Amyloid A (SAA) levels of individual mice, and Mean (bar), from surviving animals at week 9 (11 and 9 mice in compound **6** or vehicle-treated groups, respectively). Student's t-test was used to test for statistical differences between groups;

Fig. 17 Are representative hematoxylin and eosin-stained sections from distal colons from IL10$^{--/--}$ mice treated for 9 weeks with vehicle or compound **6**; and

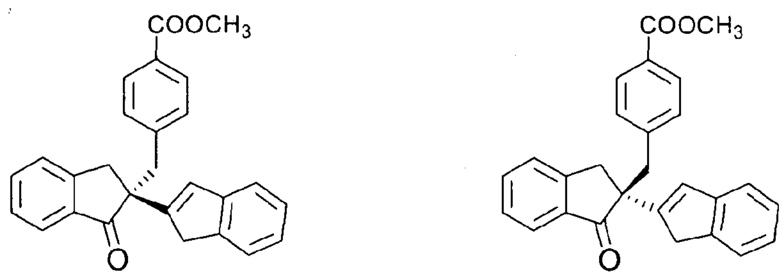
Fig. 18 Is a scatter graph showing histology scores of distal colons of IL10$^{-/--}$ mice treated with vehicle or compound **6**. Scatter graph representing histology score of individual mice, and Mean (bar), from surviving animals at week 9 (11 and 9 mice in compound **6** or vehicle-treated groups, respectively). Student's t-test was used to test for statistical differences between groups.

Detailed description of the invention

Compound 1 represents a pair of diastereoisomers that result from the reduction and demethylation of the ketone compound A which has a chiral centre at C-2, and is, as a result, a pair of enantiomers.

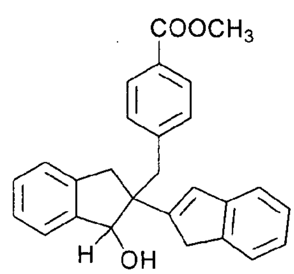


Compound A

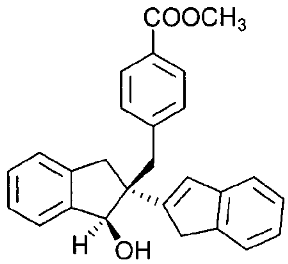


Enantiomers of compound A

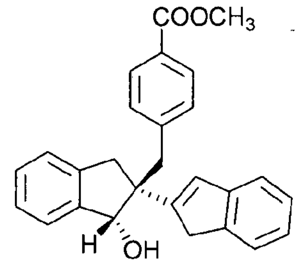
Reduction of this compound with $LiAlH_4$ yields a compound of the formula



This compound comprises two diastereoisomers:-

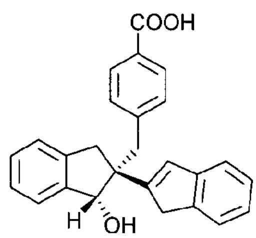


Diastereoisomer B

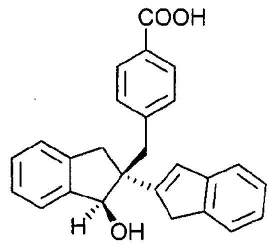


Diastereoisomer C

Hydrolysis of Diastereoisomer B gives rise to compounds **2** and **3**

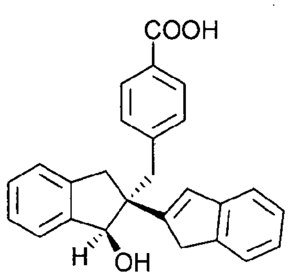


Compound **2**

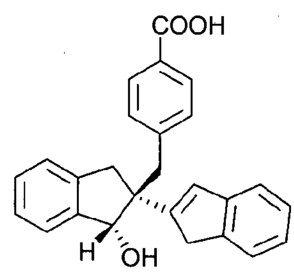


Compound **3**

Hydrolysis of Diastereoisomer C gives rise to compounds 4 and 5.



Compound **4**



Compound **5**

The diastereoisomers can be resolved chemically or chromatographically into their constituent enantiomers.

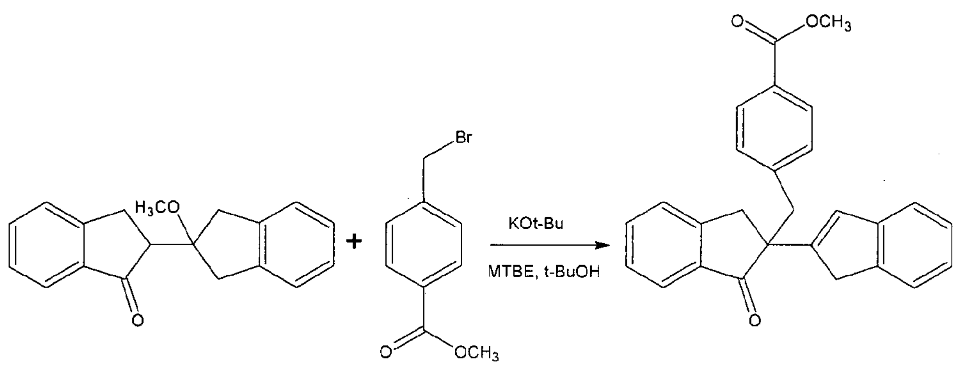
The absolute stereochemistry of compound **4** has been established by single crystal X-ray of compound 4 (R)-(+)-methylbenzylamine salt (compound **9**) (Fig. 1).

The absolute stereochemistry of compound **2** was confirmed by single crystal X-ray of compound 2 (S)-(-)-methylbenzylamine salt (compound **8**) (Fig. 2 and 2A).

General reaction procedures

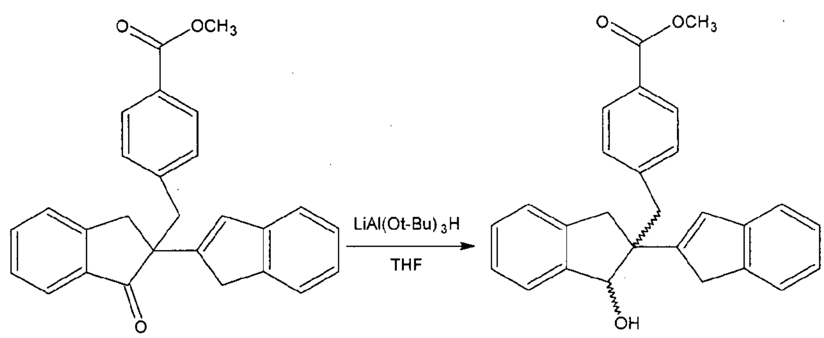
General synthetic procedures for the coupling of enantiomeric mixtures as exemplified below are described in WO9720806A, the entire contents of which are herein incorporated by reference.

General preparation of acid derivative compound A



To a stirred solution of the coupled product (4 mmol, 1.00 g) in *tert*-butanol (5 mL) and diethyl ether (30 mL) under nitrogen was added methyl (4-bromomethyl)benzoate (6 mmol, 1.41 g). To this was added a solution of potassium *tert*-butoxide in *tert*-butanol (30 mL) and diethyl ether (5 mL), slowly drop wise. With each drop, the mixture turned a yellow colour and it then reverted to its original grey colour. The mixture was stirred for a further 3 hours until the TLC (80:20 ,hexane:ethyl acetate) showed no more starting material. The reaction was quenched by the addition of sat. NH_4Cl. The layers were separated and the aqueous layer extracted with diethyl ether (2 x 120 mL). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated. The solid product precipitated from the crude on removal of most of the solvent. This was filtered off and washed with cold diethyl ether to give 0.98 g (62%) of a cream solid.

Reduction of methyl benzoate compound



To a stirred solution of the methyl benzoate compound (1.27 mmol, 0.50 g) in THF (15 mL) was added lithium tri-*tert*-butoxyaluminohydride (1.9 mmol, 0.48 g), slowly portion wise. The reaction was monitored by TLC (80:20, hexane:ethyl acetate) and after 3h, all of the starting material had been consumed.

The reaction was quenched by pouring onto ice and the crude product extracted into ethyl acetate by stirring the aqueous mixture for 10-15 min with ethyl acetate then pouring into a separatory funnel and then allowing it to separate. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated to give 0.34 g (68%) of a cream-tan solid. The product was isolated as a mixture of two diastereoisomers in an approximately 2:1 ratio.

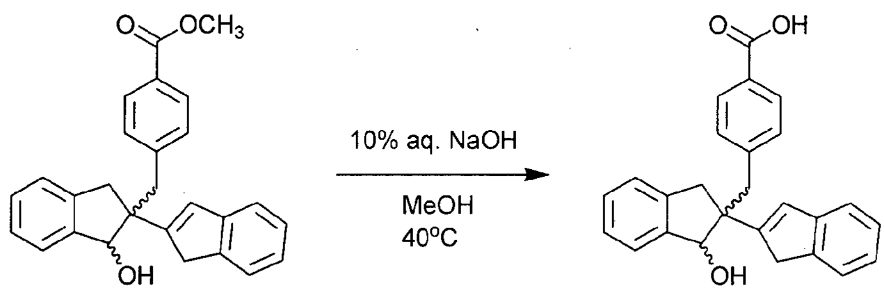
Analytical results for the mixture of two diastereoisomers

Purity (HPLC):	94.9% (as a 2:1 ratio of diastereoisomers)
δ_H(300 MHz, $CDCl_3$):	2.77-3.60 (6H, *m*, 3 x CH_2), 3.85 (3H, *s*, CH_3), [5.02 (1H, s, C**H**-OH)] 5.18 (1H, *s*, C**H**-OH), [6.23 (1H, *s*, C**H**=C)] 6.43 (1H, *s*, C**H**=C), 6.90-6.98 (2H, *m*, Ar-H), 7.11-7.21 (1H, *m*, Ar-H), 7.22-7.31 (5H, *m*, Ar-H), 7.36-7.42 (2H, *m*, Ar-H), 7.78-7.84 (2H, *m*, Ar-H).

Where possible, the value for the minor diastereoisomer is given in brackets.

δ_C(75.5 MHz, $CDCl_3$): 38.3(<u>C</u>H_2), 38.4(<u>C</u>H_2), 38.6(<u>C</u>H_2), 39.9(<u>C</u>H_2), 40.3(<u>C</u>H_2), 43.4 (<u>C</u>H_2), 51.9 (COO<u>C</u>H_3), 52.0 (COO<u>C</u>H_3), 55.9 (quat. <u>C</u>), 56.3 (quat. <u>C</u>), 82.0 (<u>C</u>H-OH), 82.8 (<u>C</u>H-OH), 120.5 (tert. <u>C</u>), 120.7 (tert. <u>C</u>), 123.5 (tert. <u>C</u>), 123.6 (tert. <u>C</u>), 124.0 (tert. <u>C</u>), 124.2 (tert. <u>C</u>), 124.5 (tert. <u>C</u>), 124.6 (tert. <u>C</u>), 124.8 (tert. <u>C</u>), 124.9 (tert. <u>C</u>), 125.1 (tert. <u>C</u>), 125.2 (tert. <u>C</u>), 126.1 (tert. <u>C</u>), 126.4 (tert. <u>C</u>), 127.0 (quat. <u>C</u>), 127.1 (quat. <u>C</u>), 128.0 (tert. <u>C</u>), 128.2 (tert. <u>C</u>), 128.5 (tert. <u>C</u>), 128.8 (tert. <u>C</u>), 129.0 (tert. <u>C</u>), 129.2 (tert. <u>C</u>), 129.5 (tert. <u>C</u>), 2 x 130.0 (2 x tert. <u>C</u>), 2 x 130.2 (2 x tert. <u>C</u>), 130.7 (tert. <u>C</u>), 140.4 (quat. <u>C</u>), 141.5 (quat. <u>C</u>), 142.8 (quat. <u>C</u>), 143.2 (quat. <u>C</u>), 143.5 (quat. <u>C</u>), 143.6 (quat. <u>C</u>), 143.7 (quat. <u>C</u>), 144.2 (quat. <u>C</u>), 144.3 (quat. <u>C</u>), 144.5 (quat. <u>C</u>), 150.4 (quat. <u>C</u>), 152.6 (quat. <u>C</u>), 167.0 (<u>C</u>=O), 167.2 (<u>C</u>=O).

<u>Hydrolysis of methyl benzoate moiety</u>



The ester was placed in a round-bottomed flask and 10% aq. NaOH (1 mL) was added to it followed by sufficient methanol to form a solution (6 mL). The solution was heated at 40 °C and monitored by TLC (80:20, hexane:ethyl acetate). After *ca.* 4h, no further ester was seen.

The mixture was cooled and sat. NH_4Cl added (solution at pH 12). Dilute HCl was added to acidic pH (pH 2). The product was extracted from the cloudy solution into ethyl acetate (3 x 10 mL). The combined extracts were dried over $MgSO_4$ and evaporated *in vacuo* to give 0.15 g (quantitative) of a cream solid. The product was isolated as a mixture of two diastereoisomers in an approximately 2:1 ratio.

Analytical results for the mixture of two diastereoisomers

Purity (HPLC): 95.2% (as a 2:1 ratio of diastereoisomers)

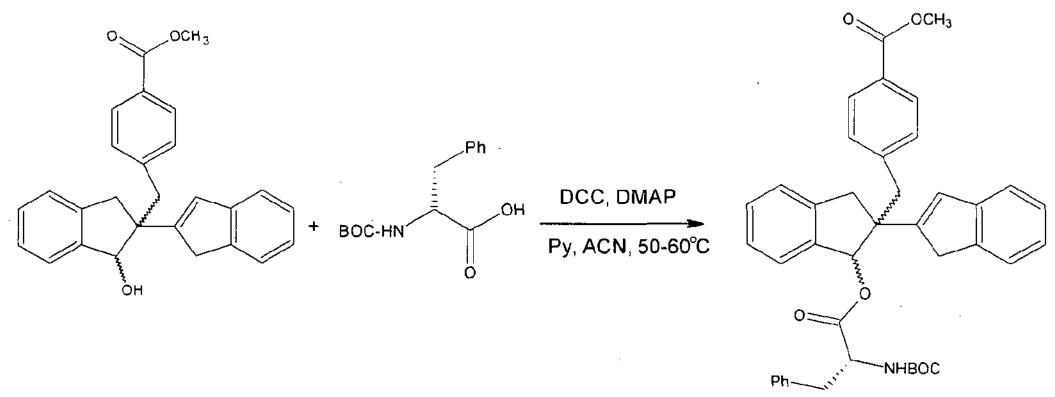
δ_H(400 MHz, $CDCl_3$): 2.81-3.59 (6H, *m*, 3 x CH_2), [5.05 (1H, *s*, C**H**-OH)], 5.23 (1H, *s*, C**H**-OH), 6.46 (1H, *s*, C**H**=C), [6.66 (1H, *s*, C**H**=C)], 6.95-7.03 (2H, *m*, Ar-H), 7.12-7.17 (1H, *m*, Ar-H), 7.21-7.29 (5H, *m*, Ar-H), 7.37-7.43 (2H, *m*, Ar-H), 7.85-7.91 (2H, *m*, Ar-H).

Where possible, the value for the minor diastereoisomer is given in brackets.

δ_C(100 MHz, $CDCl_3$): 37.9 (CH_2), 38.1 (CH_2), 38.2 (CH_2), 39.5 (CH_2), 39.9 (CH_2), 43.1 (CH_2), 55.5 (quat. C), 55.9 (quat. C), 81.6 (CH-OH), 82.4 (CH-OH), 120.2 (tert. C), 120.3 (tert. C), 123.1 (tert. C), 123.2 (tert. C), 123.5 (tert. C), 123.9 (tert. C), 124.1 (tert. C), 124.4 (tert. C), 124.5 (tert. C), 124.7 (tert. C), 125.9 (tert. C), 126.0 (tert. C), 126.5 (tert. C), 2 x 126.7 (quat. C & tert. C), 126.9 (quat. C), 128.1 (tert. C), 128.2 (tert. C), 128.4 (tert. C), 2 x 129.2 (2 x tert. C), 2 x 129.4 (2 x tert. C), 2 x 129.8 (2 x tert. C), 2 x 129.9 (2 x tert. C), 130.4 (tert. C), 140.0 (quat. C), 141.0 (quat. C), 142.3 (quat. C), 142.7 (quat. C), 143.0 (quat. C), 143.2 (quat. C), 143.8 (quat. C), 144.0 (quat. C), 144.1 (quat. C), 144.7 (quat. C), 150.0 (quat. C), 152.0 (quat. C), 170.8 (C=O), 171.1 (C=O).

Chemical separation of enantiomers

Preparation of N-BOC D-phenylalanine derivative of methyl benzoate diastereoisomer and/or separation of subsequent diastereoisomers α1 and α2 (or β1 and β2)



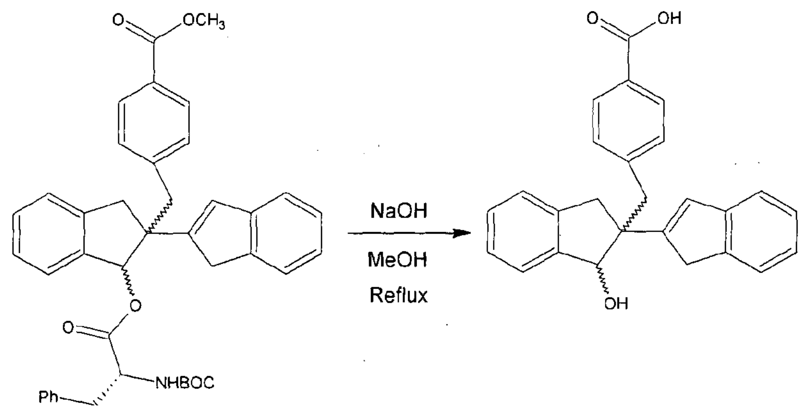
Note: procedure applicable to both diastereoisomers but the example given is for the first diastereoisomer.

Diastereoisomer A (2.5 mmol, 1.0 g) and N-BOC D-phenylalanine (3.1 mmol, 0.8 g) were placed in a round bottom flask fitted with a condenser and suspended in CH_3CN (25 mL) under nitrogen. To this suspension was added pyridine (3.1 mmol, 0.3 mL) followed by a solution of DCC (3.1 mmol, 0.7 g) and DMAP (10% mol, 0.25 mmol, 0.05 g) in CH_3CN (2 mL). The mixture was stirred for 20 h at 50°C, and then allowed to reach room temperature.

The white solid was filtered off and the solvent removed *in vacuo*. Ethyl acetate was added and the solution obtained was washed with 10% H_2SO_4, sat. $NaHCO_3$, dried over $MgSO_4$ and evaporated to give 2.1 g of a yellow oil (83% pure by HPLC, yield: quantitative).

The diastereoisomers α1 and α2 were separated by flash chromatography (90 g of silica/g of product) using hexane/MTBE 90:10. From 4.17 g of mixture, 1.3 g of α2, derivative was obtained (as well as 1.71 g of the α1 derivative and 0.3 g as a mixture of both).

<u>Hydrolysis of N-BOC D-phenylalanine derivative of methyl benzoate compound (α1, α2, β1 or β2)</u>



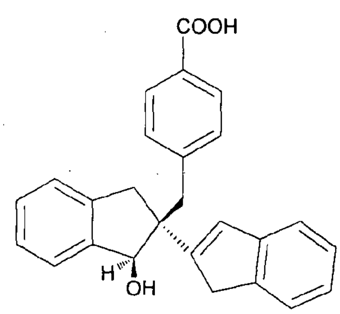
The diastereoisomer α2 (2.3 mmol, 1.45 g) was dissolved in methanol (25mL) and NaOH (11.5 mmol, 0.45 g) was added and the mixture stirred at reflux temperature and monitored by TLC. After 20h, the starting material was consumed.

The reaction was cooled to room temperature and quenched by addition of sat. NH_4Cl. The methanol was removed *in vacuo* and the aqueous solution acidified to pH 1 with conc. HCl. The product was extracted with ethyl acetate, dried over $MgSO_4$ and evaporated to give 1.6 g of a yellow gum, which was purified by a short silica column with hexane:MTBE 80:20 as eluent. 0.44 g of acid derivative compound 5 (50% yield) was obtained which was 97.2% pure by HPLC.

Note: An alternative hydrolysis was also carried out using 10% aqueous NaOH in methanol at 40-50 °C. This procedure took almost 5 days to go to completion.

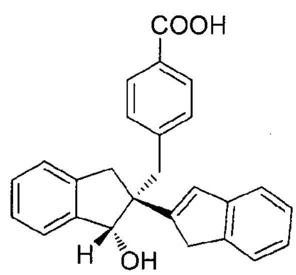
Analytical results for enantiomers α1, α2, β1, β2

Enantiomer β1 from diastereoisomer B - compound 3



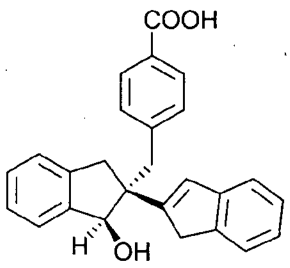
Description:	Cream amorphous solid
Melting point	195-196°C
[α] D:	+98.51 (1.07%, MeOH)
Purity:	99.0%
δ_H(400 MHz, $CDCl_3$):	2.87 (1H, *d*, J=13.28 Hz, C<u>H</u>$_2$), 3.00-3.09 (2H, *m*, C<u>H</u>$_2$), 3.29 (1H, *d*, J=13.36 Hz, C<u>H</u>$_2$), 3.43-3.61 (2H, *m*, C<u>H</u>$_2$), 5.27 (1H, *s*, C<u>H</u>-OH), 6.49 (1H, *s*, C<u>H</u>=C), 7.00 (2H, *d*, J=7.88 Hz, Ar-<u>H</u>), 7.16-7.32 (6H, *m*, Ar-<u>H</u>), 7.44 (2H, *d*, J=7.24 Hz, Ar-<u>H</u>), 7.90 (2H, *d*, J=7.92 Hz, Ar-<u>H</u>).

Enantiomer β2 from diastereoisomer B - compound 2



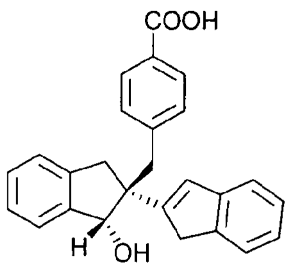
Description:	Cream amorphous solid
Melting point	184-185°C
[α] D:	-114.44 (0.18%, MeOH)
Purity:	99.8%
δ_H(400 MHz, $CDCl_3$):	2.87 (1H, *d*, J=13.32 Hz, C<u>H</u>$_2$), 3.00-3.09 (2H, *m*, C<u>H</u>$_2$), 3.29 (1H, *d*, J=13.28 Hz, C<u>H</u>$_2$), 3.46 (1H, *d*, J=22.64 Hz, C<u>H</u>$_2$), 3.58 (1H, *d*, J=22.56 Hz, C<u>H</u>$_2$), 5.27 (1H, *s*, C<u>H</u>-OH), 6.49 (1H, *s*, C<u>H</u>=C), 7.00 (2H, *d*, J=8.04 Hz, Ar-<u>H</u>), 7.15-7.34 (6H, *m*, Ar-<u>H</u>), 7.44 (2H, *d*, J=7.20 Hz, Ar-<u>H</u>), 7.90 (2H, *d*, J=8.04 Hz, Ar-<u>H</u>).

Enantiomer α1 from diastereoisomer C - compound 4



Description:	Cream solid
Melting point	136 -140°C
[α] D:	-39.3 (0.66%, MeOH)
Purity:	94.0%
δ_H(400 MHz, $CDCl_3$):	2.90-3.59 (6H, *m*, 3 x C$\underline{H_2}$), 5.08 (1H, *s*, C$\underline{H}$-OH), 6.70 (1H, *s*, C$\underline{H}$=C), 7.05 (2H, *d*, J=8.08 Hz, Ar-$\underline{H}$), 7.19 (1H, *t*, J=7.34 Hz, Ar-$\underline{H}$), 7.26-7.47 (7H, 2 x *m*, Ar-$\underline{H}$), 7.93 (2H, *d*, J=8.08 Hz, Ar-$\underline{H}$).

Enantiomer α2 from diastereoisomer C - compound 5



Description:	Cream amorphous solid
Melting point	195-196°C
[α] D:	+32.1 (1.18%, MeOH)
Purity:	97.2%

δH(400 MHz, $CDCl_3$): 2.94-3.59 (6H, *m*, 3 x C<u>H</u>$_2$), 5.08 (1H, *s*, C<u>H</u>-OH), 6.70 (1H, *s*, C<u>H</u>=C), 7.05 (2H, *d*, J=8.12 Hz, Ar-<u>H</u>), 7.19 (1H, *t*, J=7.34 Hz, Ar-<u>H</u>), 7.26-7.47 (7H, 2 x *m*, Ar-<u>H</u>), 7.93 (2H, *d*, J=8.12 Hz, Ar-<u>H</u>).

<u>HPLC method</u>

Achiral and Chiral HPLC methods were established for the qualitative and quantitative separation of enantiomers compounds **2, 3, 4, 5**.

<u>HPLC resolution of enantiomers</u>

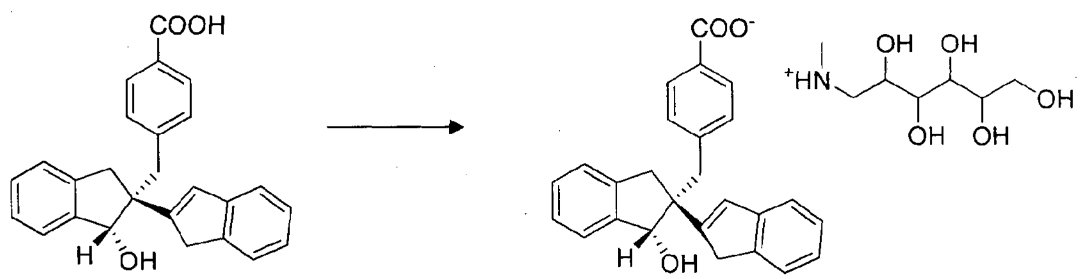
Reverse phase method	
Column	Hypersil BDS C18, 5μ, 250 x 4.6 mm Phenomenex Luna C18, 5μ, 250 x 4.6 mm, N:32
Wavelength	210 nm
Flow rate	1 mL/min (for ketone and esters) 0.6 mL/min (for acids and salts)
Mobile phase	70:30 CH_3CN : 0.1% aq. Acetic acid
Sample	1 mg/mL, made up in mobile phase (or CH_3CN:dIW=50:50 for acids/salts)
Retention times	Compound 1 - 20 min
	Diastereoisomers C (compounds 4/5) 9 min
	Diastereoisomers B (compounds 2/3) 10 min
Chiral method	
Column	ChiralPack IC, 5 μ, 250 x 4.6 mm
Wavelength	210 nm

Temperature	25 °C
Flow rate	0.35 mL/min
Mobile phase	n-Heptane/IPA/HOAc (or TFA)=90/10/0.1
Sample	1 mg/mL, made up in mobile phase (or nHeptane/IPA/MeOH = 81/9/10 for salts)
Retention times	Compound A 54 min and >60 min
	Compound **4** - 30 min
	Compound **5** – 37 min
	Compound **3** - 18 min
	Compound **2** - 19 min

Salt formation

Salts were prepared by dissolving the free acid of compounds **2, 3, 4** and **5** in aqueous or aqueous organic solvent in the presence of the appropriate base and isolating the salt by evaporation of solvent.

Compound 6: The N-Methyl-(D)-Glucamine salt (NMDG) of compound **2**.



Compound 2 Compound 6

Compound 6 physiochemical properties:

Appearance:	Off-white solid
Molecular Weight:	577 (free acid: 382)
Molecular Formula:	$C_{33}H_{39}O_8N$ (free acid: $C_{26}H_{22}O_3$)
Melting Point:	165-167 °C
Compound 6:	$[\alpha]_D$:-76.5 (sample concentration: 200 mg/10 ml in Water)
Mass (Da):	ES+ only [NMDG + Na] was visible
Elemental analysis:	Calc: C (68.61), H (6.80), N (2.42), O (22.16). Found: C (68.44), H (6.80), N (2.50), O (21.98)
δ_H(400 MHz, DMSO):	2.48 (3H, apparent *s*, NC<u>H</u>$_3$), 2.65 (1H, *d*, J=13.56 Hz, HC<u>H</u>), 2.84-3.02 (4H, *m*), 3.16 (1H, *d*, J=13.60 Hz, HC<u>H</u>), 3.40-3.70 (7H, *m*), 3.85-3.92 (1H, *m*), 5.06 (1H, *s*, C<u>H</u>-OH), 5.93 (1H, broad *s*, CH-O<u>H</u>), 6.41 (1H, *s*, C<u>H</u>=C), 6.80 (2H, *d*, J=7.92 Hz, Ar-<u>H</u>), 7.06-7.41 (8H, *m*, Ar-<u>H</u>), 7.64 (2H, *d*, J=7.80 Hz, Ar-<u>H</u>).
δ_C(100 MHz, DMSO):	33.8 (<u>C</u>H$_3$), 37.9 (<u>C</u>H$_2$), 38.2 (<u>C</u>H$_2$), 39.5 (<u>C</u>H$_2$), 51.6 (<u>C</u>H$_2$-N), 55.8 (quat. <u>C</u>), 63.5 (<u>C</u>H$_2$-O), 69.0 (<u>C</u>H-O), 70.3 (<u>C</u>H-O), 70.6 (<u>C</u>H-O), 71.3 (<u>C</u>H-O), 81.1 (<u>C</u>H-OH), 120.1 (tert. <u>C</u>), 123.4 (tert. <u>C</u>), 123.7 (tert. <u>C</u>), 124.3 (tert. <u>C</u>), 124.4 (tert. <u>C</u>), 126.1 (tert. <u>C</u>), 126.3 (tert. <u>C</u>), 127.0 (tert. <u>C</u>), 127.5 (tert. <u>C</u>), 2 x 128.5 (2 x tert. <u>C</u>), 2 x 129.1 (2 x tert. <u>C</u>), 140.4 (quat. <u>C</u>), 141.1 (quat. <u>C</u>), 142.9 (quat. <u>C</u>), 144.5 (quat. <u>C</u>), 145.2 (quat. <u>C</u>), 154.3 (quat. <u>C</u>), 170.4 (<u>C</u>=O).

<u>X – ray studies</u>

The absolute stereochemistry of compound **2** was established by single crystal X-ray analysis of its (*S*)-(-)-methylbenzylamine salt (compound **8**). The results are given in Appendix 2. The results were in agreement with the stereochemistry shown in Fig. 2. The absolute stereochemistry of compounds **4** and **5** were established by conversion of the alcohols (compounds **2-5**) to their ketenes and by correlation of their optical rotations.

<u>Inflammatory Bowel Disease (IBD)</u>

Inflammatory Bowel Disease (IBD) consists of two idiopathic inflammatory diseases, Ulcerative Colitis (UC) and Crohn's Disease (CD). The greatest distinction between CD and UC is the range of inflamed bowel tissue. Inflammation in CD is discontinuously segmented, known as regional enteritis, while UC is superficial inflammation extending proximally and continuously from the rectum. At present the cause of IBD is unknown. The disease seems to be related to an exaggerated mucosal immune response to infection of the intestinal epithelium because of an imbalance of pro-inflammatory and immune-regulatory molecules. The inheritance of patterns of IBD, suggest a complex genetic component of pathogenesis that may consist of several combined genetic mutations. Currently no specific diagnosis exists for IBD, but as an understanding of pathogenesis improves so will testing methods. Treatment of IBD consists of inducing and maintaining remission. IBD patients may be maintained on remission by use of a 5-aminosalycilate. However, while the use of aminosalycilates in UC provides considerable benefit, both in inducing remission in mild to moderate disease and in preventing relapse, the usefulness of these drugs to maintain remission in CD is questionable and is no longer recommended. The mainstay of treatment of active disease is a corticosteroid, commonly used for limited periods to return both UC and CD patients to remission, though budesonide, designed for topical administration with limited systemic absorption, has no benefit in maintaining remission. Alternatives, such as the immunosuppressive drugs azathioprine and mercaptopurine, together with methotrexate and cyclosporine have limited efficacy and the capability of inducing grave adverse effects. Anti-TNFα antibodies such as infliximab and adalimubab may be used in those patients unresponsive to standard immunosuppressive therapy. However, many patients fail to respond to anti-TNFα therapy, either due to their particular phenotype or by the production of autoantibodies.

Acute murine DSS colitis model

The dextran sodium sulphate (DSS) colitis model is an experimental mouse model that exhibits many of the symptoms observed in human UC, such as diarrhoea, bloody faeces, mucosal ulceration, shortening of the colon, weight loss and alterations in certain colon cytokines. The study is widely used as a model for studying the pathogenesis of UC and also for screening new therapeutic interventions for the treatment of UC.

In these studies, an acute colitis model was used, with 5% DSS administered in the drinking water of BALB/c mice. This dosage regime induces severe acute colitis, by days 7-8 mice had overt rectal bleeding and marked weight loss; unless sacrificed beforehand, all mice would have died by days 10-12.

Mice

Specific Pathogen-Free BALB/c mice, 6-8 weeks of age, were obtained from a commercial supplier (Harlan UK). Mice were fed irradiated diet and housed in individually ventilated cages (Tecniplast UK) under positive pressure.

DSS treatment

DSS (5%) was dissolved in drinking water. Compounds were administered orally at a dose of 10 mg/kg or 30 mg/kg on days 0-7, and mice were culled on day 8 or day 9, depending on the severity of the disease. The mice were checked each day for morbidity and the weight of individual mice was recorded. Induction of colitis was determined upon autopsy, length of colon and histology. Colons were recovered and stored at -20 °C for immunological analysis. All of the compounds and experimental groups are randomly alphabetically labelled. Throughout experiments all data recording was performed in a blind manner. The codes on boxes / groups were not broken until after the data was analysed i.e. boxes labelled A, B, C etc were identified as untreated, DSS-treated, or DSS + compound-treated.

To quantify the extent of colitis, a disease activity index (DAI) was determined based on weight loss, faecal blood and stool consistency. A score was given for each parameter, with the sum of the scores used as the DAI. For each treatment group n=8.

Description of DAI			
Score	***Weight loss %***	***Stool consistency***	***Faecal blood***
0	*None*	*Normal*	*None*
1	*1-3*		
2	*3-6*	*Loose stool*[1]	*Visible in stool*
3	*6-9*		
4	*>9*	*Diarrhea*[2]	*Gross bleeding*[3]

Definitions:

[1]Loose stool – stool not formed, but becomes a paste on handling.

[2]Diarrhea – no stool formation, fur stained around the anus.

[3]Gross bleeding- fresh blood on fur around the anus with excessive blood in the stool.

Administration of compounds

All compounds were prepared for oral gavage (0.1 mL per os (p.o.) per 10 g body weight) as a suspension in 0.5% carboxymethyl cellulose/2% Tween 80, at a dose of 3-30 mg/Kg. Compounds as free acid were initially dissolved in absolute alcohol and diluted with 14+1 with 0.5% carboxymethyl cellulose/2% Tween 80; this resulted in a fine precipitate in suspension while N-Methyl-(D)-Glucamine salts were soluble in the vehicle alone.

Effect of individual enantiomers compounds 2, 3, 4 and 5 in 5% DSS murine colitis

BALB/c given 5% DSS in drinking water were administered compounds 2, 3, 4 and 5 at 30 mg/kg p.o. as a suspension in 0.5% carboxymethyl cellulose/ 2% Tween 80 daily for 7 days. DAI measures the extent of the disease in this model. Compound **4** was without activity on this variable, there not being any significant (P>0.05) difference in DAI at any time point (Fig. 3). At day 7, both compound **2** and compound **5** significantly (P<0.5) reduced DAI by a considerable margin, from 9.0 ± 0.53 for vehicle controls to 3.2 ± 0.73 for compound **5** and 2.5 ± 0.71 for compound **2**, there being no significant difference between the two (Fig.4). In comparison, compound **3** reduced DAI to only 5.3 ± 0.6. This was significantly (P>0.05) less potent than either compound **2** or compound **5**. Further, while the DAI in compound **3**-treated mice was statistically (P<0.05) less than vehicle controls at day 7 (Fig. 4), at day 6 there was no statistical (P>0.05) difference between compound **3** and vehicle (Fig. 3). In conclusion, of the four enantiomers, compounds **2, 3, 4** and **5** both compounds **2** and **5** are highly active in this model at 30 mg/kg. Compound **3** has minimal activity which is significantly (P<0.05) less than compound **2** and compound **5**. Compound **4** is almost devoid of activity in this 5% DSS murine colitis model.

Selection of a salt of compounds **2** and **5**

As a consequence of the limited aqueous solubility of the enantiomers compound **2** and compound **5**, we attempted the synthesis of five salts of compound **5**. The sodium salt, potassium salt, calcium salt, α-methylbenzylamine salt and N-Methyl-(D)-Glucamine salt were synthesised.

The sodium and calcium salt were unsuccessful. The three salts of compound 5, named potassium salt, α-methylbenzylamine salt and N-Methyl-(D)-Glucamine salt were used for solubility and partition coefficient (logP) studies.

The solubility of the four compounds was determined:

Compound	Milli-RO H_2O µg/mL	pH 4.0 Buffer µg/mL	pH 7.0 Buffer µg/mL	pH 9.0 Buffer µg/mL
Compound **5**	1.38	0.33	320.1	369.6
Compound **5** Potassium salt	217.0	0.15	54.71	340.3
Compound **5** Methyl-benzylamine salt	413.9	0.20	227.4	311.0
Compound **5** N-Methyl-D-Glucamine salt	>60,000 *	0.14	>60,000*	>60,000*

*Estimated value

Compound **5** N-Methyl-(D)-Glucamine salt (compound **7**) was determined, surprisingly, to be the most soluble compound from this group of analogous compounds by a considerable margin, with a solubility of >60,000 µg/mL in Milli-RO water, 0.14 µg/mL in pH 4 buffer, >60,000 µg/mL in pH 7.0 and >3,000 µg/mL in pH 9.0 buffer. Almost identical values were obtained with compound **2** N-Methyl-(D)-Glucamine (compound **6**) with a solubility of >60,000 µg/mL in Milli-RO water, 0.5 µg/mL in pH4 buffer, >60,000 µg/mL in pH 7.0 and >3,000 µg/mL in pH 9.0 and buffer.

The partition coefficient of compound **5** and related analogous compounds was investigated using the HPLC method (reverse phase C18 HPLC column) at neutral, acidic and alkaline pH.

The partition coefficient of the four compounds was determined:

Compound	Neutral	Basic	Acid

	Log10 POW	Log10 POW	Log10 POW
Compound **5**	3.7	3.7	3.9
Compound **5** Potassium salt	3.7	3.7	3.9
Compound **5** Methyl-benzylamine salt	3.6	3.6	3.9
Compound **5** N-Methyl-D-Glucamine salt	3.5	3.5	3.8

The partition coefficient of each salt of compound **5** was found to be similar. It is suggested that this is happening because when the salt is in solution the compound dissociates into the parent compound **5** and the associated salt ion. As a result of this the measured partition coefficient was from the parent ion rather than the salt molecules.

The partition coefficient (Log10 POW) of compound **2** N-Methyl-D-Glucamine salt (compound **6**) was successfully determined in neutral, basic and acidic conditions as 3.5, 4.3 and 2.6 respectively.

N-Methyl-(D)-Glucamine was chosen as the salt candidate for both compound **2** and compound **5**.

<u>Effect of enantiomers compound **2** and compound **5** and their N-Methyl-(D)-Glucamine salts (compounds **6** and **7**) at 10 mg/kg in 5% DSS murine colitis</u>

Given that both compounds **2** and **5** show considerable activity in the 5% DSS model at 30 mg/kg, we then re-examined their activity, together with their N-Methyl-(D)-Glucamine salts at the lower dose of 10 mg/kg, given daily for 7 days as a suspension or solution in 0.5% carboxymethyl cellulose/2% Tween 80. No adjustment was made in the dosages of the salts to compensate for their increased molecular weight. Both compounds **5** and **7**, at 10 mg/kg, had no significant (P>0.05) effect on DAI in the 5% DSS murine colitis model when compared to vehicle control (see Fig. 5). In contrast, at day 7, both compound **2** and compound **6**, the N-

Methyl-(D)-Glucamine salt, at 10 mg/kg significantly ($P<0.05$) and potently reduced DAI from 9.3 ± 0.51 (vehicle) to 2.1 ± 0.7 and 3.3 ± 0.52 respectively (Fig. 6).

In conclusion, compound **2** (and its N-Methyl-(D)-Glucamine salt, compound **6**) is the most potent of the four enantiomers by a considerable margin, and the only enantiomer to retain activity at the lower dose level of 10 mg/kg.

<u>Effect of a range of doses of compound **6** and a comparison with Prednisolone on 5% DSS murine colitis</u>

Compound **6** was selected as the most favoured enantiomer. The activity of compound **6** in the 5% DSS murine model of colitis at varying dose levels was tested to ascertain if there was a dose/response relationship and to make a comparison with a potent oral steroid, Prednisolone, commonly used to return patients suffering from acute exacerbations of IBD to remission.

Mice were administered compound **6** at dose levels 3, 10 and 30 mg/Kg (equivalent to 6.6-20 mg/Kg of the compound **2**). A group of DSS-treated mice was also treated with prednisolone, 5 mg/Kg. Prednisolone is a corticosteroid in clinical use in the treatment of human IBD and the quantity used in this study is the optimal dose of prednisolone for this model. After 3 days of treatment of BALB/c mice with 5% DSS in the drinking water signs of colitis were apparent. This was manifested as weight loss (Fig. 7) and an increase in the disease DAI (Fig. 8). However, following oral administration daily for 7 days, compound **6** at three doses (3, 10 and 30 mg/Kg) caused no overt reactions in mice. Compound **6** ameliorated the severity of colitis following acute DSS treatment in multiple parameters of disease examined. The capacity of compound **6** to ameliorate disease in the DSS model was dose-dependent. Compound **6** at 30 mg/Kg was therapeutic in the DSS model at a comparable, or better, efficacy relative to prednisolone at 5 mg/Kg.

The severity of these symptoms are progressive; by day 7 the DSS-treated mice have lost up to 15% of their body weight and all mice have perfuse rectal bleeding. The DAI values on the day of autopsy showed that mice treated with compound **6** 3-30 mg/kg had at each dose level, a significantly ($P<0.05$ – $P<0.01$) lower DAI than vehicle controls. Prednisolone (5 mg/kg) also significantly ($P<0.01$; ANOVA; Dunnett Multiple Comparison Test), reduced DAI scores (Fig. 9).

At autopsy on day 7, there was significant shortening of colon length ($P<0.05$ – $P<0.01$; ANOVA; Dunnett Multiple Comparison Test) in all DSS treated groups compared to colons from mice not treated with DSS (Fig. 10). The lowest dose of 3 mg/kg of compound **6** did not have a significant effect in inhibiting colon shortening when compared to vehicle controls whereas the 10 and 30 mg/kg groups and the Prednisolone group did have a significant effect. Compound **6** at 30 mg/kg was significantly better than Prednisolone ($P<0.05$; ANOVA; Dunnett Multiple Comparison Test) (Fig. 10).

Following DSS treatment histology sections of the distal colon showed extensive crypt damage and cell infiltration (Fig. 11).

The extent of colon damage was quantified using an arbitrary scoring system. Compound **6** at both 10 and 30 mg/Kg, caused a dose-dependent and highly statistically significant reduction ($P<0.01$; Kruksal-Wallis ANOVA; Dunnett Multiple Comparison Test) in colon pathology relative to the vehicle group. In contrast, there was no significant improvement in histology scores with the prednisolone (5 mg/Kg) treated group relative to vehicle-treated mice (Fig. 12).

Consistent with the histology results showing inflammation in the colons of mice, there was a significant ($P<0.001$; Kruksal-Wallis ANOVA; Dunnett Multiple Comparison Test) elevation in colon myeloperoxidase (MPO) activity in DSS-treated mice administered vehicle only. Colonic myeloperoxidase activity (MPO), representing the level of inflammatory neutrophil cell infiltration into the gut wall which was increased by almost 8-fold by DSS treatment but was significantly ($P<0.05$) reduced by both compound **6** at 30 mg/kg and Prednisolone, at 63% and 54% respectively by day 7 (Fig. 13).

Quantification of levels of colon cytokines showed that DSS-treatment induces elevated IL1β (Fig. 14(a)), TNFα (Fig. 14(b)) and IL6 (Fig. 14(c)), to 0.744 ± 0.076 ng/mg, 1.478 ± 0.378 ng/mg and 1.057 ± 0.1784 ng/mg respectively. In each case, compound **6** caused a significant

(P<0.05, 30 mg/kg) and dose-dependant reduction in these cytokine levels. Prednisolone (5 mg/kg) also reduced (p<0.05) these increases in cytokine levels; for each cytokine there was no significant difference between the effect of prednisolone 5 mg/kg and compound **6** at the higher dose level of 30 mg/kg at day 7

In summary, following oral administration daily for 7 days, compound **6** at three doses (3, 10 and 30 mg/Kg) caused no overt reactions in mice. Compound **6** ameliorated the severity of colitis following acute 5% DSS treatment by multiple parameters of disease examined and the capacity to ameliorate the disease is dose-dependent. Further, compound **6** at 30 mg/Kg was therapeutic in the DSS model at a comparable or better efficacy, relative to prednisolone (5 mg/Kg).

Chronic $IL10^{-/-}$ Model

Mice with a deletion in the $IL10^{-/-}$ gene spontaneously develop chronic colitis, with the age of onset and the severity of the disease being dependent on background mouse strain and the conditions in which the animals are housed. The onset of colitis in $IL10^{-/-}$ mice housed under the conditions used in this study was also strain dependent, with an earlier onset and greater severity, in terms of mortality, in BALB/c strain mice relative to C57BL/6 strain animals. In this experiment, animals received oral treatment on a MWF regime over 9 weeks. Initially, both groups of mice progressively gain weight (Fig. 15). Vehicle treated mice stopped gaining weight from week 5 of treatment, whereas compound **6**-treated mice maintained weight gain until week 8. By week 9 animals had marked weight loss, with one moribund animal humanely killed on day 60 in each group. As other mice were losing weight and developing clinical symptoms of disease, both groups were culled at week 9 (day 63) and analysed. While there were greater mortalities in the vehicle-treated group (25%) relative to compound **6** treated mice (9.2%) by Kaplan-Meier analysis, there was no statistical difference in survival of $IL10^{-/-}$ mice over the 9 weeks.
Serum was recovered from mice and Serum Amyloid A (SAA) and was analysed as a marker for severity of colitis. There were significantly (*P<0.05*; Student's t-test) reduced SAA levels in compound **6** treated mice relative to vehicle treated $IL10^{-/-}$ mice (Fig. 16).

Histology sections of colons from $IL10^{-/-}$ mice treated with vehicle or compound **6** are shown in Fig. 17.

Histology sections of colons from IL10$^{-/-}$ mice treated with vehicle or compound **6** were scored. The extent of colon pathology was significantly reduced (*P<0.05*; Student's t-test) in IL10$^{-/-}$ mice receiving compound relative to mice treated with vehicle (Fig. 18).

In summary, oral treatment with compound **6** (300 mg/kg/week) in IL10$^{-/-}$ BALB/c strain mice, using a MWF regime over 9 weeks, delayed weight loss and reduced deaths from colitis relative to vehicle-treated mice. In this model of chronic colitis, compound **6** significantly reduced disease indices with respect to a serum marker of colon inflammation and the degree of inflammation and damage to the colon. This is particularly noteworthy in view of the fact that the plasma half-life ($t_{1/2}$) for compound **6** is 3 hours in the rat. With the standard MWF dosing schedule, mice will have been unexposed to compound **6** for substantial periods during the experiment.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.

Appendix 1

List of abbreviations used

aq	aqueous
b.p.	boiling point
$CDCl_3$	chloroform-d
$CH(OCH_3)_3$	trimethylsilyl orthoformate
CO_2	carbon dioxide
DCM	dichloromethane
dIW	distilled ionized water
DMSO	dimethyl sulphoxide
Et_2O	ether
EtOH	ethanol
H_2O	water
HCl	hydrochloric acid
IR	infra red
IPA	isopropyl alcohol
KCl	potassium chloride
M	molar
min	minutes
μl	microlitres
mM	milli-molar
m.p.	melting point
N_2	nitrogen
$NaBH_4$	sodium borohydride
NaOH	sodium hydroxide
Na_2SO_4	sodium sulphate
NMR	nuclear magnetic resonance
O_2	oxygen
RT	room temperature
$^{t}BuOH$	*tert* butanol

^{t}BuOK	potassium *tert* butoxide
S.E.M.	standard error of mean
THF	tetrahydrofuran
TLC	thin layer chromatography
μl	microliters
Triflic Acid	trifluoromethanesulfonic acid
TMS Triflate	trimethyl silyl trifluoromethanesulfonate
v/v	volume per volume
w/v	weight per volume
λ_{em}	emission wavelength
λ_{exc}	excitation wavelength

Appendix 2

X-ray studies

A single crystal X-ray analysis was carried out on compound 2 (*S*)-(-)-methylbenzylamine salt (compound **8**), using a SuperNova, Dual, Cu at zero, Atlas Diffractometer and the parameters outlined in Table 1.

Table 1. Data collection and structure refinement for compound 8, the (*S*)-(-)-methylbenzylamine salt of compound 2.

Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Radiation source	SuperNova (Cu) X-ray Source, Cu Kα
Data collection method	Omega scans
Theta range for data collection	3.74 to 76.22°
Index ranges	$-13 \le h \le 13, -11 \le k \le 12, -14 \le l \le 14$
Reflections collected	12753
Independent reflections	5263 [R(int) = 0.0196]
Coverage of independent reflections	99.4 %
Variation in check reflections	N/A
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.90238
Structure solution technique	direct
Structure solution program	Bruker SHELXTL
Refinement technique	Full-matrix least-squares on F^2
Refinement program	Bruker SHELXTL
Function minimized	$\Sigma w(F_o^2-F_c^2)^2$
Data / restraints / parameters	5263 / 1 / 363
Goodness-of-fit on F^2	1.007
Δ/σ_{max}	0.001
Final R indices	
5161 data; $I>2\sigma(I)$	R1 = 0.0321, wR2 = 0.0857
all data	R1 = 0.0327, wR2 = 0.0865
Weighting scheme	$w=1 / [\sigma^2 (F_o^2)+(0.0600P)^2+0.2200P]$ where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	0.04(14)

Extinction coefficient	0.0035(5)
Largest diff. peak and hole	0.214 and -0.154 e$Å^{-3}$

Refinement summary:

Ordered Non-H atoms, XYZ	Freely refining
Ordered Non-H atoms, U	Anisotropic
H atoms (on carbon), XYZ	Idealized positions riding on attached atoms
H atoms (on carbon), U	Appropriate multiple of U(eq) for bonded atom
H atoms (on heteroatoms), XYZ	Freely refining
H atoms (on heteroatoms), U	Isotropic
Disordered atoms, OCC	Refined with a two part model constrained to a total of unity
Disordered atoms, XYZ	freely refining
Disordered atoms, U	freely refining

- The single crystal X-ray data establishes that the structure of compound **6** is monoclinic, space group $P2_1$, with one molecule of compound **6** in the asymmetric unit (Table 2).

Table 2. Sample and crystal data for compound 8

Crystallization solvents	Diethyl ether, MeOH, THF	
Crystallization method	Slow evaporation	
Empirical formula	$C_{34}H_{33}N_1O_3$	
Formula weight	503.61	
Temperature	100(1) K	
Wavelength	1.54178 Å	
Crystal size	0.50 x 0.50 x 0.50 mm	
Crystal habit	Colourless Block	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 11.0344(2) Å	α= 90°
	b = 10.1727(2) Å	β= 93.682(2)°
	c = 11.8532(2) Å	γ = 90°
Volume	1327.77(4) $Å^3$	

Z	2
Density (calculated)	1.260 Mg/m^3
Absorption coefficient	0.627 mm^{-1}
F(000)	536

- The absolute stereochemistry was determined as *S, S* at C9 and C10 for compound **2** and *S* at C33 for the methylbenzylamine cation. The assignment was made from consideration of both the Flack parameter which was determined to be 0.04(14) and from the *a priori* knowledge of the stereochemistry of the salt former.

- The absolute stereochemistry was also determined using Bayesian statistics on the Bijvoet pair differences which resulted in a probability of the stereochemistry at the chiral centers C9, C10 and C33 being *S, S* and *S* respectively as 1.000 and *R, R* and *R* as 0.000. This supports the assignment of *S, S* and *S* for C9, C10 and C33 respectively from the Flack parameter measurement.

- The calculated X-ray powder diffraction pattern from the single crystal X-ray structure was in agreement with the stereochemistry shown in Figure 2 (or the following).

Table 3. Atomic coordinates and equivalent isotropic, atomic displacement parameters, (Å^2), for compound 8. U(eq) is defined as one third of the trace of the orthogonalised U_{ij} tensor.

	x/a	y/b	z/c	U(eq)
O1	0.02763(10)	0.17316(11)	1.16556(8)	0.0228(2)
O2	0.07430(9)	-0.03465(10)	1.12294(7)	0.0194(2)
O3	0.10561(8)	0.01057(10)	1.90142(8)	0.0184(2)
C1	0.08315(12)	-0.12167(14)	1.47373(12)	0.0198(3)
C2	0.07248(13)	-0.09752(14)	1.35802(12)	0.0192(3)
C3	0.08014(11)	0.02912(13)	1.31666(11)	0.0158(3)
C4	0.05975(11)	0.05851(14)	1.19195(11)	0.0164(3)
C5	0.10196(12)	0.13219(14)	1.39262(11)	0.0184(3)
C6	0.11261(13)	0.10790(14)	1.50817(11)	0.0197(3)
C7	0.10101(11)	-0.01884(14)	1.55106(10)	0.0164(3)

C8	0.09988(12)	-0.04205(14)	1.67717(10)	0.0177(3)
C9	0.22568(11)	-0.05199(14)	1.74191(10)	0.0160(3)
C10	0.20981(12)	-0.06390(14)	1.87231(10)	0.0173(3)
C11	0.32285(12)	-0.00001(14)	1.92450(11)	0.0183(3)
C12	0.36695(13)	-0.00323(15)	2.03747(11)	0.0217(3)
C13	0.46523(13)	0.07703(16)	2.07062(12)	0.0263(3)
C14	0.51796(13)	0.15733(16)	1.99312(13)	0.0271(3)
C15	0.47368(13)	0.16061(15)	1.87974(13)	0.0237(3)
C16	0.37476(12)	0.08173(14)	1.84684(11)	0.0188(3)
C17	0.30303(12)	0.07486(14)	1.73362(11)	0.0189(3)
C18	0.29536(12)	-0.17122(14)	1.70380(10)	0.0170(3)
C19	0.24493(13)	-0.29849(15)	1.68674(11)	0.0224(3)
C20	0.34284(13)	-0.38466(15)	1.64945(10)	0.0202(3)
C21	0.34340(15)	-0.51740(16)	1.62093(12)	0.0279(3)
C22	0.45250(18)	-0.57426(17)	1.59308(13)	0.0363(8)
C23	0.55837(16)	-0.50075(18)	1.59165(13)	0.0317(4)
C24	0.55735(14)	-0.36697(17)	1.61785(12)	0.0269(3)
C25	0.44911(13)	-0.31016(15)	1.64729(11)	0.0212(3)
C26	0.42215(14)	-0.17370(16)	1.68241(12)	0.0238(3)
C18A	0.29536(12)	-0.17122(14)	1.70380(10)	0.0170(3)
C19A	0.24493(13)	-0.29849(15)	1.68674(11)	0.0224(3)
C20A	0.34284(13)	-0.38466(15)	1.64945(10)	0.0202(3)
C21A	0.34340(15)	-0.51740(16)	1.62093(12)	0.0279(3)
C22A	0.45250(18)	-0.57426(17)	1.59308(13)	0.0279(3)
C23A	0.55837(16)	-0.50075(18)	1.59165(13)	0.0317(4)
C24A	0.55735(14)	-0.36697(17)	1.61785(12)	0.0269(3)
C25A	0.44911(13)	-0.31016(15)	1.64729(11)	0.0212(3)
C26A	0.42215(14)	-0.17370(16)	1.68241(12)	0.0238(3)
N1	-0.09024(11)	-0.21952(13)	1.02800(10)	0.0194(2)
C27	-0.18541(12)	0.06679(15)	0.92258(12)	0.0220(3)
C28	-0.19466(13)	0.15069(16)	0.82981(13)	0.0256(3)
C29	-0.23606(14)	0.10317(17)	0.72421(13)	0.0273(3)
C30	-0.26855(15)	-0.02757(18)	0.71195(13)	0.0301(3)
C31	-0.26063(14)	-0.11089(16)	0.80481(13)	0.0255(3)
C32	-0.21928(12)	-0.06417(15)	0.91135(11)	0.0200(3)
C33	-0.21444(12)	-0.15827(15)	1.01084(12)	0.0205(3)
C34	-0.24587(14)	-0.09613(16)	1.12172(13)	0.0256(3)

Table 4. Selected bond lengths, (Å), for compound 8

O1-C4	1.2528(18)	O2-C4	1.2688(17)
O3-C10	1.4373(16)	O3-H3A	0.88(2)
C1-C2	1.3909(19)	C1-C7	1.3964(19)
C2-C3	1.383(2)	C3-C5	1.3929(19)
C3-C4	1.5108(17)	C5-C6	1.3893(18)
C6-C7	1.395(2)	C7-C8	1.5141(16)

C8-C9	1.5457(17)	C9-C18	1.5203(19)
C9-C17	1.5537(19)	C9-C10	1.5713(16)
C10-C11	1.5040(18)	C11-C16	1.391(2)
C11-C12	1.3953(17)	C12-C13	1.394(2)
C13-C14	1.385(2)	C14-C15	1.401(2)
C15-C16	1.390(2)	C16-C17	1.5152(18)
C18-C19	1.418(2)	C18-C26	1.4380(19)
C19-C20	1.481(2)	C20-C21	1.392(2)
C20-C25	1.398(2)	C21-C22	1.394(2)
C22-C23	1.388(3)	C23-C24	1.396(2)
C24-C25	1.391(2)	C25-C26	1.485(2)
N1-C33	1.5073(18)	N1-H1B	0.91(2)
N1-H1C	0.93(2)	N1-H1D	0.90(2)
C27-C32	1.388(2)	C27-C28	1.390(2)
C28-C29	1.391(2)	C29-C30	1.383(3)
C30-C31	1.387(2)	C31-C32	1.398(2)
C32-C33	1.5172(19)	C33-C34	1.518(2)

Table 5. Selected bond angles, (°), for compound 8

C10-O3-H3A	107.0(15)	C2-C1-C7	120.96(13)
C3-C2-C1	120.72(13)	C2-C3-C5	118.95(12)
C2-C3-C4	121.50(12)	C5-C3-C4	119.48(12)
O1-C4-O2	125.44(12)	O1-C4-C3	116.78(12)
O2-C4-C3	117.77(12)	C6-C5-C3	120.23(13)
C5-C6-C7	121.32(13)	C6-C7-C1	117.75(12)
C6-C7-C8	120.71(12)	C1-C7-C8	121.43(13)
C7-C8-C9	115.87(10)	C18-C9-C8	111.09(11)
C18-C9-C17	110.70(11)	C8-C9-C17	113.19(11)
C18-C9-C10	108.74(10)	C8-C9-C10	109.89(10)
C17-C9-C10	102.86(10)	O3-C10-C11	109.18(11)
O3-C10-C9	109.69(10)	C11-C10-C9	103.26(10)
C16-C11-C12	121.07(13)	C16-C11-C10	110.61(11)
C12-C11-C10	127.84(13)	C13-C12-C11	118.29(14)
C14-C13-C12	120.72(13)	C13-C14-C15	120.99(14)
C16-C15-C14	118.34(14)	C15-C16-C11	120.58(13)
C15-C16-C17	129.14(13)	C11-C16-C17	110.16(12)
C16-C17-C9	103.90(11)	C19-C18-C26	109.64(13)
C19-C18-C9	124.70(12)	C26-C18-C9	125.65(13)
C18-C19-C20	107.21(12)	C21-C20-C25	120.31(14)
C21-C20-C19	131.41(14)	C25-C20-C19	108.27(13)
C20-C21-C22	118.51(15)	C23-C22-C21	121.31(15)
C22-C23-C24	120.24(15)	C25-C24-C23	118.68(15)
C24-C25-C20	120.94(14)	C24-C25-C26	130.48(14)
C20-C25-C26	108.57(13)	C18-C26-C25	106.29(13)
C33-N1-H1B	108.3(13)	C33-N1-H1C	112.0(13)

H1B-N1-H1C	107.4(18)	C33-N1-H1D	111.6(13)
H1B-N1-H1D	112.5(18)	H1C-N1-H1D	105.0(17)
C32-C27-C28	120.51(14)	C27-C28-C29	120.09(15)
C30-C29-C28	119.78(14)	C29-C30-C31	120.10(14)
C30-C31-C32	120.61(15)	C27-C32-C31	118.89(14)
C27-C32-C33	122.36(13)	C31-C32-C33	118.74(13)
N1-C33-C32	110.61(11)	N1-C33-C34	108.16(11)
C32-C33-C34	114.30(12)		

Table 6. Selected torsion angles, (°), for compound 8

C7-C1-C2-C3	0.4(2)	C1-C2-C3-C5	1.7(2)
C1-C2-C3-C4	-175.50(12)	C2-C3-C4-O1	156.41(13)
C5-C3-C4-O1	-20.75(18)	C2-C3-C4-O2	-22.38(18)
C5-C3-C4-O2	160.46(12)	C2-C3-C5-C6	-1.7(2)
C4-C3-C5-C6	175.57(12)	C3-C5-C6-C7	-0.5(2)
C5-C6-C7-C1	2.5(2)	C5-C6-C7-C8	-173.65(12)
C2-C1-C7-C6	-2.52(19)	C2-C1-C7-C8	173.64(12)
C6-C7-C8-C9	-83.92(16)	C1-C7-C8-C9	100.03(15)
C7-C8-C9-C18	-64.43(16)	C7-C8-C9-C17	60.83(15)
C7-C8-C9-C10	175.19(12)	C18-C9-C10-O3	-155.49(11)
C8-C9-C10-O3	-33.70(15)	C17-C9-C10-O3	87.10(12)
C18-C9-C10-C11	88.22(13)	C8-C9-C10-C11	-149.99(11)
C17-C9-C10-C11	-29.19(13)	O3-C10-C11-C16	-96.36(13)
C9-C10-C11-C16	20.29(15)	O3-C10-C11-C12	75.67(18)
C9-C10-C11-C12	-167.68(14)	C16-C11-C12-C13	-0.5(2)
C10-C11-C12-C13	-171.75(13)	C11-C12-C13-C14	-0.3(2)
C12-C13-C14-C15	0.3(2)	C13-C14-C15-C16	0.4(2)
C14-C15-C16-C11	-1.2(2)	C14-C15-C16-C17	174.32(14)
C12-C11-C16-C15	1.2(2)	C10-C11-C16-C15	173.88(13)
C12-C11-C16-C17	-175.05(12)	C10-C11-C16-C17	-2.40(16)
C15-C16-C17-C9	167.34(14)	C11-C16-C17-C9	-16.79(15)
C18-C9-C17-C16	-88.09(12)	C8-C9-C17-C16	146.44(11)
C10-C9-C17-C16	27.92(13)	C8-C9-C18-C19	-44.46(16)
C17-C9-C18-C19	-171.10(11)	C10-C9-C18-C19	76.60(15)
C8-C9-C18-C26	137.25(13)	C17-C9-C18-C26	10.60(17)
C10-C9-C18-C26	-101.70(15)	C26-C18-C19-C20	-1.81(14)
C9-C18-C19-C20	179.67(11)	C18-C19-C20-C21	-179.77(14)
C18-C19-C20-C25	1.34(15)	C25-C20-C21-C22	1.5(2)
C19-C20-C21-C22	-177.24(14)	C20-C21-C22-C23	-1.1(2)
C21-C22-C23-C24	-0.3(2)	C22-C23-C24-C25	1.2(2)
C23-C24-C25-C20	-0.7(2)	C23-C24-C25-C26	177.73(14)
C21-C20-C25-C24	-0.6(2)	C19-C20-C25-C24	178.39(12)
C21-C20-C25-C26	-179.41(12)	C19-C20-C25-C26	-0.38(15)
C19-C18-C26-C25	1.57(15)	C9-C18-C26-C25	-179.92(11)
C24-C25-C26-C18	-179.32(14)	C20-C25-C26-C18	-0.71(15)

C32-C27-C28-C29	-1.2(2)	C27-C28-C29-C30	0.3(2)
C28-C29-C30-C31	0.4(2)	C29-C30-C31-C32	-0.3(2)
C28-C27-C32-C31	1.2(2)	C28-C27-C32-C33	-178.07(13)
C30-C31-C32-C27	-0.5(2)	C30-C31-C32-C33	178.85(14)
C27-C32-C33-N1	-86.99(16)	C31-C32-C33-N1	93.72(15)
C27-C32-C33-C34	35.36(18)	C31-C32-C33-C34	-143.93(14)

Table 7. Anisotropic atomic displacement parameters, (Å^2), for compound 8 The anisotropic atomic displacement factor exponent takes the form: $-2\pi^2$ [h^2a^{*2} U_{11} + ... + 2hka* b* U_{12}

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0325(5)	0.0206(5)	0.0153(4)	0.0025(4)	0.0015(4)	0.0044(4)
O2	0.0255(5)	0.0206(5)	0.0123(4)	-0.0017(4)	0.0024(3)	-0.0014(4)
O3	0.0205(4)	0.0232(5)	0.0116(4)	-0.0002(4)	0.0027(3)	0.0016(4)
C1	0.0257(7)	0.0179(7)	0.0159(6)	0.0017(5)	0.0018(5)	-0.0028(5)
C2	0.0267(7)	0.0171(7)	0.0139(6)	-0.0035(5)	0.0024(5)	-0.0019(5)
C3	0.0160(6)	0.0187(7)	0.0128(6)	-0.0004(5)	0.0017(4)	0.0011(5)
C4	0.0166(5)	0.0193(7)	0.0134(6)	0.0000(5)	0.0014(4)	-0.0018(5)
C5	0.0234(6)	0.0155(7)	0.0159(6)	-0.0001(5)	-0.0011(5)	0.0020(5)
C6	0.0251(6)	0.0175(7)	0.0158(6)	-0.0030(5)	-0.0024(5)	0.0028(5)
C7	0.0150(5)	0.0213(7)	0.0129(6)	0.0000(5)	0.0009(4)	0.0028(5)
C8	0.0188(6)	0.0217(7)	0.0124(6)	-0.0007(5)	0.0000(4)	0.0018(5)
C9	0.0186(6)	0.0177(7)	0.0117(5)	0.0004(5)	0.0007(4)	-0.0002(5)
C10	0.0206(6)	0.0190(7)	0.0121(6)	0.0000(5)	0.0005(4)	0.0022(5)
C11	0.0201(6)	0.0185(7)	0.0163(6)	-0.0030(5)	0.0004(5)	0.0033(5)
C12	0.0234(6)	0.0249(8)	0.0166(6)	-0.0018(5)	-0.0015(5)	0.0056(5)
C13	0.0237(7)	0.0322(9)	0.0216(7)	-0.0074(6)	-0.0074(5)	0.0074(6)
C14	0.0196(7)	0.0284(8)	0.0324(8)	-0.0099(6)	-0.0049(6)	0.0015(6)
C15	0.0199(6)	0.0229(7)	0.0282(7)	-0.0035(6)	0.0008(5)	0.0012(6)
C16	0.0186(6)	0.0198(7)	0.0178(6)	-0.0023(5)	0.0007(5)	0.0028(5)
C17	0.0213(6)	0.0203(7)	0.0151(6)	0.0004(5)	0.0018(5)	-0.0008(5)
C18	0.0200(6)	0.0206(7)	0.0101(5)	0.0011(5)	-0.0009(4)	0.0004(5)
C19	0.0245(7)	0.0249(8)	0.0176(6)	-0.0024(5)	0.0008(5)	0.0029(5)
C20	0.0256(7)	0.0227(7)	0.0124(6)	0.0001(5)	0.0015(5)	0.0027(5)
C21	0.0392(8)	0.0237(8)	0.0215(6)	-0.0032(6)	0.0059(6)	-0.0017(7)
C22	0.063(2)	0.0236(16)	0.0226(13)	-0.0024(11)	0.0090(13)	0.0165(15)
C23	0.0359(8)	0.0356(9)	0.0240(7)	-0.0034(6)	0.0049(6)	0.0140(7)
C24	0.0253(7)	0.0331(9)	0.0225(7)	-0.0050(6)	0.0034(5)	0.0047(6)
C25	0.0253(7)	0.0253(8)	0.0129(5)	0.0003(5)	0.0016(5)	0.0035(5)
C26	0.0277(7)	0.0248(8)	0.0197(6)	-0.0005(6)	0.0069(5)	0.0012(6)
C18A	0.0200(6)	0.0206(7)	0.0101(5)	0.0011(5)	-0.0009(4)	0.0004(5)
C19A	0.0245(7)	0.0249(8)	0.0176(6)	-0.0024(5)	0.0008(5)	0.0029(5)

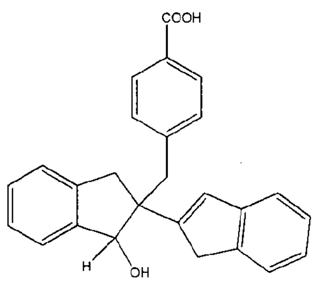
C20A	0.0256(7)	0.0227(7)	0.0124(6)	0.0001(5)	0.0015(5)	0.0027(5)
C21A	0.0392(8)	0.0237(8)	0.0215(6)	-0.0032(6)	0.0059(6)	-0.0017(7)
C22A	0.0392(8)	0.0237(8)	0.0215(6)	-0.0032(6)	0.0059(6)	-0.0017(7)
C23A	0.0359(8)	0.0356(9)	0.0240(7)	-0.0034(6)	0.0049(6)	0.0140(7)
C24A	0.0253(7)	0.0331(9)	0.0225(7)	-0.0050(6)	0.0034(5)	0.0047(6)
C25A	0.0253(7)	0.0253(8)	0.0129(5)	0.0003(5)	0.0016(5)	0.0035(5)
C26A	0.0277(7)	0.0248(8)	0.0197(6)	-0.0005(6)	0.0069(5)	0.0012(6)
N1	0.0248(6)	0.0191(6)	0.0143(5)	-0.0013(5)	0.0005(4)	-0.0007(5)
C27	0.0216(6)	0.0233(7)	0.0213(7)	-0.0001(5)	0.0017(5)	-0.0030(5)
C28	0.0250(7)	0.0228(8)	0.0293(7)	0.0035(6)	0.0038(6)	-0.0021(6)
C29	0.0265(7)	0.0298(9)	0.0254(7)	0.0087(6)	-0.0001(5)	-0.0017(6)
C30	0.0326(8)	0.0357(9)	0.0214(7)	0.0019(6)	-0.0041(6)	-0.0050(7)
C31	0.0286(7)	0.0238(8)	0.0234(7)	0.0001(6)	-0.0034(6)	-0.0053(6)
C32	0.0169(6)	0.0233(7)	0.0198(6)	0.0024(5)	0.0007(5)	-0.0024(5)
C33	0.0196(6)	0.0205(7)	0.0212(6)	0.0023(5)	0.0001(5)	-0.0031(5)
C34	0.0280(7)	0.0264(8)	0.0232(7)	0.0029(6)	0.0065(6)	0.0024(6)

Appendix 3

Compound **1**	4-((-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoic acid
Compound **2**	4-(((1S,2S)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoic acid
Compound **3**	4-(((1R,2R)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoic acid
Compound **4**	4-(((1R,2S)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoic acid
Compound **5**	4-(((1S,2R)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoic acid
Compound **6**	6-(Methylamino)hexane-1,2,3,4,5-pentanol 4-(((1S,2S)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoate
Compound **7**	6-(Methylamino)hexane-1,2,3,4,5-pentanol 4-(((1S,2R)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoate
Compound **8**	(S)-1-Phenylethylammonium 4-(((1S,2S)-1-hydroxy-2,3-dihydro-1H,-1'H-2,2'-biinden-2-yl)methyl)benzoate
Compound **9**	(R)-1-Phenylethylammonium 4-(((1R,2S)-1-hydroxy-2,3-dihydro-1H,1'H-2,2'-biinden-2-yl)methyl)benzoate

Claims

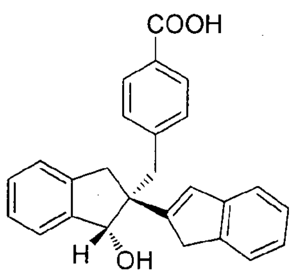
1. A compound of the formula I



(I)

and isomers and pharmaceutically acceptable salts thereof.

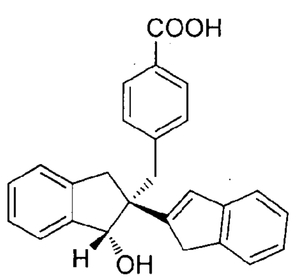
2. A compound of the relative stereochemistry and formula II



(II)

and pharmaceutically acceptable salts thereof.

3. A compound of the absolute stereochemistry and formula II

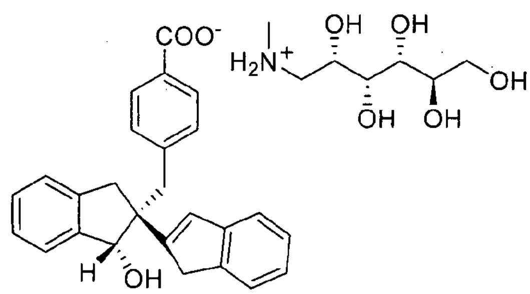


(II)

and pharmaceutically acceptable salts thereof.

4. The N-Methyl-(D)-Glucamine salt of a compound as claimed in any of claims 1 to 3.

5. The N-Methyl-(D)-Glucamine salt of the compound of formula III:



(III)

6. A pharmaceutical composition comprising an effective amount of a compound as claimed in any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

7. A method for the prophylaxis or treatment of inflammatory bowel disease, comprising administering to a subject an effective amount of a compound as claimed in any one of claims 1 to 5.

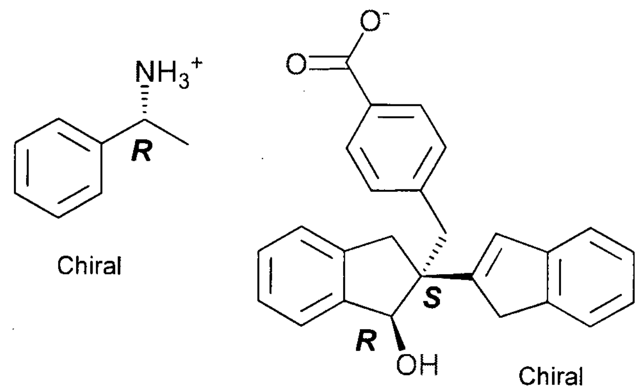
8. A method for the prophylaxis or treatment of ulcerative colitis, comprising administering to a subject an effective amount of a compound as claimed in any one of claims 1 to 5.

9. A method for the prophylaxis or treatment of Crohn's disease, comprising administering to a subject an effective amount of a compound as claimed in any one of claims 1 to 5.

10. A compound as claimed in any of claims 1 to 5 for use in the prophylaxis or treatment of inflammatory bowel disease.

11. A compound as claimed in any of claims 1 to 5 for use as in the prophylaxis or treatment of ulcerative colitis.

12. A compound as claimed in any of claims 1 to 5 for use in the prophylaxis or treatment of Crohn's disease.



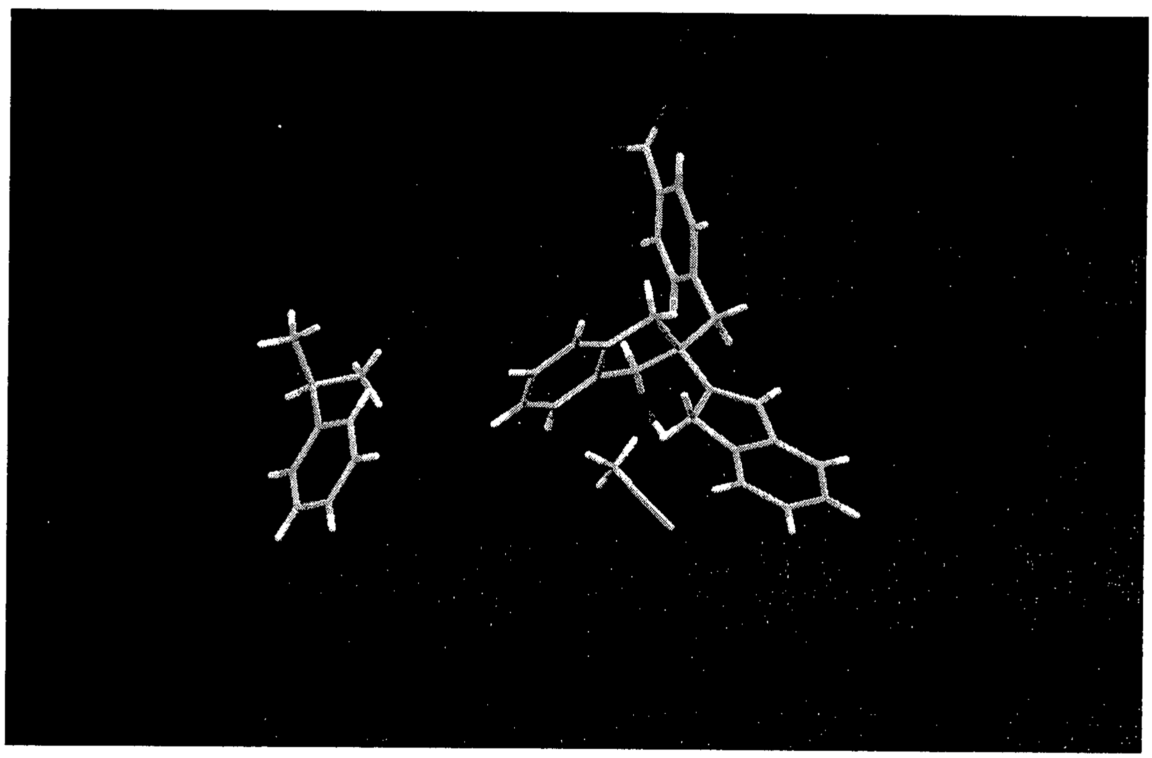
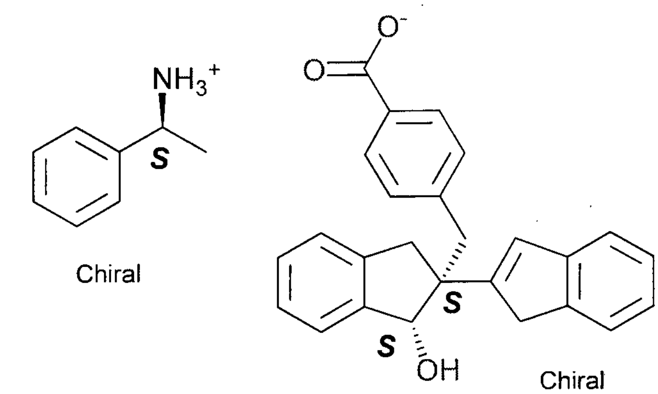


Fig. 1.


NH3+
S
Chiral
O-
O
S
S
OH
Chiral

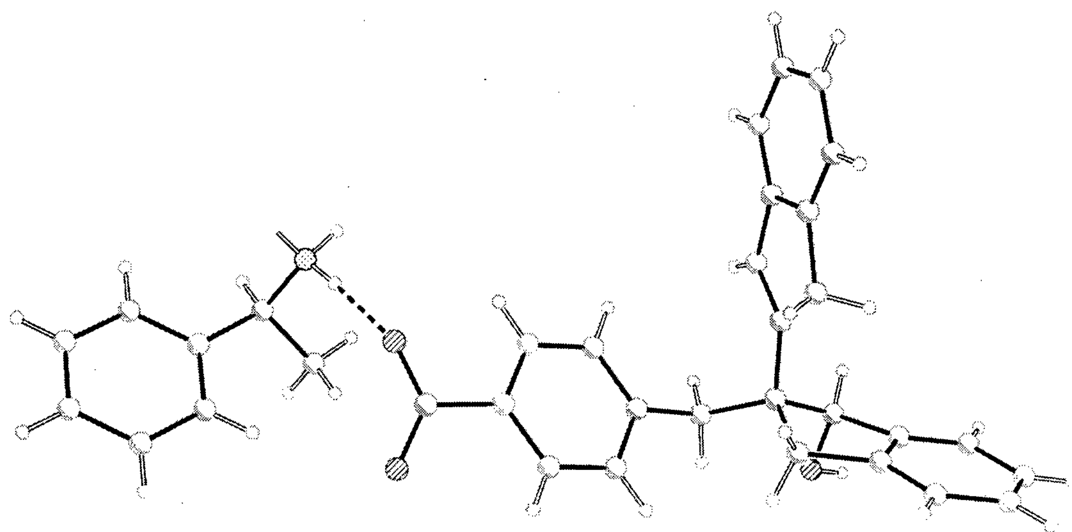


Fig. 2

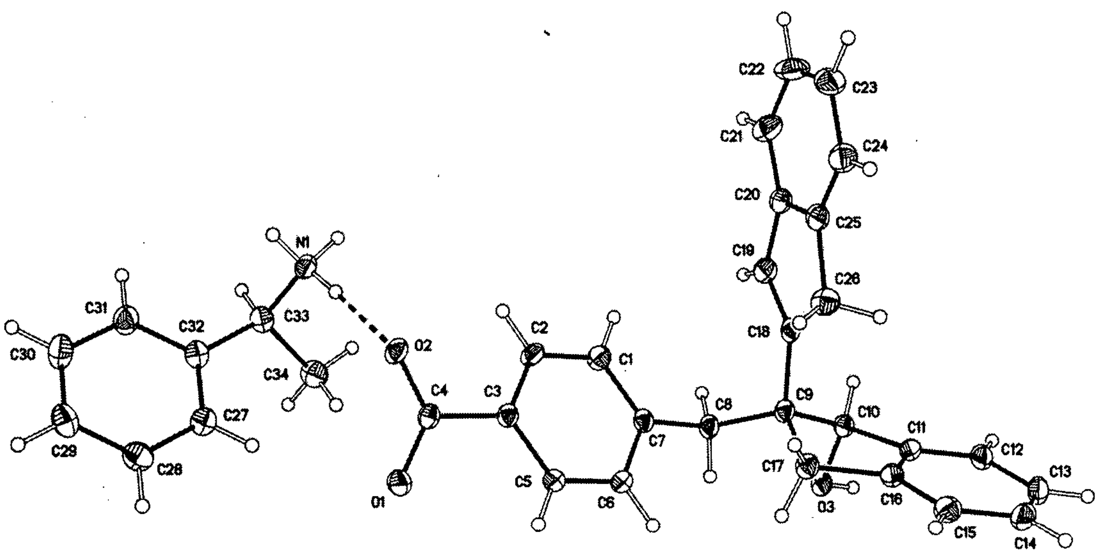

C22
C23
C21
C24
C20
C25
N1
C19
C26
C31
C32
C33
C2
C1
C18
C30
O2
C34
C4
C3
C8
C9
C10
C11
C27
C7
C12
C17
C13
C29
C28
C5
O1
C6
O3
C16
C15
C14

Fig. 2A

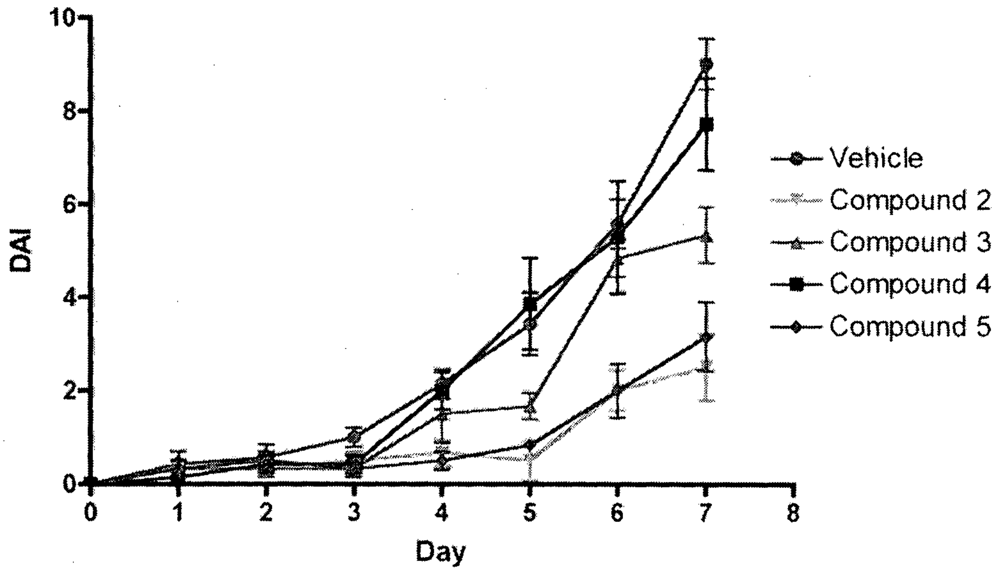

10
8
6
4
2
0
DAI
0
1
2
3
4
5
6
7
8
Day
Vehicle
Compound 2
Compound 3
Compound 4
Compound 5

Fig. 3.

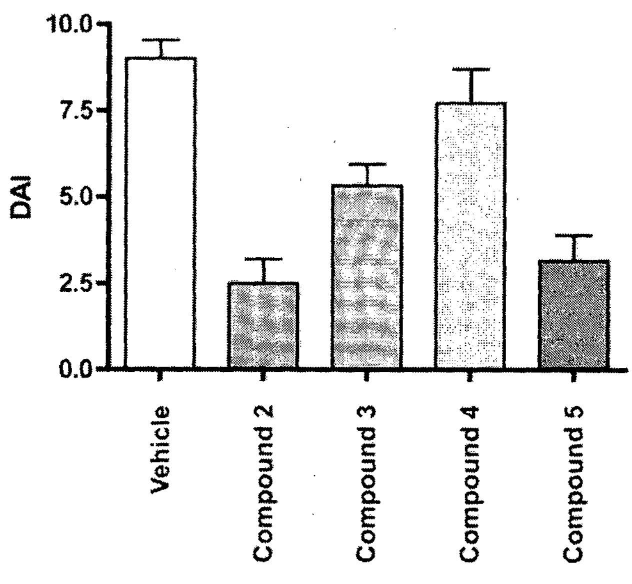

10.0
7.5
5.0
2.5
0.0
DAI
Vehicle
Compound 2
Compound 3
Compound 4
Compound 5

Fig. 4

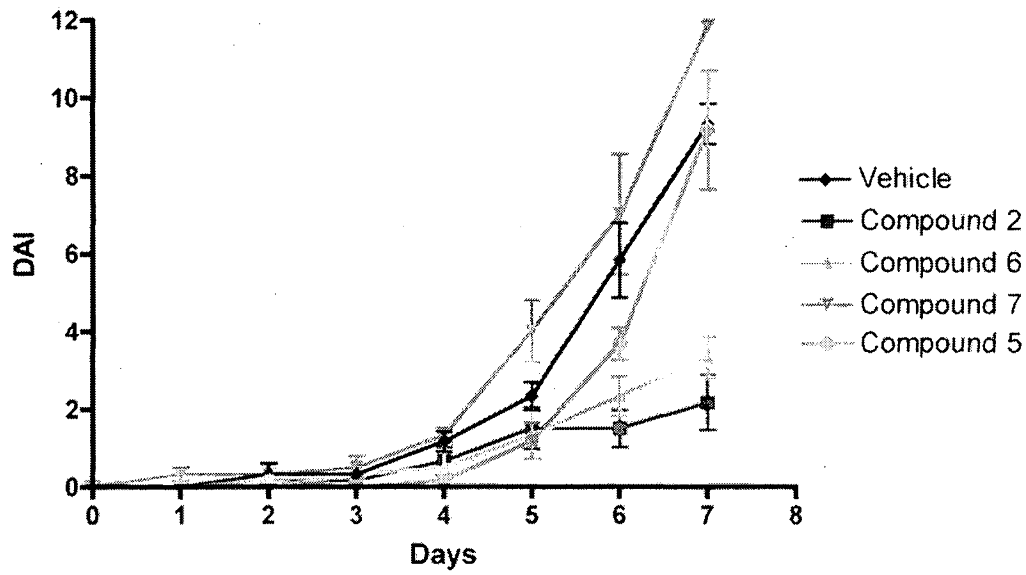

12
10
8
6
4
2
0
DAI
0
1
2
3
4
5
6
7
8
Days
Vehicle
Compound 2
Compound 6
Compound 7
Compound 5

Fig. 5

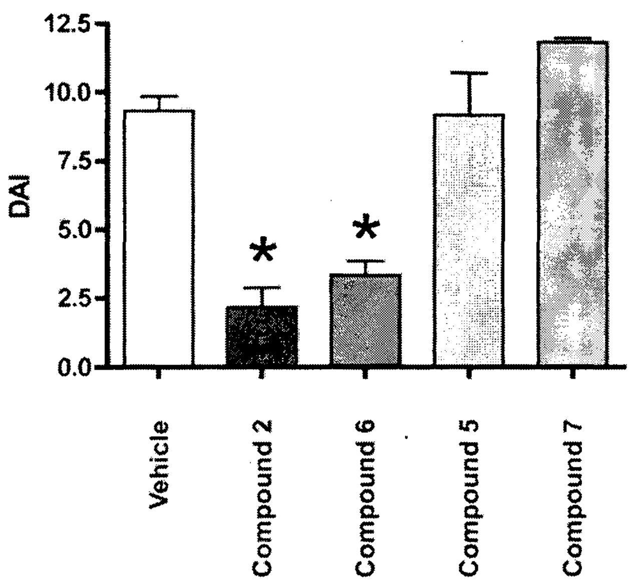

12.5
10.0
7.5
5.0
2.5
0.0
DAI
*
*
Vehicle
Compound 2
Compound 6
Compound 5
Compound 7

Fig. 6

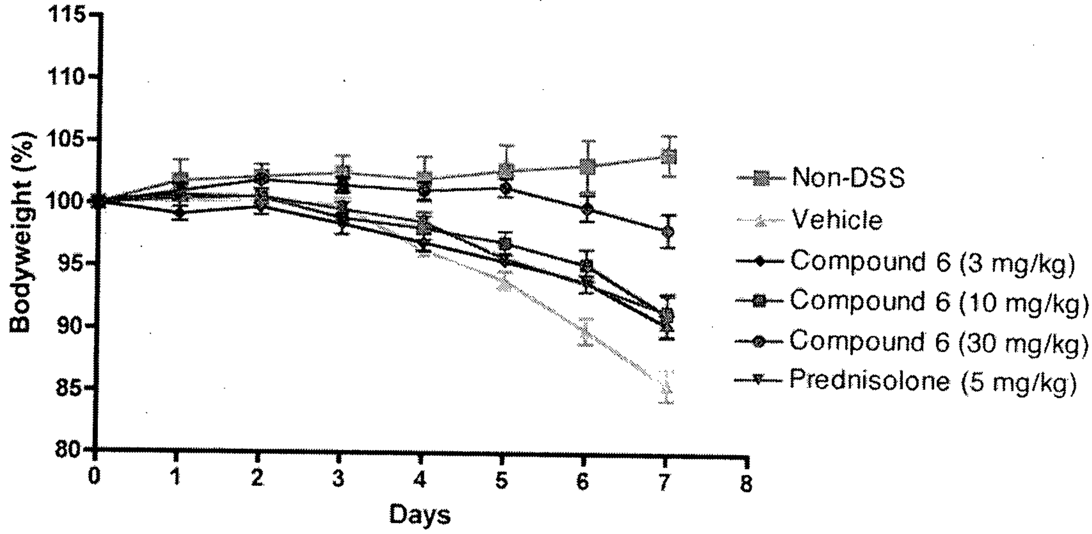

115
110
105
100
95
90
85
80
Bodyweight (%)
0
1
2
3
4
5
6
7
8
Days
Non-DSS
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 7

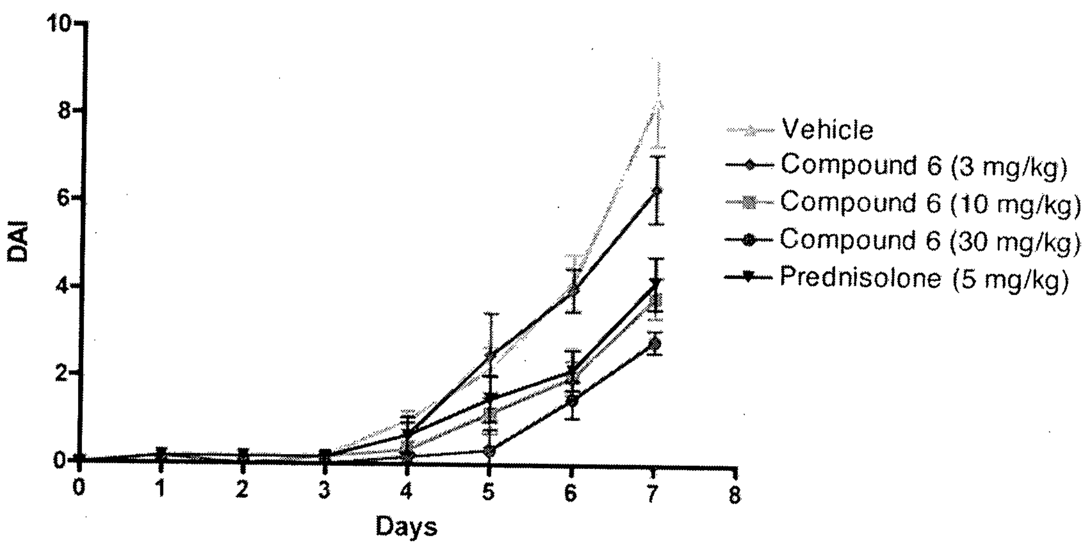

10
8
6
4
2
0
DAI
0
1
2
3
4
5
6
7
8
Days
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 8

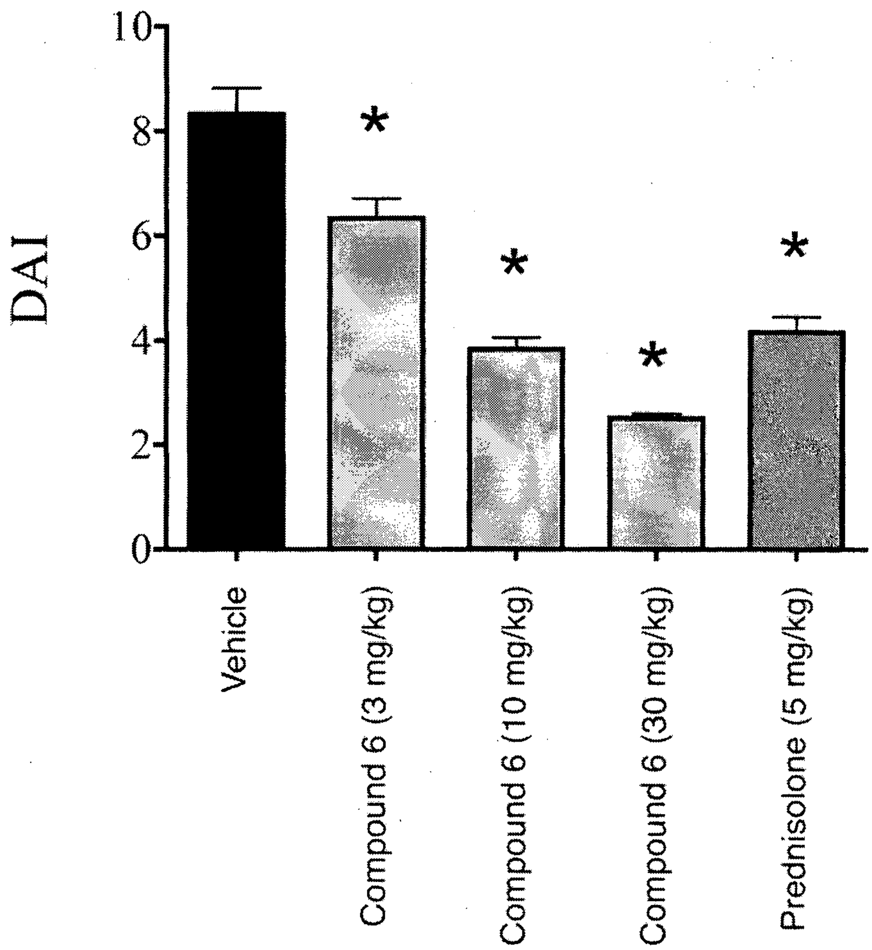
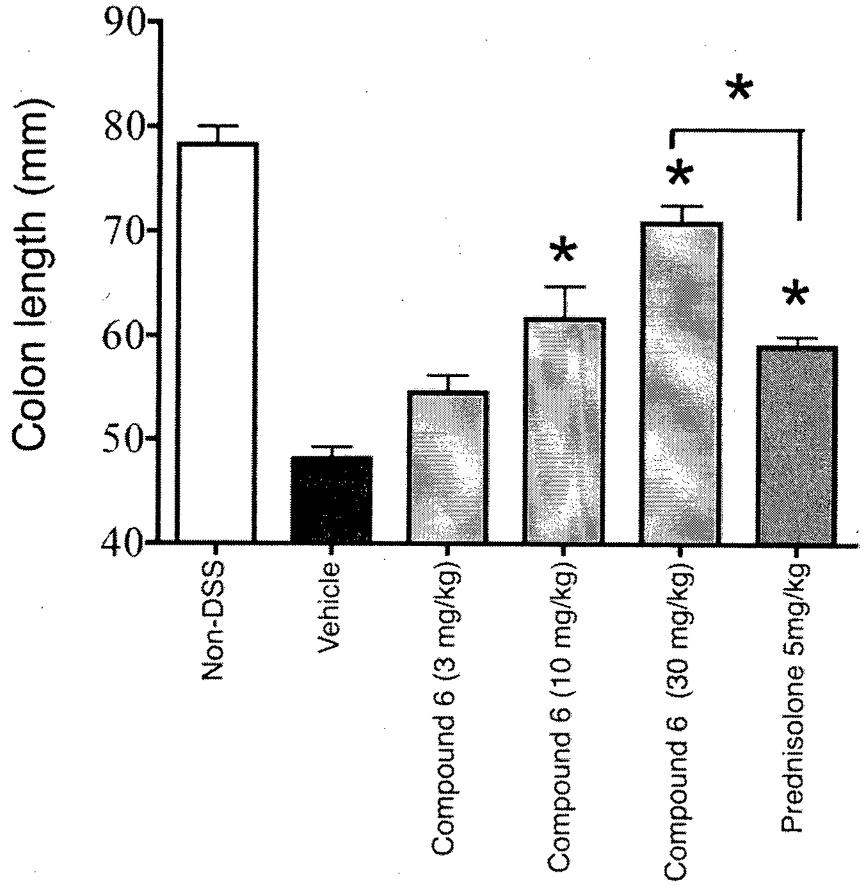

DAI
10
8
6
4
2
0
*
*
*
*
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 9



90
80
70
60
50
40
Colon length (mm)
*
*
*
*
Non-DSS
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone 5mg/kg

Fig. 10

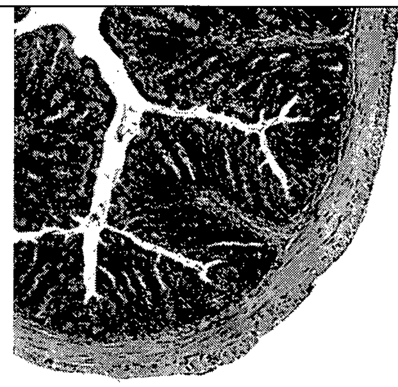
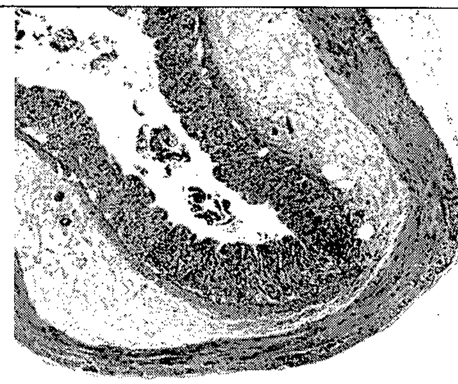
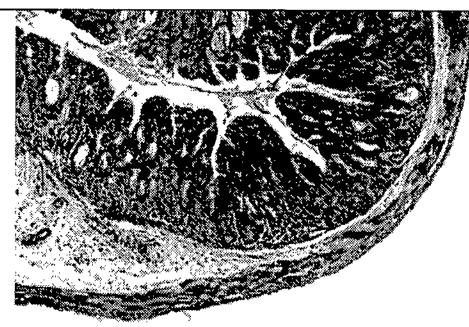
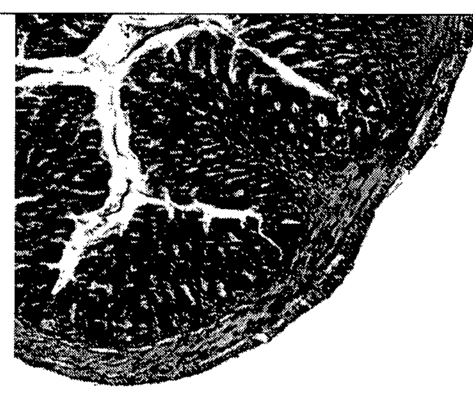
Untreated	
DSS + vehicle	
DSS + Prednisolone (5mg/Kg)	
DSS + Compound 6 (30mg/Kg)	

Fig. 11

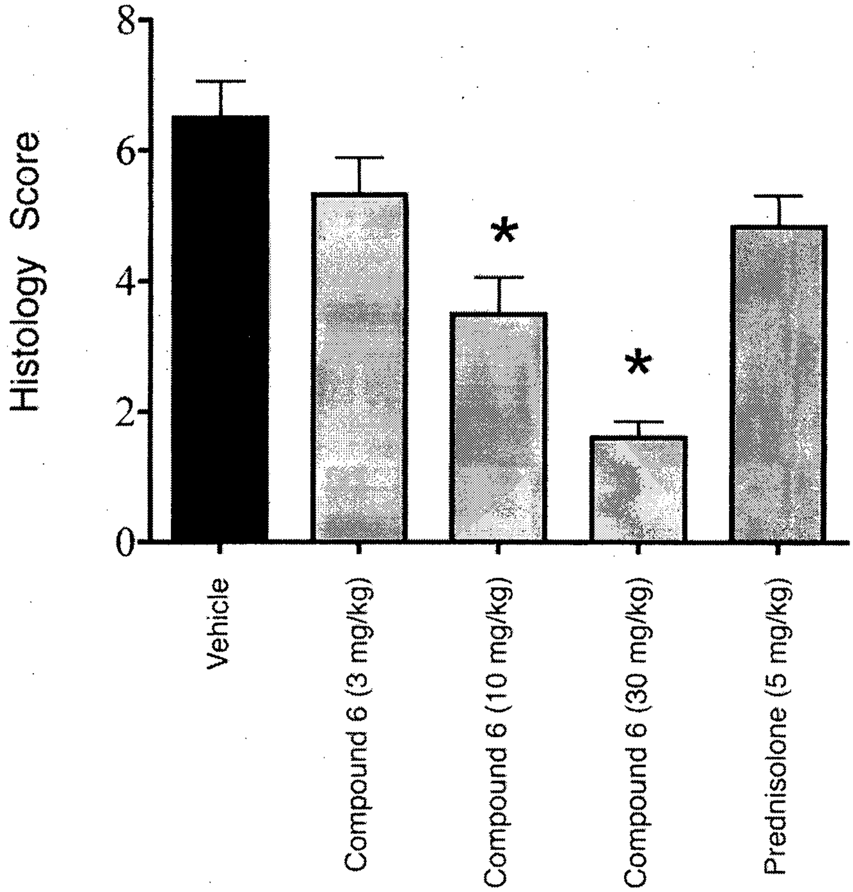

Histology Score
8
6
4
2
0
*
*
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 12

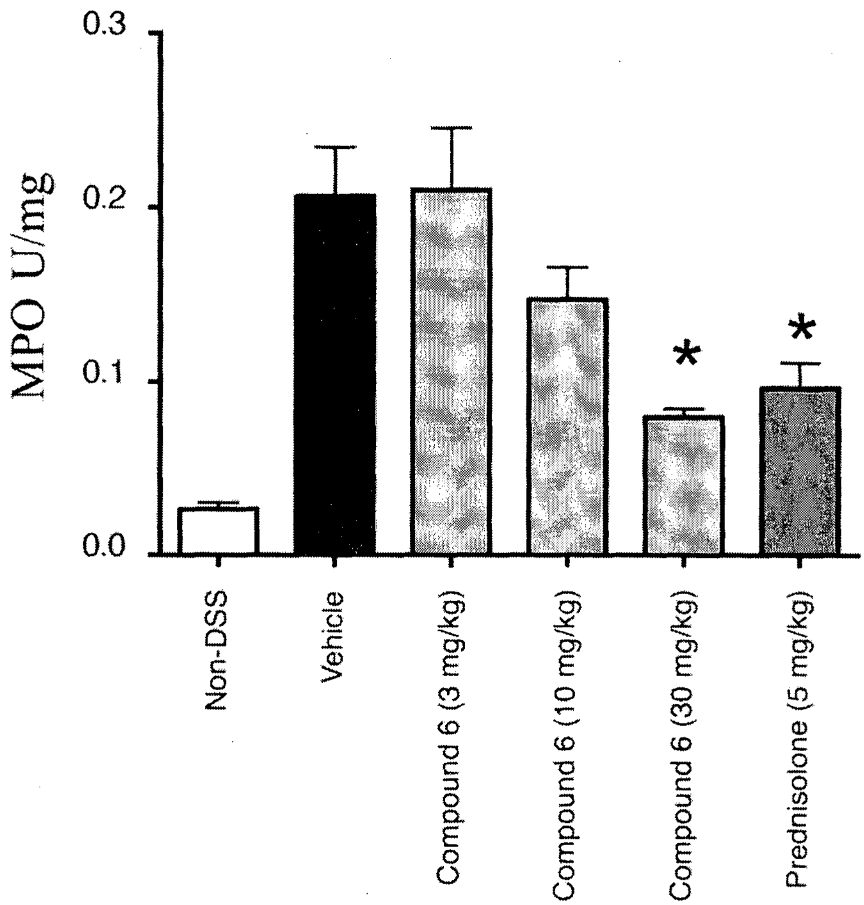

MPO U/mg
0.3
0.2
0.1
0.0
*
*
Non-DSS
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 13

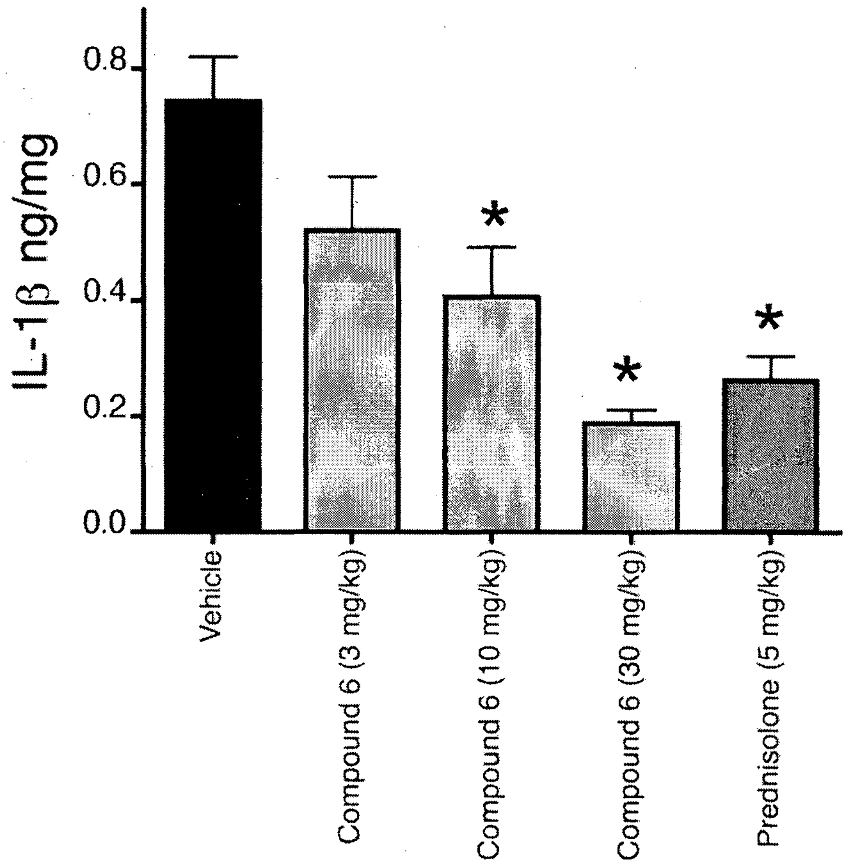

IL-1β ng/mg
0.8
0.6
0.4
0.2
0.0
*
*
*
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 14(a)

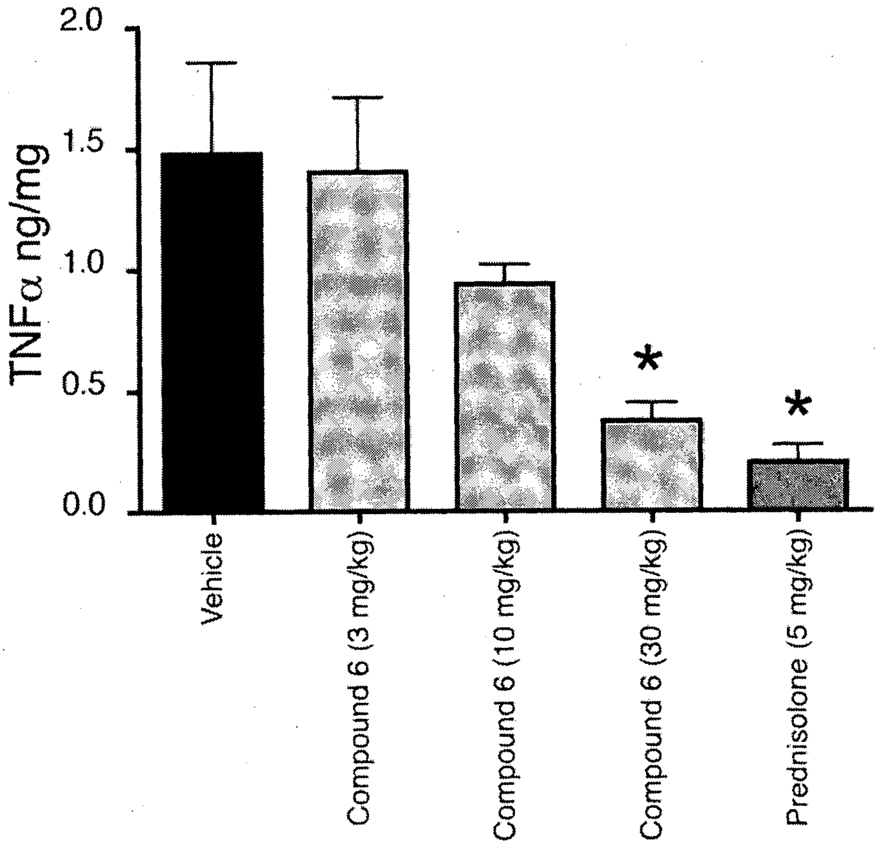

TNFα ng/mg
2.0
1.5
1.0
0.5
0.0
*
*
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 14(b)

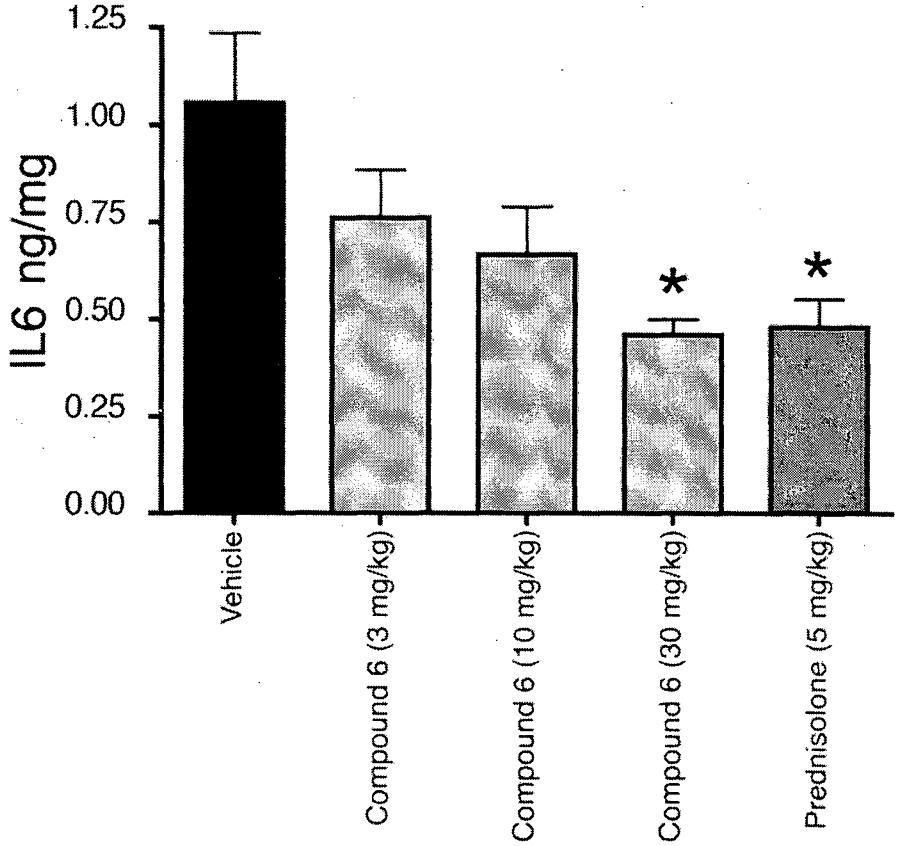

IL6 ng/mg
1.25
1.00
0.75
0.50
0.25
0.00
*
*
Vehicle
Compound 6 (3 mg/kg)
Compound 6 (10 mg/kg)
Compound 6 (30 mg/kg)
Prednisolone (5 mg/kg)

Fig. 14(c)

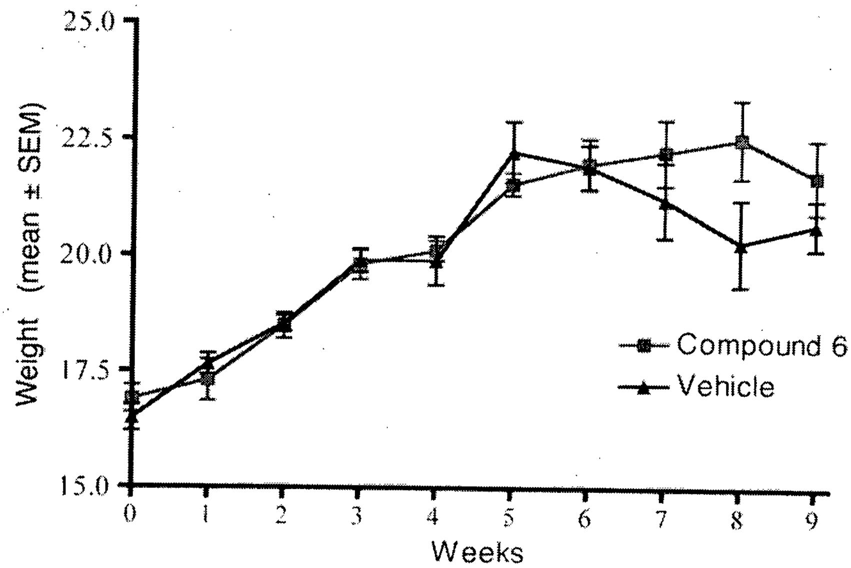

25.0
22.5
20.0
17.5
15.0
Weight (mean ± SEM)
0
1
2
3
4
5
6
7
8
9
Weeks
Compound 6
Vehicle

Fig 15

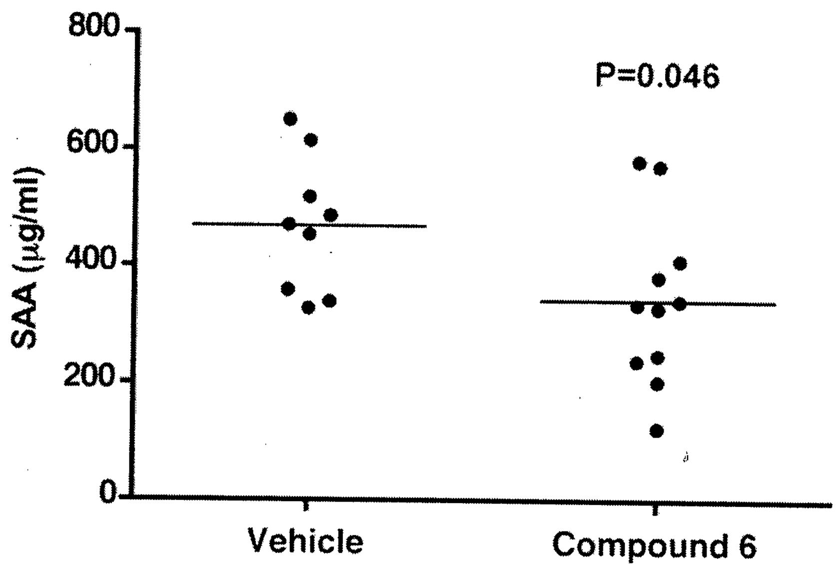

800
600
400
200
0
SAA (μg/ml)
P=0.046
Vehicle
Compound 6

Fig. 16

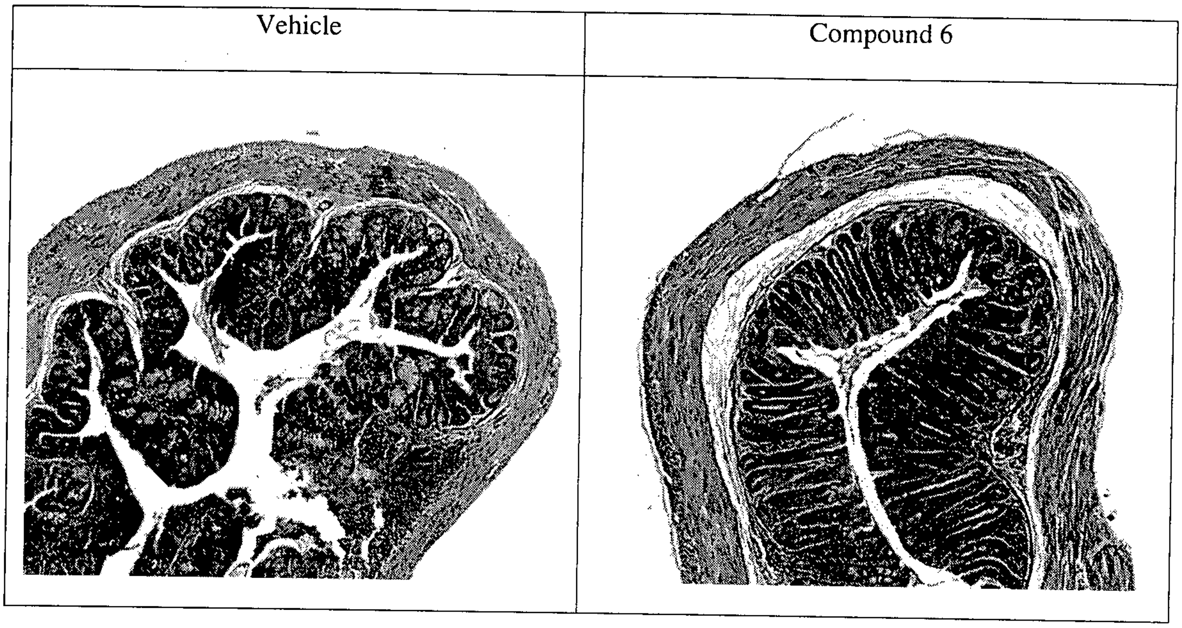

Vehicle
Compound 6

Fig. 17

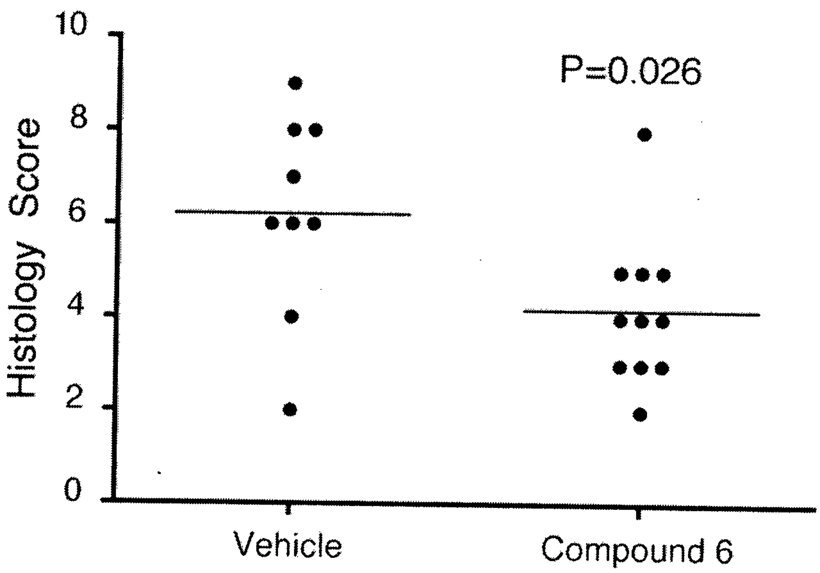

10
8
6
4
2
0
Histology Score
P=0.026
Vehicle
Compound 6

Fig. 18

INTERNATIONAL SEARCH REPORT

International application No
PCT/IE2012/000037

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C65/19 A61K31/192 A61P1/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/128256 A1 (BRUGNARA CARLO [US] ET AL) 12 September 2002 (2002-09-12) claim 7, paragraph [0032] -----	1-12
X,P	FRANKISH, NEIL ET AL: "6-(Methylamino)hexane-1,2,3,4,5-pentanol 4-(((1S,2S)-1-Hydroxy-2,3-dihydro-1H,1'H-[2,2-biinden]-2- yl)methyl)benzoate (PH46A): A Novel Small Molecule With Efficacy In Murine Models Of Colitis", JOURNAL OF MEDICINAL CHEMISTRY, vol. 55, no. 11, 4 June 2012 (2012-06-04), pages 5497-5505, XP009163235, the whole document -----	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"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	Date of mailing of the international search report
23 October 2012	19/11/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Grammenoudi, S

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IE2012/000037

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002128256 A1	12-09-2002	NONE	