THIONINIUM COMPOUNDS AND THEIR USE

RELATED APPLICATION

This application is related to United States patent application number 60/786,699 filed 29 March 2006; the contents of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

This invention pertains generally to processes, uses, methods and materials utilising particular diaminophenothiazinium compounds. These compounds are useful as drugs, for example, in the treatment of tauopathies, such as Alzheimer's disease.

BACKGROUND

A number of patents and publications are cited herein in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Each of these references is incorporated herein by reference in its entirety into the present disclosure, to the same extent as if each individual reference was specifically and individually indicated to be incorporated by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and "comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges are often expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment.

Conditions of dementia such as Alzheimer's disease (AD) are frequently characterised by a progressive accumulation of intracellular and/or extracellular deposits of proteinaceous

structures such as β-amyloid plaques and neurofibrillary tangles (NFTs) in the brains of affected patients. The appearance of these lesions largely correlates with pathological neurofibrillary degeneration and brain atrophy, as well as with cognitive impairment (see, e.g., Mukaetova-Ladinska, E.B., et al., 2000, Am. J. Pathol., Vol. 157, No. 2, pp. 623-636).

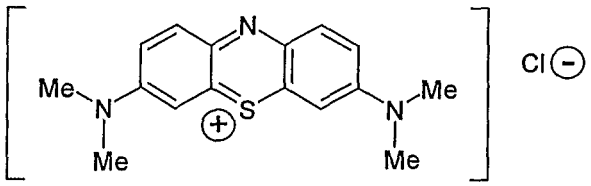
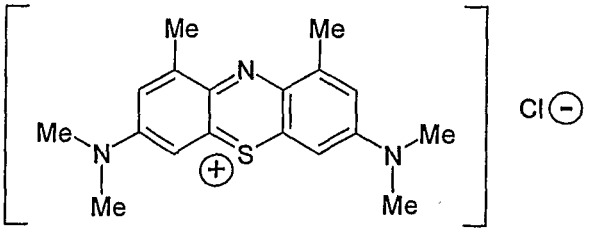
In AD, both neuritic plaques and NFTs contain paired helical filaments (PHFs), of which a major constituent is the microtubule-associated protein tau (see, e.g., Wischik et al., 1988, PNAS USA, Vol. 85, pp. 4506-4510). Plaques also contain extracellular β-amyloid fibrils derived from the abnormal processing of amyloid precursor protein (APP) (see, e.g., Kang et al., 1987, Nature, Vol. 325, p. 733). An article by Wischik et al. (in 'Neurobiology of Alzheimer's Disease', 2nd Edition, 2000, Eds. Dawbarn, D. and Allen, S.J., The Molecular and Cellular Neurobiology Series, Bios Scientific Publishers, Oxford) discusses in detail the putative role of tau protein in the pathogenesis of neurodegenerative dementias. Loss of the normal form of tau, accumulation of pathological PHFs, and loss of synapses in the mid-frontal cortex all correlate with associated cognitive impairment. Furthermore, loss of synapses and loss of pyramidal cells both correlate with morphometric measures of tau-reactive neurofibrillary pathology, which parallels, at a molecular level, an almost total redistribution of the tau protein pool from a soluble to a polymerised form (i.e., PHFs) in Alzheimer's disease.

Tau exists in alternatively-spliced isoforms, which contain three or four copies of a repeat sequence corresponding to the microtubule-binding domain (see, e.g., Goedert, M., et al., 1989, EMBO J., Vol. 8, pp. 393-399; Goedert, M., et al., 1989, Neuron, Vol. 3, pp. 519-526). Tau in PHFs is proteolytically processed to a core domain (see, e.g., Wischik, C.M., et al., 1988, PNAS USA, Vol. 85, pp. 4884-4888; Wischik et al., 1988, PNAS USA, Vol. 85, pp. 4506-4510; Novak, M., et al., 1993, EMBO J., Vol. 12, pp. 365-370) which is composed of a phase-shifted version of the repeat domain; only three repeats are involved in the stable tau-tau interaction (see, e.g., Jakes, R., et al., 1991, EMBO J., Vol. 10, pp. 2725-2729). Once formed, PHF-like tau aggregates act as seeds for the further capture and provide a template for proteolytic processing of full-length tau protein (see, e.g., Wischik et al., 1996, PNAS USA, Vol. 93, pp. 11213-11218).

The phase shift which is observed in the repeat domain of tau incorporated into PHFs suggests that the repeat domain undergoes an induced conformational change during incorporation into the filament. During the onset of AD, it is envisaged that this conformational change could be initiated by the binding of tau to a pathological substrate, such as damaged or mutated membrane proteins (see, e.g., Wischik, C.M., et al., 1997, in "Microtubule-associated proteins: modifications in disease", Eds. Avila, J., Brandt, R. and Kosik, K. S. (Harwood Academic Publishers, Amsterdam) pp.185-241).

In the course of their formation and accumulation, PHFs first assemble to form amorphous aggregates within the cytoplasm, probably from early tau oligomers which become truncated prior to, or in the course of, PHF assembly (see, e.g., Mena, R., et al., 1995, Acta Neuropathol., Vol. 89, pp. 50-56; Mena, R., et al., 1996, Acta Neuropathol., Vol. 91, pp. 633-641). These filaments then go on to form classical intracellular NFTs. In this state, the PHFs consist of a core of truncated tau and a fuzzy outer coat containing full-length tau (see, e.g., Wischik et al., 1996, PNAS USA, Vol. 93, pp. 11213-11218). The assembly process is exponential, consuming the cellular pool of normal functional tau and inducing new tau synthesis to make up the deficit (see, e.g., Lai, R. Y. K., et al., 1995, Neurobiology of Ageing, Vol. 16, No. 3, pp. 433-445). Eventually, functional impairment of the neurone progresses to the point of cell death, leaving behind an extracellular NFT. Cell death is highly correlated with the number of extracellular NFTs (see, e.g., Wischik et al., in 'Neurobiology of Alzheimer's Disease', 2nd Edition, 2000, Eds. Dawbarn, D. and Allen, S.J., The Molecular and Cellular Neurobiology Series, Bios Scientific Publishers, Oxford). As tangles are extruded into the extracellular space, there is progressive loss of the fuzzy outer coat of the neurone with corresponding loss of N-terminal tau immunoreactivity, but preservation of tau immunoreactivity associated with the PHF core (see, e.g., Bondareff, W. et al., 1994, J. Neuropath. Exper. Neurol., Vol. 53, No. 2, pp. 158-164).

Diaminophenothiazines have previously been shown to inhibit tau protein aggregation and to disrupt the structure of PHFs, and reverse the proteolytic stability of the PHF core (see, e.g., WO 96/30766, F Hoffman-La Roche). Such compounds were disclosed for use in the treatment or prophylaxis of various diseases, including Alzheimer's disease. These included, amongst others:

MTC	 methyl-thioninium chloride
DMMTC	 1,9-dimethyl-methyl-thioninium chloride

Additionally, WO 02/055720 (The University Court of the University of Aberdeen) discusses the use of reduced forms of diaminophenothiazines specifically for the treatment of a variety of protein aggregating diseases, although the disclosure is primarily concerned with tauopathies.

WO 2005/030676 (The University Court of the University of Aberdeen) discusses radiolabelled phenothiazines, and their use in diagnosis and therapy, for example, of tauopathies.

Notwithstanding these disclosures, it will be appreciated that the provision of one or more further compounds, not previously specifically identified as being effective tau protein aggregation inhibitors, would provide a contribution to the art.

PCT/GB2005/003634 (TauRx Therapeutics Pte. Ltd) was filed, but not published, prior to the filing of the present application (WO 2006/032879 A2 was published on 30 March 2006). That application relates *inter alia* to methods of synthesizing and purifying certain 3,7-diamino-phenothiazin-5-ium compounds (referred to as "diaminophenothiazinium compounds") including MTC and other compounds described herein. It further discloses therapeutic uses of these compounds.

DESCRIPTION OF THE INVENTION

The present inventors have now identified certain thioninium compounds as being effective tau protein aggregation inhibitors and in preferred forms having certain other desirable properties, for example by comparison with the compounds of the prior art discussed above.

As discussed above, tau proteins are characterised as being one among a larger number of protein families which co-purify with microtubules during repeated cycles of assembly and disassembly (Shelanski *et al.* (1973) Proc. Natl. Acad. Sci. USA, 70., 765-768), and are known as microtubule-associated-proteins (MAPs). Members of the tau family share the common features of having a characteristic N-terminal segment, sequences of approximately 50 amino acids inserted in the N-terminal segment, which are developmentally regulated in the brain, a characteristic tandem repeat region consisting of 3 or 4 tandem repeats of 31-32 amino acids, and a C-terminal tail.

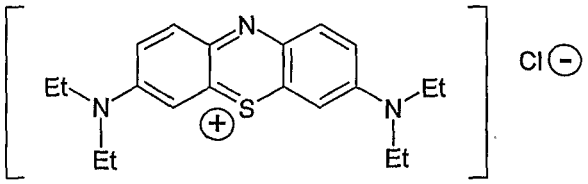
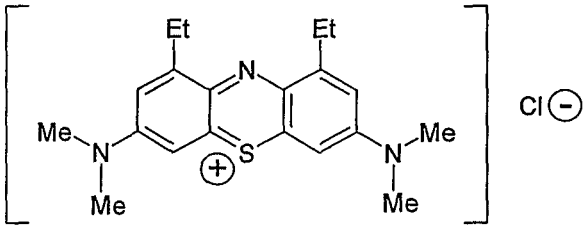
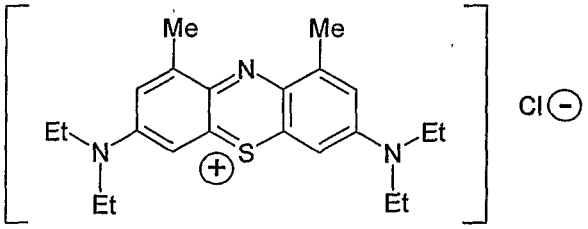
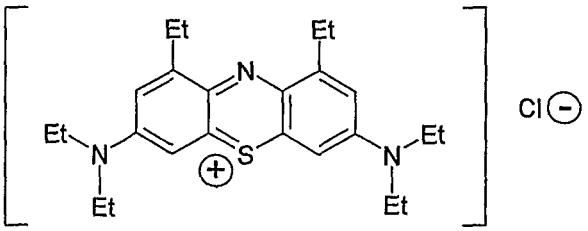
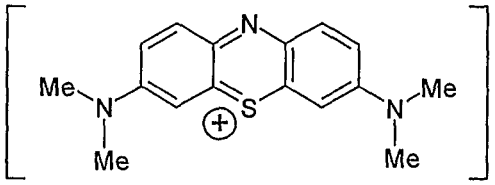
One or more of these specific compounds are known in the art – for example MTZ is described in Fierz-David and Blangley, 1949, "F. Oxazine and Thiazine Dyes," in: Fundamental Processes of Dye Chemistry, published by Interscience (London, UK), pp. 308-314. However it is believed that none of these have previously been disclosed in the prior art as tau protein aggregation inhibitors.

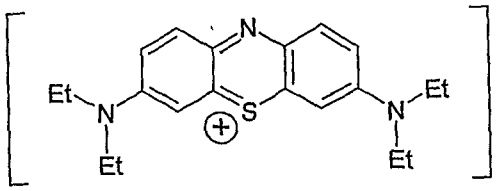
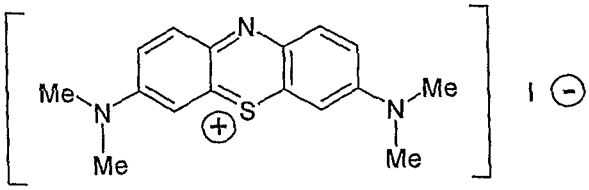
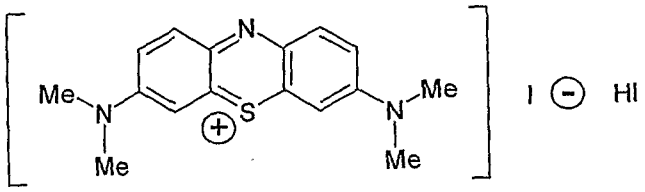
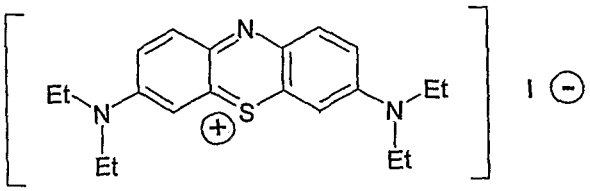
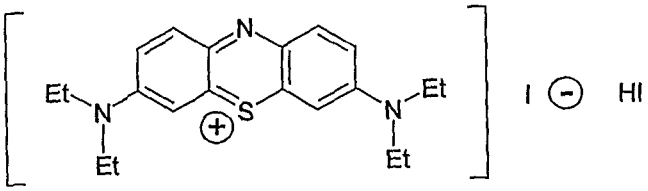
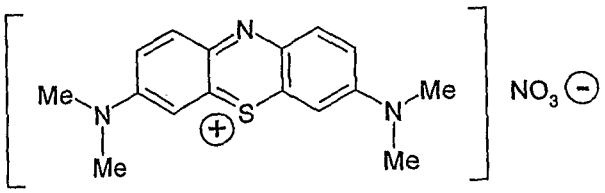
The invention therefore relates to methods, uses, compositions and other materials employing these compounds as tau protein aggregation inhibitors and as therapeutics or prophylactics of diseases associated with tau protein aggregation ("tauopathies"). The invention further provides processes for making these compounds.

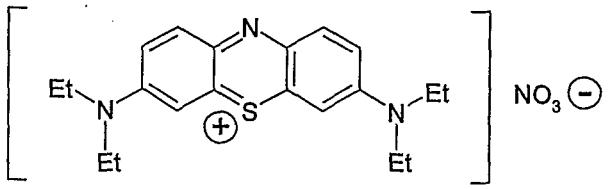
These and other aspects of the invention are discussed in more detail hereinafter.

Compounds

In general, the present invention relates to one or more compounds selected from the following diaminophenothiazinium compounds:

A	ETC	 ethyl-thioninium chloride
B	DEMTC	 1,9-diethyl-methyl-thioninium chloride
C	DMETC	 1,9-dimethyl-ethyl-thioninium chloride
D	DEETC	 1,9-diethyl-ethyl-thioninium chloride
E	MTZ	 methyl-thioninium chloride zinc chloride mixed salt

F	ETZ	 ethyl-thioninium chloride zinc chloride mixed salt
G	MTI	 methyl-thioninium iodide
H	MTI.HI	 methyl-thioninium iodide hydrogen iodide mixed salt
I	ETI	 ethyl-thioninium iodide
J	ETI.HI	 ethyl-thioninium iodide hydrogen iodide mixed salt
K	MTN	 methyl-thioninium nitrate

L	ETN	 ethyl-thioninium nitrate

These compounds A-L are described herein as "diaminophenothiazinium compounds" or "DAPT compounds" or "compounds of the invention" or (unless context demands otherwise) "active compounds".

Isotopic Variation

In one embodiment, one or more of the carbon atoms of the compound is ^{11}C or ^{13}C or ^{14}C.

In one embodiment, one or more of the carbon atoms of the compound is ^{11}C.
In one embodiment, one or more of the carbon atoms of the compound is ^{13}C.
In one embodiment, one or more of the carbon atoms of the compound is ^{14}C.
In one embodiment, one or more of the nitrogen atoms of the compound is ^{15}N.

In one embodiment, one or more or all of the carbon atoms of one or more or all of the groups -Me (or -Et) is ^{11}C.

In one embodiment, one or more or all of the carbon atoms of one or more or all of the groups $-NMe_2$ (or $-NEt_2$) is ^{11}C.

In one embodiment, the groups $-NMe_2$ are $-N(^{11}CH_3)_2$.

Uses to reverse or inhibit the aggregation of tau protein.

One aspect of the invention is the use of a diaminophenothiazinium compound to reverse or inhibit the aggregation of tau protein. This aggregation may be *in vitro*, or *in vivo*, and may be associated with a tauopathy disease state as discussed herein. Also provided are methods of reversing or inhibiting the aggregation of tau protein comprising contacting the aggregate or protein with a compound as described herein.

Preferred compounds

In **this and all other** aspects of the invention, unless context demands otherwise, the compound is selected from the list consisting of A, B, C, D, E, F, G, H, I, J, K, and L (in each case optionally being an isotopic variant thereof as described above).

In one embodiment, it is compound A.
In one embodiment, it is compound B.
In one embodiment, it is compound C.
In one embodiment, it is compound D.
In one embodiment, it is compound E.
In one embodiment, it is compound F.
In one embodiment, it is compound G.
In one embodiment, it is compound H.
In one embodiment, it is compound I.
In one embodiment, it is compound J.
In one embodiment, it is compound K.
In one embodiment, it is compound L.

In one embodiment the diaminophenothiazinium compound may be one which is *obtained by, or is obtainable by,* a method as described herein (see "Methods of Synthesis" below).

Preferred compounds of the present invention are those which show high activity in the assays described herein, particularly the *in vitro* assay described below. Preferred compounds have a B50 of less than 500, more preferably less than 300, 200, 100, 90, 80, 70, 60, 50, 40, 30 or 20 μM, as determined with reference to the Examples herein.

In one embodiment the diaminophenothiazinium compound has a RxIndex (RxI) value obtained as determined with reference to the Examples herein of greater than or equal to 150, more preferably greater than or equal to 160, 170, 180, 190, 200, 500, 1000, 1500, or 2000.

Methods of treatment or prophylaxis and 1st & 2nd medical uses

One aspect of the present invention pertains to a method of treatment or prophylaxis of a tauopathy condition in a patient, comprising administering to said patient a therapeutically-effective amount of a diaminophenothiazinium compound, as described herein.

Aspects of the present invention relate to "tauopathies". As well as Alzheimer's disease (AD), the pathogenesis of neurodegenerative disorders such as Pick's disease and Progressive Supranuclear Palsy (PSP) appears to correlate with an accumulation of pathological truncated tau aggregates in the dentate gyrus and stellate pyramidal cells of the neocortex, respectively. Other dementias include fronto-temporal dementia (FTD); parkinsonism linked to chromosome 17 (FTDP-17); disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC); pallido-ponto-nigral degeneration (PPND); Guam-ALS

syndrome; pallido-nigro-luysian degeneration (PNLD); cortico-basal degeneration (CBD) and others (see Wischik *et al.* 2000, loc. cit, for detailed discussion - especially Table 5.1). All of these diseases, which are characterized primarily or partially by abnormal tau aggregation, are referred to herein as "tauopathies" or "diseases of tau protein aggregation".

In this and all other aspects of the invention relating to tauopathies, preferably the tauopathy is selected from the list consisting of the indications above, i.e., AD, Pick's disease, PSP, FTD, FTDP-17, DDPAC, PPND, Guam-ALS syndrome, PNLD, and CBD.

In one preferred embodiment the tauopathy is Alzheimer's disease (AD).

One aspect of the present invention pertains to a diaminophenothiazinium compound, as described herein, for use in a method of treatment or prophylaxis (e.g., of a tauopathy condition) of the human or animal body by therapy.

One aspect of the present invention pertains to use of a diaminophenothiazinium compound, as described herein, in the manufacture of a medicament for use in the treatment or prophylaxis of a tauopathy condition.

A further embodiment is a method of treatment or prophylaxis of a disease of tau protein aggregation as described herein, which method comprises administering to a subject a diaminophenothiazinium compound, or therapeutic composition comprising the same, such as to inhibit the aggregation of the tau protein associated with said disease state.

Other methods and uses

In a further embodiment there is disclosed a diaminophenothiazinium compound, or therapeutic composition comprising the same, for use in a method of treatment or prophylaxis of a disease of tau protein aggregation as described above, which method comprises administering to a subject the diaminophenothiazinium compound or composition such as to inhibit the aggregation of the tau protein associated with said disease state.

In a further embodiment there is disclosed use of a diaminophenothiazinium compound in the preparation of a medicament for use in a method of treatment or prophylaxis of a disease of tau protein aggregation as described above, which method comprises administering to a subject the medicament such as to inhibit the aggregation of the tau protein associated with said disease state.

In one embodiment there is disclosed a method of regulating the aggregation of a tau protein in the brain of a mammal, which aggregation is associated with a disease state as described above, the treatment comprising the step of administering to said mammal in need of said treatment, a prophylactically or therapeutically effective amount of an inhibitor of said aggregation, wherein the inhibitor is a diaminophenothiazinium compound.

One aspect of the invention is a method of inhibiting production of protein aggregates (e.g. in the form of paired helical filaments (PHFs), optionally in neurofibrillary tangles (NFTs)) in the brain of a mammal, the treatment being as described herein.

In one aspect the invention provides a drug product for the treatment of a disease state associated with tau protein aggregation in a mammal suffering therefrom, comprising a container labeled or accompanied by a label indicating that the drug product is for the treatment of said disease, the container containing one or more dosage units each comprising at least one pharmaceutically acceptable excipient and, as an active ingredient, an isolated pure diaminophenothiazinium compound of the invention.

Compositions, formulations and purity

Compositions and formulations are discussed in more detail hereinafter.

However, in one embodiment, the diaminophenothiazinium compound may be provided or used in a composition which is equal to or less than 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, or 90% pure. In one embodiment the compound of the invention is <u>not</u> one which is obtained or obtainable by the high purity methods in disclosed PCT/GB2005/003634 (TauRx Therapeutics Pte. Ltd) which was filed, but not published, prior to the filing of the present application.

One aspect of the present invention pertains to a dosage unit (e.g., a pharmaceutical tablet or capsule) comprising 20 to 300 mg of a diaminophenothiazinium compound as described herein (e.g., obtained by, or obtainable by, a method as described herein; having a purity as described herein; etc.).

Dosage units (e.g., a pharmaceutical tablet or capsule) comprising 20 to 300 mg of a diaminophenothiazinium compound as described herein and a pharmaceutically acceptable carrier, diluent, or excipient are discussed in more detail hereinafter.

In one embodiment, the amount is about 25 mg.
In one embodiment, the amount is about 35 mg.
In one embodiment, the amount is about 50 mg.

In one embodiment, the amount is about 70 mg.
In one embodiment, the amount is about 125 mg.
In one embodiment, the amount is about 175 mg.
In one embodiment, the amount is about 250 mg.

Preferred dosage regimes

Dosage regimes are discussed in more detail hereinafter.

However in one embodiment, the diaminophenothiazinium compound is administered to a human patient according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

In one embodiment, the diaminophenothiazinium compound is administered to a human patient according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

Preferred combination therapies

Combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously, are discussed in more detail hereinafter. Thus it will be understood that any of the medical uses or methods described herein may be used in a combination therapy.

In one embodiment, a treatment of the invention (e.g., employing a compound of the invention) is in combination with a cholinesterase inhibitor such as Donepezil (Aricept™), Rivastigmine (Exelon™) or Galantamine (Reminyl™).

In one embodiment, a treatment of the invention (e.g., employing a compound of the invention) is in combination with an NMDA receptor antagonist such as Memantine (Ebixa™, Namenda™).

In one embodiment, a treatment of the invention (e.g. employing a compound of the invention) is in combination with a muscarinic receptor agonist.

In one embodiment, a treatment of the invention (e.g. employing a compound of the invention) is in combination with an inhibitor of amyloid precursor protein tobeta-amyloid (e.g., an inhibitor of amyloid precursor protein processing that leads to enhanced generation of beta-amyloid).

Ligands and labels

Diaminophenothiazinium compounds discussed herein that are capable of inhibiting the aggregation of tau protein will also be capable of acting as ligands or labels of tau protein (or aggregated tau protein). Thus, in one embodiment, the diaminophenothiazinium compound is a ligand of tau protein (or aggregated tau protein).

Such diaminophenothiazinium compounds (ligands) may incorporate, be conjugated to, be chelated with, or otherwise be associated with, other chemical groups, such as stable and unstable detectable isotopes, radioisotopes, positron-emitting atoms, magnetic resonance labels, dyes, fluorescent markers, antigenic groups, therapeutic moieties, or any other moiety that may aid in a prognostic, diagnostic or therapeutic application.

For example, as noted above, in one embodiment, the diaminophenothiazinium compound is as defined above, but with the additional limitation that the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with one or more (e.g., 1, 2, 3, 4, etc.) isotopes, radioisotopes, positron-emitting atoms, magnetic resonance labels, dyes, fluorescent markers, antigenic groups, or therapeutic moieties.

In one embodiment, the diaminophenothiazinium compound is a ligand as well as a label, e.g., a label for tau protein (or aggregated tau protein), and incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels.

For example, in one embodiment, the diaminophenothiazinium compound is as defined above, but with the additional limitation that the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels.

Labelled diaminophenothiazinium compounds (e.g., when ligated to tau protein or aggregated tau protein) may be visualised or detected by any suitable means, and the skilled person will appreciate that any suitable detection means as is known in the art may be used.

For example, the diaminophenothiazinium compound (ligand-label) may be suitably detected by incorporating a positron-emitting atom (e.g., ^{11}C) (e.g., as a carbon atom of one or more alkyl group substituents, e.g., methyl group substituents) and detecting the compound using positron emission tomography (PET) as is known in the art.

Suitable methods for preparing these and similar ^{11}C labelled diaminophenothiaziniums are shown, for example, in WO 02/075318 (see Figures 11a, 11b, 12) and WO 2005/030676.

Thus, in one aspect, the present invention provides a method of labelling tau protein (or aggregated tau protein) comprising the step of: contacting the tau protein (or aggregated tau protein) with a diaminophenothiazinium compound that incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels.

In another aspect, the present invention provides a method of detecting tau protein (or aggregated tau protein) comprising the steps of: contacting the tau protein (or aggregated tau protein) with a diaminophenothiazinium compound that incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels, and detecting the presence and\or amount of said compound bound to tau protein (or aggregated tau protein).

In another aspect, the present invention provides a method of diagnosis or prognosis of a tau proteinopathy in a subject believed to suffer from the disease, comprising the steps of:
(i) introducing into the subject a diaminophenothiazinium compound capable of labelling tau protein or aggregated tau protein, particularly tau protein (e.g., a diaminophenothiazinium compound that incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels),
(ii) determining the presence and\or amount of said compound bound to tau protein or aggregated tau protein in the brain of the subject,
(iii) correlating the result of the determination made in (ii) with the disease state of the subject.

In another aspect, the present invention provides a diaminophenothiazinium compound capable of labelling tau protein or aggregated tau protein (e.g., a diaminophenothiazinium compound that incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels), for use in a method of diagnosis or prognosis of a tau proteinopathy.

In another aspect, the present invention provides use of a diaminophenothiazinium compound capable of labelling tau protein or aggregated tau protein, particularly tau protein (e.g., a diaminophenothiazinium compound that incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more (e.g., 1, 2, 3, 4, etc.) detectable labels), in a method of manufacture of a diagnostic or prognostic reagent for use in the diagnosis or prognosis of a tau proteinopathy.

Those skilled in the art will appreciate that instead of administering diaminophenothiazinium ligands/labels directly, they could be administered in a precursor form, for conversion to the active form (e.g., ligating form, labelling form) by an activating agent present in, or administered to, the same subject.

The ligands disclosed herein may be used as part of a method of diagnosis or prognosis. It may be used to select a patient for treatment, or to assess the effectiveness of a treatment or a therapeutic (e.g. an inhibitor of tau protein aggregation) administered to the subject.

Methods of Synthesis

Methods for the chemical synthesis of compounds of the present invention are described in the Examples herein. These and/or other well known methods may be modified and/or adapted in known ways in order to facilitate the synthesis of other compounds of the present invention.

Thus one aspect of the invention provides a method of synthesising a compound of the invention as described herein, described, or substantially as described, with reference to any of the Examples hereinafter.

The invention further provides a diaminophenothiazinium compound of the invention which is *obtained by* or *is obtainable by,* a method as described herein.

Some aspects of the invention will now be explained in more detail:

Treatment

The term "treatment," as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included.

The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect,

commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

Similarly, the term "prophylactically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired prophylactic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

The term "treatment" includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g., drugs, antibodies (e.g., as in immunotherapy), prodrugs (e.g., as in photodynamic therapy, GDEPT, ADEPT, etc.); surgery; radiation therapy; and gene therapy.

Routes of Administration

The diaminophenothiazinium compound, or pharmaceutical composition comprising it, may be administered to a subject/patient by any convenient route of administration, whether systemically/peripherally or topically (i.e., at the site of desired action).

Routes of administration include, but are not limited to, oral (e.g., by ingestion); buccal; sublingual; transdermal (including, e.g., by a patch, plaster, etc.); transmucosal (including, e.g., by a patch, plaster, etc.); intranasal (e.g., by nasal spray); ocular (e.g., by eyedrops); pulmonary (e.g., by inhalation or insufflation therapy using, e.g., via an aerosol, e.g., through the mouth or nose); rectal (e.g., by suppository or enema); vaginal (e.g., by pessary); parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal (including, e.g., intracatheter injection into the brain); by implant of a depot or reservoir, for example, subcutaneously or intramuscularly.

The Subject/Patient

The subject/patient may be an animal, mammal, a placental mammal, a marsupial (e.g., kangaroo, wombat), a monotreme (e.g., duckbilled platypus), a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), a lagomorph (e.g., a rabbit), avian (e.g., a bird), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), porcine (e.g., a pig), ovine (e.g., a sheep), bovine (e.g., a cow), a primate,

simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), an ape (e.g., gorilla, chimpanzee, orangutang, gibbon), or a human.

Furthermore, the subject/patient may be any of its forms of development, for example, a foetus.

In one preferred embodiment, the subject/patient is a human.

Suitable subjects for the method may be selected on the basis of conventional factors. Thus the initial selection of a patient may involve any one or more of: rigorous evaluation by experienced clinician; exclusion of non-AD diagnosis as far as possible by supplementary laboratory and other investigations; objective evaluation of level of cognitive function using neuropathologically validated battery.

In one embodiment, the subject/patient is not a human.

Formulations

While it is possible for the diaminophenothiazinium compound to be used (e.g., administered) alone, it is often preferable to present it as a composition or formulation.

In one embodiment, the composition is a pharmaceutical composition (e.g., formulation, preparation, medicament) comprising a diaminophenothiazinium compound, as described herein, and a pharmaceutically acceptable carrier, diluent, or excipient.

In one embodiment, the composition is a pharmaceutical composition comprising at least one diaminophenothiazinium compound, as described herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, including, but not limited to, pharmaceutically acceptable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, colouring agents, flavouring agents, and sweetening agents.

In one embodiment, the composition further comprises other active agents, for example, other therapeutic or prophylactic agents.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, Handbook of Pharmaceutical Additives, 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse Information Resources, Inc., Endicott, New York, USA), Remington's Pharmaceutical Sciences, 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994.

Another aspect of the present invention pertains to methods of making a pharmaceutical composition comprising admixing at least one [^{11}C]-radiolabelled phenothiazine or phenothiazine-like compound, as defined herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, e.g., carriers, diluents, excipients, etc. If formulated as discrete units (e.g., tablets, etc.), each unit contains a predetermined amount (dosage) of the active compound.

The term "pharmaceutically acceptable," as used herein, pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

The formulations may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with carriers (e.g., liquid carriers, finely divided solid carrier, etc.), and then shaping the product, if necessary.

The formulation may be prepared to provide for rapid or slow release; immediate, delayed, timed, or sustained release; or a combination thereof.

Formulations suitable for parenteral administration (e.g., by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (e.g., solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (e.g., in a liposome or other microparticulate). Such liquids may additional contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active ingredient in the liquid is from about 1 ng/ml to about 10 µg/ml, for example from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) 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 tablets.

Examples of Formulations

One aspect of the present invention pertains to a dosage unit (e.g., a pharmaceutical tablet or capsule) comprising 20 to 300 mg of a diaminophenothiazinium compound as described herein (e.g., obtained by, or obtainable by, a method as described herein; having a purity as described herein; etc.), and a pharmaceutically acceptable carrier, diluent, or excipient.

In one embodiment, the dosage unit is a tablet.
In one embodiment, the dosage unit is a capsule.

In one embodiment, the amount is 30 to 200 mg.
In one embodiment, the amount is about 30 mg.
In one embodiment, the amount is about 60 mg.
In one embodiment, the amount is about 100 mg.
In one embodiment, the amount is about 150 mg.
In one embodiment, the amount is about 200 mg.

In one embodiment, the pharmaceutically acceptable carrier, diluent, or excipient is or comprises one or both of a glyceride (e.g., Gelucire 44/14 ®; lauroyl macrogol-32 glycerides PhEur, USP) and colloidal silicon dioxide (e.g., 2% Aerosil 200 ®; Colliodal Silicon Dioxide PhEur, USP).

Dosage

It will be appreciated by one of skill in the art that appropriate dosages of the diaminophenothiazinium compound, and compositions comprising the diaminophenothiazinium compound, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at

the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician, veterinarian, or clinician.

In general, a suitable dose of the active compound is in the range of about 100 ng to about 25 mg (more typically about 1 µg to about 10 mg) per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, an amide, a prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 100 mg, 3 times daily.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 150 mg, 2 times daily.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 200 mg, 2 times daily.

The invention will now be further described with reference to the following non-limiting Examples. Other embodiments of the invention will occur to those skilled in the art in the light of these.

The disclosure of all references cited herein, inasmuch as it may be used by those skilled in the art to carry out the invention, is hereby specifically incorporated herein by cross-reference.

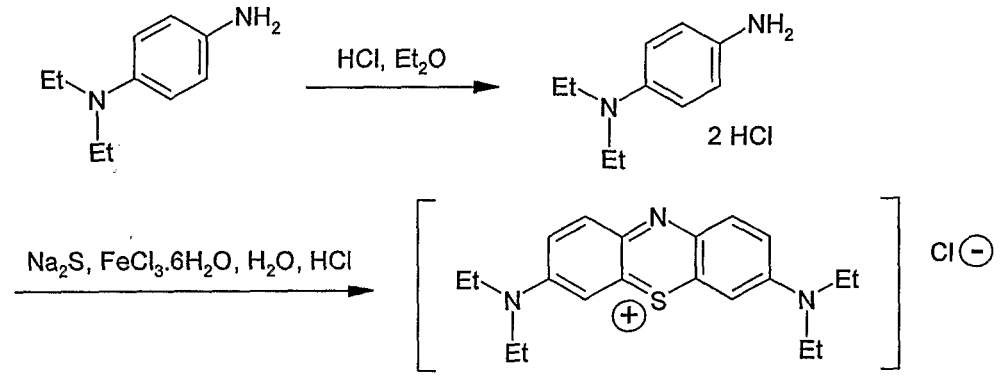
EXAMPLES

Example 1 – Methods of Synthesis

The following syntheses are provided solely for illustrative purposes and are not intended to limit the scope of the invention, as described herein.

Synthesis 1

Ethyl-thioninium chloride (ETC)



N,N-diethyl-p-phenylenediamine dihydrochloride

N,N-diethyl-*p*-phenylenediamine (5 g, 30.4 mmol) was dissolved in diethyl ether (25 cm^3) and hydrochloric acid (6 cm^3, 10 M) was added and the mixture was concentrated to give the title compound (7.22 g, 100%) as a red/brown solid. δ_H (250 MHz; D_2O): 7.68 (4H, m, ArH), 3.69 (4H, q, 7.32, NCH_2), 1.11 (6H, t, 7.32, CH_3); δ_C (62.9 MHz; D_2O): 12.1 (CH_3), 56.4 (NCH_2), 126.8 (ArC), 127.6 (ArC), 135.5 (ArC), 139.1 (ArC).

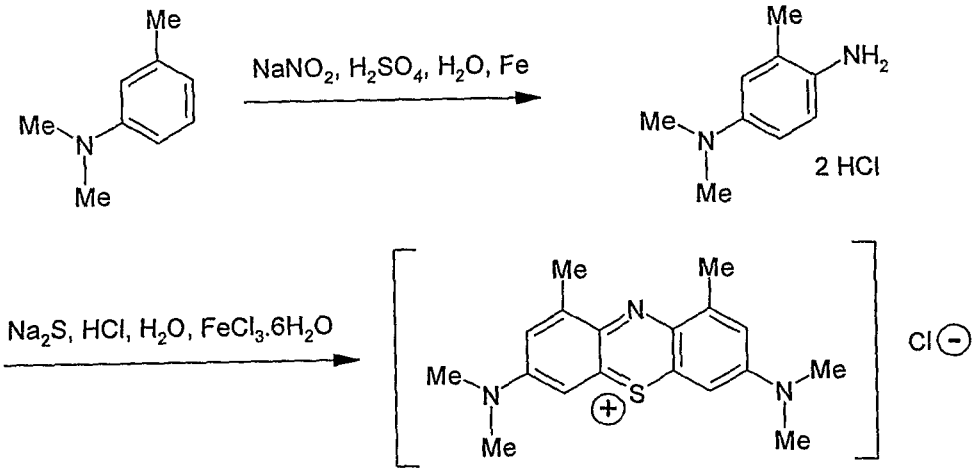
Ethyl-thioninium chloride

N,N-diethyl-*p*-phenylenediamine dihydrochloride (7.22 g, 30.4 mmol) was dissolved in water (250 cm^3) and the pH adjusted to 1.6 with HCl, to which sodium sulphide (>60%) (3.95 g, 30.4 mmol) was added portionwise. The suspension was stirred until all the sodium sulphide had dissolved. A solution of iron (III) chloride (27.15 g, 100 mmol) in water (200 cm^3) was prepared and half the solution was added to the mixture. An immediate colour change from light yellow to blue occurred. The solution was then aerated for 1 hour before the remaining iron (III) chloride solution was added. The mixture was cooled to 5°C and filtered to remove a light green sludge. Aqueous HCl (15 cm^3, 6 M) was added to the filtrate, followed by sodium chloride (60 g), and the suspension stirred for 5 minutes before filtering to give a solid product, which was dissolved in DCM, dried over magnesium sulphate, filtered, and concentrated to give a purple/green solid (1.28 g, 22%). This purple/green solid was loaded onto a prepared C18 reverse phase column and washed with water (1 L) or until the yellow colour ceased.

The product was washed off the column with MeOH/HCl (pH 2) and concentrated to give the title compound (0.64 g, 11%) as a sticky purple solid. δ_H (250 MHz; D_2O): 1.26(12H, t, 6.5, CH_3), 3.56 (8H, q, 6.5, NCH_2), 7.01 (2H, s, ArH), 7.20 (2H, d, 9.25, ArH), 7.54 (2H, d, 9.25, ArH); m/z (ESI) 340.2 (100%, $[M\text{-}Cl]^+$).

Synthesis 2

1,9-Dimethyl-methyl-thioninium chloride (DMMTC)



3-Methyl-N,N-dimethylphenylenediamine dihydrochloride

To a 250cm^3 round bottom flask was added water (100 cm^3) and the temperature was reduced to 5°C with an ice bath. To this cooled solution was carefully added sulphuric acid (98%, 22.5 g). To this solution was added 3-methyl-*N,N*-dimethylaniline (10 g, 74 mmol) and then sodium nitrite (5.6 g, 81.4 mmol), and the solution was stirred at room temperature for 1 hour. Iron (Fe) filings (12.8 g, 229 mmol) were added and the mixture stirred for a further 2 hours. The solution was filtered and then neutralized with saturated sodium hydrogen carbonate solution and the organics were extracted into ethyl acetate (3 x 100 cm^3). The extracts were dried over magnesium sulphate, filtered, and concentrated to give a brown oil. The oil was dissolved in diethyl ether (100 cm^3) and concentrated hydrochloric acid (50 cm^3) was added. The solution was evaporated to dryness to give the title compound (10 g, 60%) as a light tan solid. ν_{max} (KBr)/cm^{-1}: 2849 (*CH*), 2821 (*CH*), 2543 (*CH*), 2444 (*CH*), 1586 (*C=N*), 1487 (*CH*), 1445 (*CH*), 1415 (*CH*), 1138 (*CH*); δ_H (250 MHz; D_2O): 7.59 (1H, s, ArH), 7.50 (2H, s, ArH), 3.24 (6H, s, CH_3), 2.39 (3H, s, CH_3); δ_C(62.9 MHz; D_2O) 18.9 (CH_3), 48.8 (CH_3), 122.1 (ArC), 126.2 (ArC), 127.6 (ArC), 133.7 (ArC), 137.4 (ArC), 144.4 (ArC).

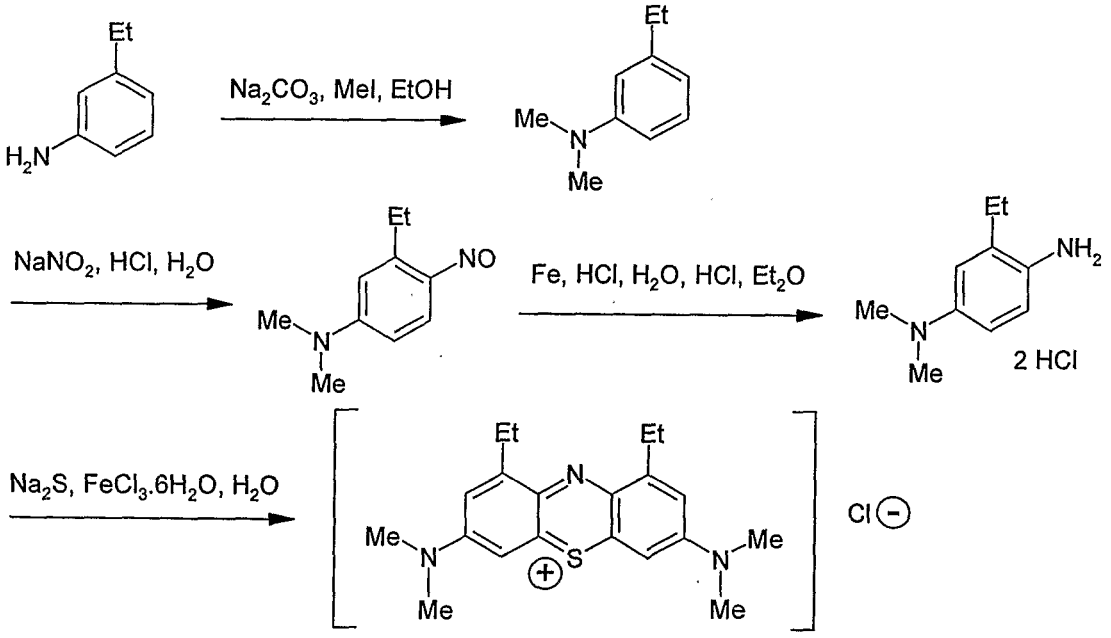
Dimethylmethythioninium Chloride

To a 500 cm^3 round bottom flask was added 3-methyl-*N,N*-dimethyl-phenylene-diamine dihydrochloride (0.9 g, 4.03 mmol) which was dissolved in aqueous hydrochloric acid

($50\ cm^3$, 3 M) before sodium sulphide (>60%) (0.52 g, 4.03 mmol) was added. Iron (III) chloride hexahydrate (7.26 g, 27 mmol) was dissolved in water ($50\ cm^3$) and half of this solution was poured into the reaction mixture, giving an immediate blue colour. The solution was then aerated for 2 hours before the remaining aqueous iron (III) chloride solution was added. The mixture was cooled to 5°C and filtered; the precipitate was dissolved in boiling water ($60\ cm^3$), filtered, and cooled. Hydrochloric acid ($10\ cm^3$, 6 M) was added to the cooled solution, which was then filtered to yield the title compound (0.22 g, 16%) as a purple/blue solid. ν_{max} (KBr)/cm^{-1}: 2926 (*CH*), 1604 (*C=N*), 1535, 1496, 1444 (*CH*), 1404 (*CH*), 1315 (*CH*), 1185 (*CH*); δ_H (250 MHz; DMSO): 7.29 (2H, s, ArH), 7.23 (2H, s, ArH), 3.29 (12H, s, CH_3), 2.55 (6H, s, CH_3); δ_C (62.9 MHz; DMSO): 18.9 (CH_3), 41.5 (CH_3), 105.7 (ArC), 118.7 (ArC), 133.6 (ArC), 134.5 (ArC), 147.2 (ArC), 154.2 (ArC); Anal. Calcd. for $C_{18}H_{22}N_3S.3H_2O$: C, 51.98; H, 6.74; N, 10.11; S, 7.70. Found: C, 52.03; H, 6.59; N, 10.05; S, 7.66.

Synthesis 3

1,9-Diethyl-methyl-thioninium chloride (DEMTC)



N,N-Dimethyl-m-ethylaniline

To a $100\ cm^3$ round bottom flask was added 3-ethylaniline (10 g, 82.5 mmol), ethanol ($15\ cm^3$), sodium carbonate (11.81 g, 111.4 mmol). Methyl iodide (31.63 g, 222 mmol) was added dropwise. The mixture was then heated at 45°C for 10 hours before cooling to room temperature and adding water ($100\ cm^3$). The mixture was extracted into diethyl ether (3 x $100\ cm^3$) and the extracts were dried over magnesium sulphate, filtered, and concentrated to give the title compound (4.68 g, 38%) as a light yellow oil. ν_{max} (neat)/cm^{-1}: 3045 (*CH*), 2960 (*CH*), 2920 (*CH*), 2891 (*CH*), 2797 (*CH*), 1597 (*C=N*), 1494 (*CH*), 1438 (*CH*), 1352 (*CH*), 1225 (*CH*); δ_H (250 MHz; $CDCl_3$): 7.22 (1H, t, 7.75, ArH), 6.63 (3H,

m, ArH), 2.97 (6H, s, NCH_3), 2.63 (2H, q, 7.5, CH_2), 1.27 (3H, t, 7.5, CH_3); δ_C (62.9 MHz; $CDCl_3$): 15.8 (CH_3), 29.5 (NCH_2), 40.8 (NCH_3), 110.3 (ArC), 112.4 (ArC), 116.5 (ArC), 129.1 (ArC), 145.3 (ArC), 150.9 (ArC).

N,N-Dimethyl-m-ethyl-p-phenylenediamine dihydrochloride

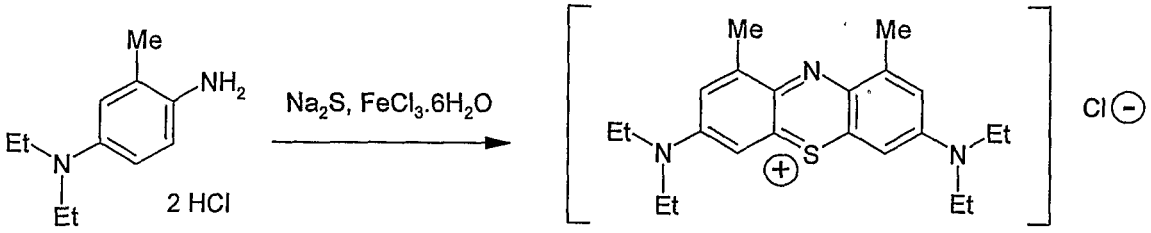
To a 250 cm^3 round bottom flask was added *N,N*-dimethyl-*m*-ethylaniline (4.68 g, 31.3 mmol), water (100 cm^3) and hydrochloric acid (8.5 cm^3, 37%) and the solution was cooled to 5°C. An aqueous (80 cm^3) solution of sodium nitrite (2.46 g, 3.57 mmol) was then added dropwise to the aniline mixture and stirred for 3 hours at room temperature. Iron (Fe) fillings (5.24 g, 94 mmol) and hydrochloric acid (8.5 cm^3, 37%) were added and the mixture was stirred at room temperature for 3 hours. The suspension was filtered and the filtrate adjusted to pH 7 with sodium bicarbonate solution before extraction into ethyl acetate (3 x 50 cm^3). The combined extracts were dried over magnesium sulphate, filtered, and concentrated to yield a brown oil. The oil was dissolved in ethanol (100 cm^3) and diethyl ether (80 cm^3) and hydrochloric acid (7cm^3, 37%) was added carefully to give the title compound (7.42 g, 72%) as a light tan solid. ν_{max} (KBr)/cm^{-1}: 2976 (*CH*), 2894 (*CH*), 2859 (*CH*), 2753 (*CH*), 1583 (*C=N*), 1508 (*CH*), 1486 (*CH*), 1459 (*CH*), 1183 (*CH*); δ_H (250 MHz; D_2O): 7.66 (1H, s, ArH), 7.56 (2H, s, ArH), 3.29 (6H, s, NCH_3), 2.74 (2H, q, 7.5, CH_2), 1.25 (3H, t, 7.5, CH_3); δ_C (62.9 MHz; $CDCl_3$): 15.5 (CH_3) 25.6 (NCH_2), 48.9 (NCH_3), 122.1 (ArC), 124.6 (ArC), 128.1 (ArC), 132.6 (ArC), 143.3 (ArC), 144.9 (ArC).

1,9-Diethyl Methylthioninium chloride

N,N-Dimethyl-*m*-ethyl-*p*-phenylenediamine dihydrochloride (1.3 g, 5.5 mmol) was dissolved in water (50 cm^3) and the solution adjusted to pH 1.6. Sodium sulphide >60% (0.71g, 5.5mmol) was then added portionwise to the pink solution. To the suspension was added an aqueous solution of iron (III) chloride (2.23 g, 8.2 mmol in 50 cm^3 of water) and there was an immediate colour change to purple. The solution was then aerated for 1 hour before a second portion of iron (III) chloride solution (2.23 g, 8.2 mmol in 50 cm^3 of water) was added. The solution was cooled to 5°C before filtering and washing the precipitate with water. To the filtrate was added sodium chloride (50 g) and the solution was stirred for 10 minutes, and the colour changed to red/purple as the product was salted out. The suspension was filtered and the solid dissolved in dichloromethane (100 cm^3) and methanol (10 cm^3) before drying over magnesium sulphate. Filtration and concentration gave the title compound (0.15 g, 15%) as a green solid. ν_{max} (KBr)/cm^{-1}: 3408 (*CH*), 2613 (*CH*), 1606 (*C=N*), 1399 (*CH*), 1316 (*CH*); δ_H (250 MHz; D_2O): 6.55 (2H, s, ArH), 6.23 (2H, s, ArH), 2.92 (12H, s, NCH_3), 2.56 (4H, q, 7.5, CH_2), 0.99 (6H, t, 7.5, CH_3); (ESI), 340.4 (100%, $[M - Cl]^+$). Optionally, flash column chromatography was performed to remove iron chloride residues, with 10% methanol: 90% dichloromethane as eluent and using silica 40-63µ 60Å.

Synthesis 4

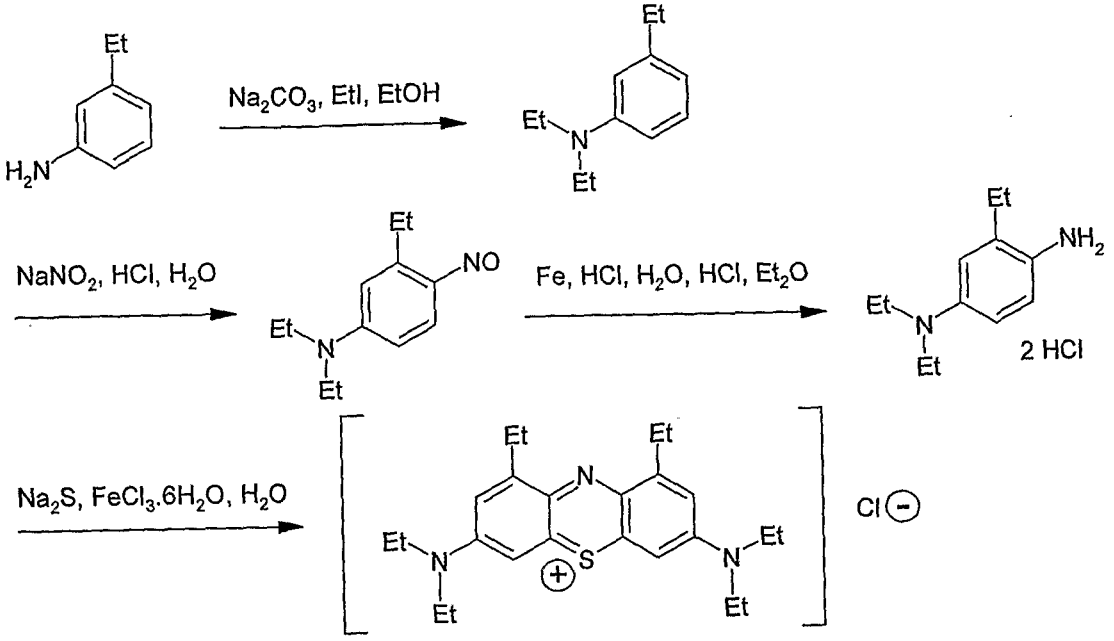
1,9-Dimethyl-ethyl-thioninium chloride (DMETC)



N,N-Diethyl-3-methyl-4-phenylenediamine dihydrochloride (10.74 g, 50 mmol) was dissolved in water (400 cm^3) and the pH adjusted to 1.6, which then had sodium sulphide (>60%) (3.90 g, 50 mmol) added. Iron (III) chloride (20.28 g, 75 mmol) was added as an aqueous solution (175 cm^3) giving an immediate colour change from yellow to deep blue. The mixture was aerated for 1 hour before a second aliquot of aqueous iron (III) chloride (20.28 g, 75 mmol in 175 cm^3) was added. The solution was cooled to 5°C and held at that temperature for 1 hour before filtering. The filtrate had sodium chloride (200 g) added and was filtered to yield the crude product as a blue/purple solid. The crude solid was purified by column chromatography (eluent being 10% MeOH, 90% DCM using silica 40-63µ 60Å) to give the title compound (0.80 g, 4%) as a green/purple solid. ν_{max} (KBr)/cm^{-1}: 2971 (*CH*), 2921 (*CH*), 2865 (*CH*), 1600 (*C=N*), 1412 (*CH*), 1326 (*CH*); δ_H (250 MHz; D_2O): 6.62 (2H, s, ArH), 6.39 (2H, s, ArH), 3.30 (8H, q, NCH_2), 1.89 (6H, s, $ArCH_3$), 1.09 (12H, t, CH_3); δ_C(62.9 MHz; D_2O) 12.6 (CH_3), 18.0 (CH_3), 46.2 (NCH_2), 103.6 (ArC), 117.1 (ArC), 132.3 (ArC), 133.9 (ArC), 147.3 (ArC), 151.9 (ArC); m/z (ESI) 368.1 (100%, $[M-Cl]^+$).

Synthesis 5

1,9-Diethyl-ethyl-thioninium chloride (DEETC)



N,N-Diethyl-m-ethylaniline

To a 100 cm^3 round bottom flask was added 3-ethylaniline (5.0 g, 41.3 mmol), ethanol (7.5 cm^3), sodium carbonate (5.9 g, 55.7 mmol). Ethyl iodide (17.38 g, 111.4 mmol) was added dropwise. The mixture was then heated at 45°C for 12 hours before cooling to room temperature and adding water (50cm^3). The mixture was extracted into diethyl ether (3 x 50 cm^3) the extracts were dried over magnesium sulphate, filtered, and concentrated to give the title compound (7.03 g, 96%) as a light yellow oil. δ_H (250 MHz; $CDCl_3$): 7.20 (1H, dd, 9, 7.25, ArH), 6.60 (3H, m, ArH), 3.43 (4H, q, 7, NCH_2), 2.69 (2H, q, 7.25, CH_2), 1.32 (3H, t, 7.5, CH_3), 1.23 (6H, t, 7, CH_3); δ_C (62.9 MHz; $CDCl_3$): 12.7 (CH_3), 15.8 (CH_3), 29.5 (CH_2), 44.4 (NCH_3), 109.4 (ArC), 111.4 (ArC), 115.1 (ArC), 129.2 (ArC), 145.4 (ArC), 147.9 (ArC).

N,N-Diethyl-m-ethyl-p-phenylenediamine dihydrochloride

To a 250 cm^3 round bottom flask was added *N,N*-diethyl-*m*-ethylaniline (5 g, 28.2 mmol), water (50 cm^3) and hydrochloric acid (9 cm^3, 37%) and the solution was cooled to 5°C. An aqueous (20 cm^3) solution of sodium nitrite (2.14 g, 31.0 mmol) was then added dropwise to the aniline mixture and stirred for 1 hour at low temperature. Iron (Fe) fillings (4.72 g, 84.6 mmol) and hydrochloric acid (9 cm^3, 37%) were added and the mixture stirred below 30°C for 2 hours. The suspension was filtered and the filtrate adjusted to pH 7 with sodium bicarbonate solution before extraction into ethyl acetate (3 x 50 cm^3). The combined extracts were dried over magnesium sulphate, filtered, and concentrated

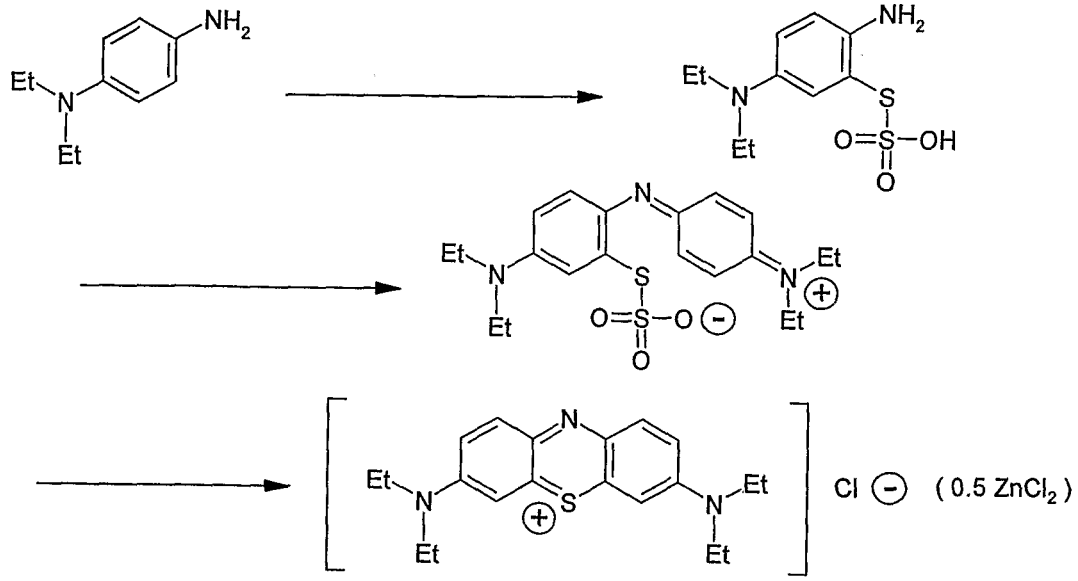
to yield a brown oil. The crude oil was purified by column chromatography (eluent being ethyl acetate using silica 40-63μ 60Å) giving the phenylenediamine as a brown oil (2.2 g, 41%). The oil was dissolved in diethyl ether (50 cm^3) and hydrochloric acid added (2.5cm^3, 37%) and the solution was concentrated to give the title compound (2.76 g, 41%) as a light brown solid. δ_H (250 MHz; D_2O): 7.50 (3H, m, ArH), 3.59 (4H, q, 7.25, NCH_2), 2.69 (2H, q, 7.5, CH_2), 1.20 (3H, t, 7.5, CH_3), 1.03 (6H, t, 7.25, CH_3); δ_C (62.9 MHz; D_2O): 12.1 (CH_3), 15.5 (CH_3), 25.5 (CH_2), 56.3 (NCH_2), 123.9 (ArC), 126.0 (ArC), 127.9 (ArC), 133.1 (ArC), 139.4 (ArC), 143.3 (ArC).

1,9-Diethyl Ethylthioninium chloride

N,N-Diethyl-*m*-ethyl-*p*-phenylenediamine dihydrochloride (2 g, 7.5 mmol) was dissolved in water (75 cm^3) and the solution adjusted to pH 1.6. The pink solution then had sodium sulphide (>60%) (1.35g, 10.4mmol) added portion-wise. To the suspension was added an aqueous solution of iron (III) chloride (4.22 g, 15.6 mmol in 35 cm^3 of water) where there was an immediate colour change to purple. The solution was then aerated for 1 hour before a second portion of iron (III) chloride (4.22 g, 15.6 mmol in 35 cm^3 of water) solution was added. The solution was cooled to 5°C before filtering and washing the precipitate with water. The precipitate was also washed with ethanol and the ethanol concentrated to give a sticky purple solid. To the aqueous filtrate was added sodium chloride (50 g) and the solution was stirred for 10 minutes whereby the colour changed to red/purple as the product was salted out. The suspension was filtered and the solid dissolved in dichloromethane (100 cm^3) and methanol (10 cm^3) before drying over magnesium sulphate. Filtering and concentration with the ethanol soluble product gave the title compound (0.06 g, 3%) as a purple solid. δ_H (250 MHz; D_2O): 6.73 (2H, s, ArH), 6.48 (2H, s, ArH), 3.45 (8H, brdq, NCH_2), 2.46 (4H, q, 7.5, CH_2), 1.17 (12H, brdt, CH_3), 0.93 (6H, t, 7.5, CH_3); m/z (ESI) 396.2 (100%, $[M\text{-}Cl]^+$). Optionally, flash column chromatography was performed to remove iron chloride residues, with 10% methanol: 90% dichloromethane as eluent and using silica 40-63μ 60Å.

Synthesis 6

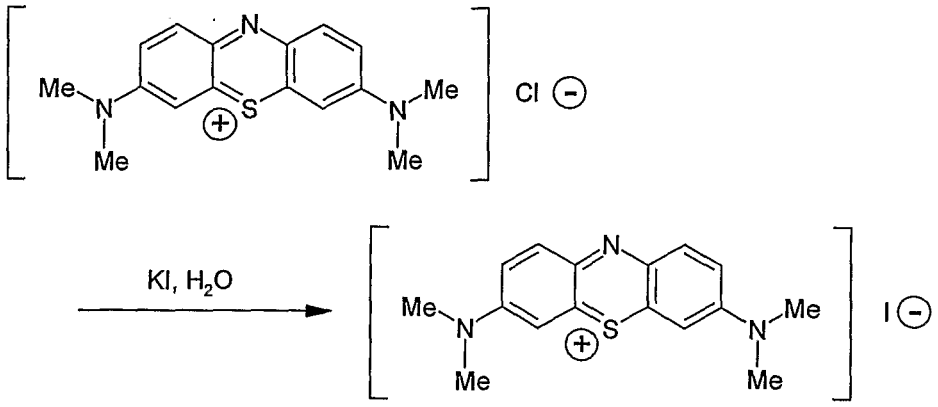
Ethyl-thioninium chloride zinc chloride double salt (ETZ)



A stirred mixture of *N,N*-diethyl-*p*-phenylenediamine (5.0 g, 30.4 mmol) in H_2O (100 cm^3) and H_2SO_4 (conc., '98 %', 1 cm^3) was treated with non-reducing $ZnCl_2$ solution ($ZnCl_2$, 7.60 g, 55 mmol in 15 cm^3 of H_2O with $Na_2Cr_2O_7.2H_2O$, 100 mg) to produce a reddish reaction mixture. Additions of $Al_2(SO_4)_3.16H_2O$ solution (5.80 g, 9.2 mmol in 10 cm^3 of H_2O), $Na_2S_2O_3.5H_2O$ solution (8.0 g, 32.2 mmol in 10 cm^3 H_2O) and one-third of a solution of $Na_2Cr_2O_7.2H_2O$ (8.7 g, 29.2 mmol in 15 cm^3 of H_2O) were followed by a rapid rise in temperature to 40°C. A solution of *N,N*-diethylaniline (3.0 g, 20.1 mmol in conc. HCl, 4 cm^3) was added, and followed by an addition of the remaining $Na_2Cr_2O_7.2H_2O$ solution. A dark green precipitate was observed. The temperature was rapidly raised to 75°C, after which a slurry of activated MnO_2 (3.80 g, 44.7 mmol in 5 cm^3 of H_2O) was added. The temperature was raised to 85°C, and left to stir at that temperature for 30 minutes. A blue solution with precipitate was observed. The reaction mixture was cooled to 50°C and H_2SO_4 (conc., 11cm^3) was slowly added. The reaction was further cooled to 20°C, and vacuum filtered to recover the precipitate, which was then washed with brine (saturated salt water). This black solid was re-dissolved in H_2O (250 cm^3) at 100°C, and cooled, followed by vacuum filtration to remove insolubles. The filtrate was treated with $ZnCl_2$ (4 g) and NaCl (23 g) and left in the refrigerator for 16 hours, after which the resulting precipitate was recovered by vacuum filtration, washed with brine (30 cm^3), and dried in a vacuum oven for 3 hours, to give the title compound (5.7 g, 71 %) as a rusty red powder. δ_H (250 MHz, D_2O): 1.20 (12H, br t, CH_3), 3.50 (8H, br q, CH_2), 6.80 (2H, s, ArH), 7.05 (2H, br d, ArH) and 7.30 (2H, br d, ArH). See, for example, Fierz-David and Blangley, 1949, "F. Oxazine and Thiazine Dyes," in: Fundamental Processes of Dye Chemistry, published by Interscience (London, UK), pp. 308-314.

Synthesis 7

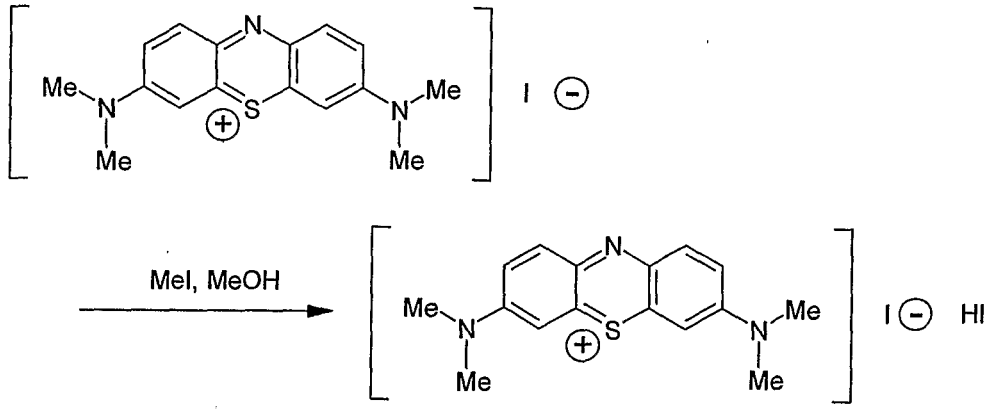
Methyl-thioninium Iodide (MTI)



Methyl-thioninium chloride (2.00 g, 6.25 mmol) was dissolved in water (50 cm^3) and potassium iodide (1.56 g, 9.4 mmol) was added with stirring. A precipitate formed, which was filtered and the solid was recrystallised from boiling water (50 cm^3) to yield the title compound (1.98 g, 77%) as fine green needles. δ_H (250 MHz; DMSO): 7.88 (2H, br d, Ar*H*), 7.49 (4H, br s, Ar*H*), 3.37 (12H, s, CH_3). Analysis for $C_{16}H_{18}N_3SI$: C, 46.72; H, 4.41; N, 10.22; S, 7.80; I, 30,85; Found: C, 46.30; H, 4.21; N, 10.14; S, 7.86; I, 29.34.

Synthesis 8

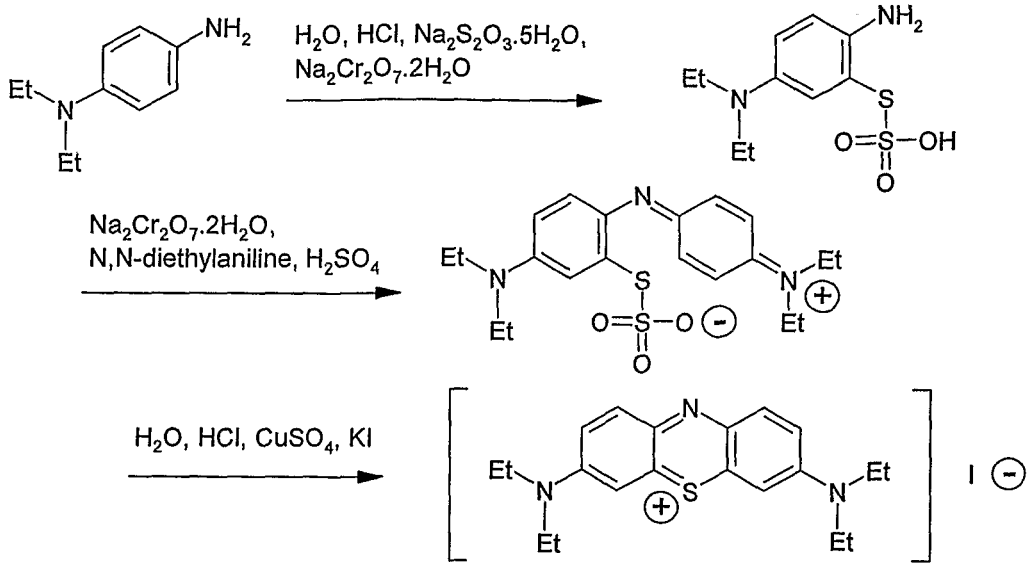
Methyl-thioninium Iodide Hydrogen Iodide Mixed Salt (MTI.HI)



Methyl-thioninium iodide (0.50 g, 1.22 mmol) was dissolved in methanol (20 cm^3) and methyl iodide (1.90 g, 13.37 mmol) was added while stirring. The mixture was heated at reflux for 18 hours before additional methyl iodide (0.42 g, 6.69 mmol) was added and the mixture was once again heated to reflux and stirred for 8 hours. The mixture was cooled to room temperature, giving a solid that was filtered and washed with methanol to yield the title compound (0.30 g, 46%) as bronze green solid. δ_H (250 MHz; DMSO): 7.82 (2H, d, J = 8.5, Ar*H*), 7.42 (4H, s, Ar*H*), 3.34 (12H, s, CH_3). δ_C (62.9 MHz; DMSO): 153.8 (Ar*C*), 137.9 (Ar*C*), 134.9 (Ar*C*), 133.5 (Ar*C*), 119.1 (Ar*C*), 118.8 (Ar*C*), 106.9 (Ar*C*), 106.6 (Ar*C*), 41.1 (NCH_3).

Synthesis 9

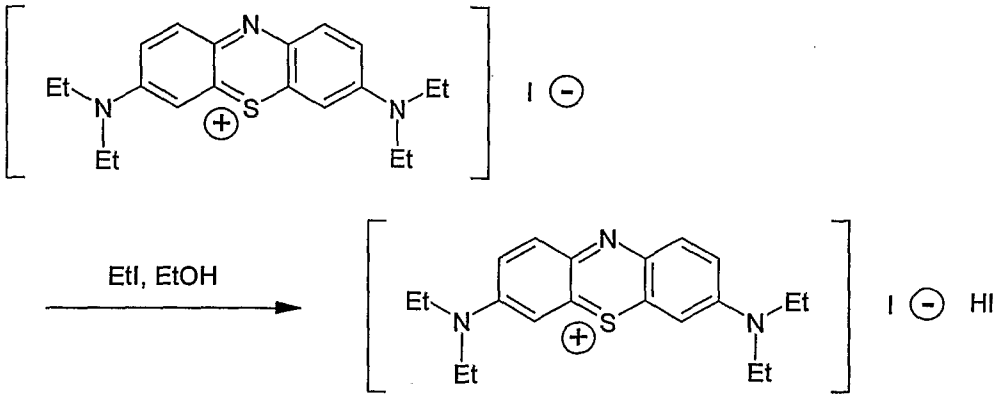
Ethyl-thioninium iodide (ETI)



A stirred mixture of *N,N*-diethyl-*p*-phenylenediamine (10.0 g, 61 mmol) in aqueous hydrochloric acid (0.5 M, 200 cm^3) was adjusted to pH 2 with aqueous sodium hydroxide (10%). The diamine solution was cooled to 5°C before the addition of aqueous $Na_2S_2O_3.5H_2O$ (16.65 g, 67 mmol in 20 cm^3 H_2O). An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (7.27 g, 24 mmol in 35 cm^3 of H_2O) was added dropwise to the mixture over a 15 minute period giving a black suspension. The suspension was stirred at 5°C for 1 hour (pH = 8.07, T = 3.7°C). A solution of *N,N*-diethylaniline (8.25 g, 61 mmol), H_2SO_4 (6 g) and water (10 cm^3) was cooled to 5°C before addition to the suspension. An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (19.09 g, 64 mmol in 50 cm^3 of H_2O) was then added dropwise to the mixture over a 20 minute period giving a thick dark green suspension. The mixture was stirred at 5°C for 2 hours (pH = 6.75, T = 6°C) before filtering. The green purple solid obtained was washed with water (2 x 50 cm^3). The solid was slurried in aqueous hydrochloric acid (300 cm^3, pH 2) giving a suspension with a pH = 6.37 at 22°C. To the suspension was added $CuSO_4$ (1.52 g, 6.1 mmol) and the mixture heated to 90°C where a deep blue solution formed. After stirring at this temperature for 1 hour the mixture was cooled to 25°C and filtered. The solid was washed with water (2 x 50 cm^3), the filtrate was adjusted from pH 6.33 to pH 2.00, T = 25°C with hydrochloric acid (5 M). The deep blue solution was heated to 80°C and potassium iodide (14 g) was added and upon cooling an orange purple precipitate was deposited. Filtration gave a purple powder (8.8 g, 31%), which was recrystallised from hot ethanol (400 cm^3) to give the title compound as fine purple needles. Mp 211°C; ν_{max} (KBr)/cm^{-1}: 3574 (*CH*), 3484 (*CH*), 3028 (*CH*), 2965 (*CH*), 1662 (*C=C*), 1539 (*CH*), 1474 (*CH*), 1346 (*CH*); δ_C (62.9 MHz, $CDCl_3$): 1.33 (12H, t, 7, CH_3), 3.72 (8H, q, 7, NCH_2), 7.23 (2H, d, 9.75, ArH), 7.41 (2H, s, ArH), 7.83 (2H, d, 9.75, ArH); δ_H (62.9 MHz, $CDCl_3$):152.4, 138.8, 135.7, 135.2, 118.3, 106.4, 46.8, 13.2.

Synthesis 10

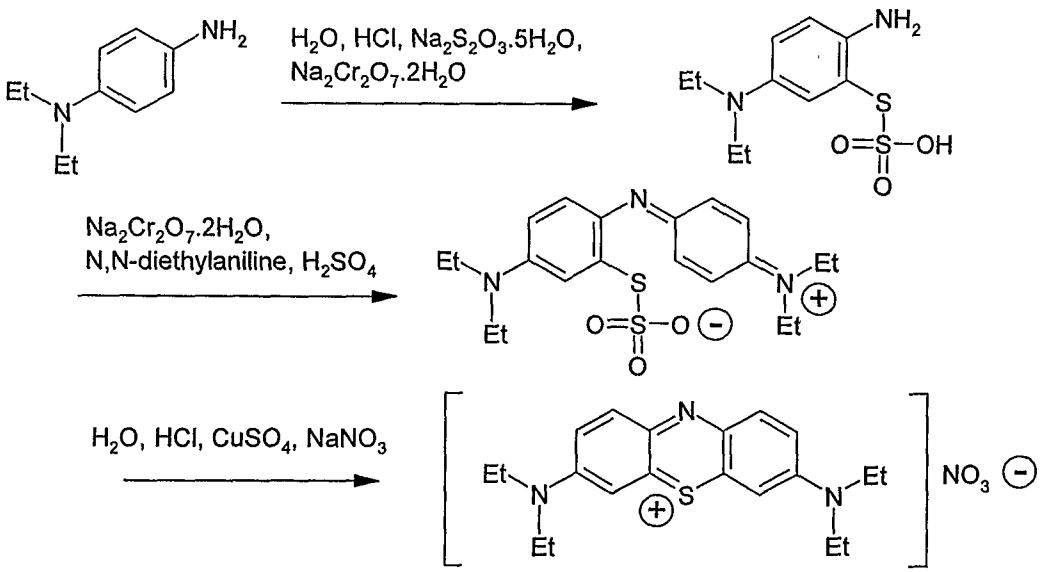
Ethyl-thioninium iodide Hydrogen Iodide Mixed Salt (ETI.HI)



Ethyl-thioninium iodide (2.00 g, 4.28 mmol) was dissolved in ethanol (100 cm^3) and ethyl iodide (27.35 g, 175 mmol) was added while stirring. The mixture was heated at reflux for 18 hours, then cooled to room temperature, giving a precipitate that was filtered and washed with ethanol to yield the title compound (1.02 g, 40%) as a bronze solid. δ_H (250 MHz; D_2O): 7.90 (2H, br d, Ar*H*), 7.42 (4H, s, Ar*H*), 2.45 (8H, br q, NCH_2), 1.23 (12H, br t, CH_3).

Synthesis 11

Ethyl-thioninium nitrate (ETN)

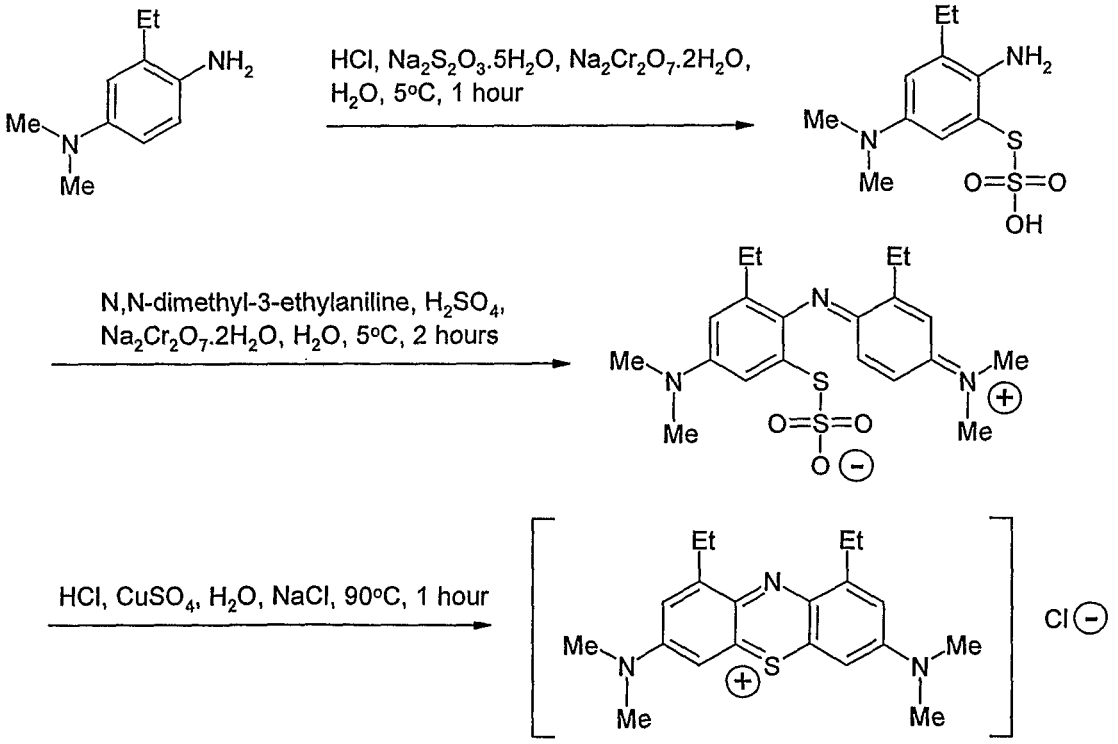


A stirred mixture of *N,N*-diethyl-*p*-phenylenediamine (10.0 g, 61 mmol) in aqueous hydrochloric acid (0.5 M, 200 cm^3) was adjusted to pH 2 with aqueous sodium hydroxide (10%). The diamine solution was cooled to 5°C before the addition of aqueous $Na_2S_2O_3.5H_2O$ (16.65 g, 67 mmol in 20 cm^3 H_2O). An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (7.27 g, 24 mmol in 35 cm^3 of H_2O) was added dropwise to the mixture over a 15 minute period giving a black suspension. The suspension was stirred at 5°C for

1 hour (pH = 8.07, T = 3.7°C). A solution of *N,N*-diethylaniline (8.25 g, 61 mmol), H_2SO_4 (6 g) and water (10 cm^3) was cooled to 5°C before addition to the suspension. An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (19.09 g, 64 mmol in 50 cm^3 of H_2O) was then added dropwise to the mixture over a 20 minute period giving a thick dark green suspension. The mixture was stirred at 5°C for 2 hours (pH = 6.75, T = 6°C) before filtering. The green purple solid obtained was washed with water (2 x 50 cm^3). The solid was slurried in aqueous hydrochloric acid (300 cm^3, pH 2) giving a suspension with a pH = 6.37 at 22°C. To the suspension was added $CuSO_4$ (1.52 g, 6.1 mmol) and the mixture heated to 90°C wherein a deep blue solution formed. After stirring at this temperature for 1 hour, the mixture was cooled to 25°C and filtered. The solid was washed with water (2 x 50 cm^3), and the filtrate was adjusted from pH 6.33 to pH 2.00, T = 25°C with hydrochloric acid (5 M). The deep blue solution was heated to 80°C and had sodium nitrate (50 g) added and was allowed to cool to 25°C slowly while stirring gently. The product was filtered as green needles (6.80 g, 28%). δ_H (250 MHz, $CDCl_3$): 1.36 (12H, t, 7, CH_3), 3.72 (8H, q, 7, NCH_2), 7.23 (2H, d, 9.5, ArH), 7.39 (2H, s, ArH), 7.89 (2H, d, 9.5, ArH); δ_H (62.9 MHz, $CDCl_3$): 152.5, 138.8, 135.7, 135.6, 118.1, 106.4, 46.6, 12.9.

Synthesis 12

1,9-Diethyl-methylthioninium chloride (DEMTC)



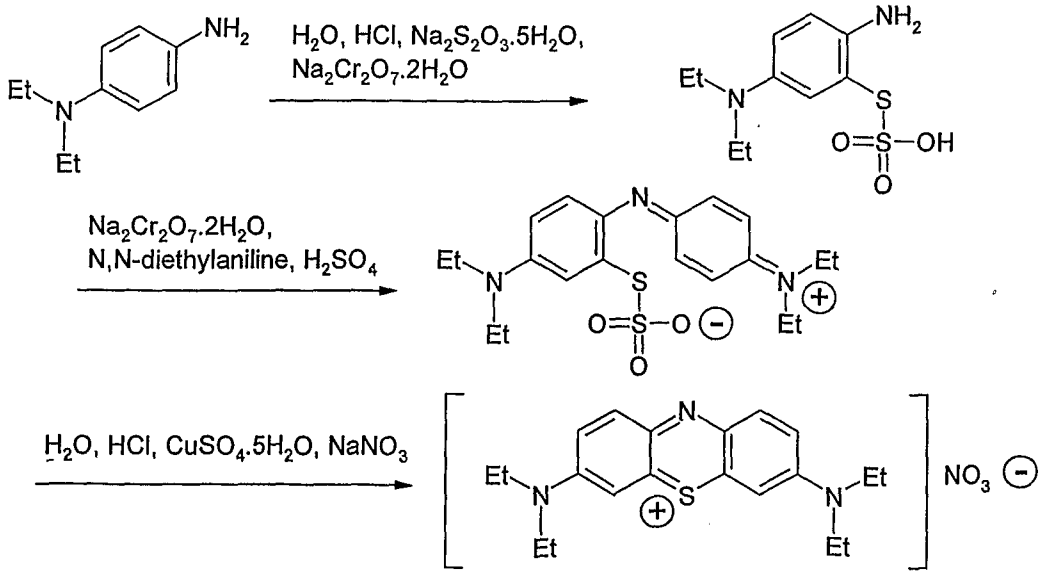
N,N-Dimethyl-3-ethyl-p-phenylenediamine (5.15 g, 31.4 mmol) was dissolved in aqueous hydrochloric acid (0.5 M, 100 cm^3). The resulting solution was adjusted to pH 2 with aqueous sodium hydroxide (10%) and cooled to 5°C. An aqueous solution of $Na_2S_2O_3.5H_2O$ (8.57 g, 34.6 mmol in 10 cm^3 of water) was added drop-wise to the stirred

solution. An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (3.74 g, 12.6 mmol in 18 cm^3 of water) was added drop-wise over a 15 minute period giving a black suspension. Stirring was continued at 5°C for 1 hour. N,N-Dimethyl-3-ethylaniline (4.68 g, 31.4 mmol) was dissolved in aqueous sulphuric acid (3.00 g in 5 cm^3 of water) and the resulting solution cooled to 5°C before addition to the reaction mixture. An aqueous solution of $Na_2Cr_2O_7.2H_2O$ (9.83 g, 33.0 mmol in 25 cm^3 of water) was added drop-wise over a 20 minute period giving a dark green suspension. Stirring was maintained at 5°C for a further 2 hours before the solid was collected by filtration. The solid was washed with water (3 x 30 cm^3) before it was slurried in aqueous hydrochloric acid (150 cm^3, pH 2). $CuSO_4$ (785 mg, 3.14 mmol) was added to the suspension and the mixture heated to 90°C for 1 hour. The resulting deep blue mixture was filtered and the solid washed with water (3 x 40 cm^3). The filtrate was adjusted to pH 2 with hydrochloric acid (1 M). The solution was heated to 80°C before NaCl (2.00 g, 34.5 mmol) was added. The solution was allowed to cool to room temperature where a precipitate was deposited. The solid was collected by filtration to give the title compound as a dark green solid (1.89 g, 16%). δ_H (250 MHz; D_2O): 6.65 (2H, s, ArH), 6.23 (2H, s, ArH), 2.92 (12H, s, NCH_3), 2.56 (4H, q, 7.5, CH_2), 0.99 (6H, t, 7.5, CH_3); ν_{max} (KBr)/cm^{-1} 3408 (*CH*), 2613 (*CH*), 1606 (C=N), 1399 (*CH*), 1316 (*CH*). MS (ESI): 340.4 [100% (M-Cl) $^+$].

This synthesis represents an alternative to Synthesis 3 above. The principal advantages of this synthesis compared to Synthesis 3 above are: (a) higher yield, (b) it can be performed at greater concentrations making it more suitable for use on an industrial scale, and (c) it does not produce H2S, and so is cheaper and simpler when performed on an industrial scale.

Synthesis 13

Ethyl-thioninium nitrate (ETN)



To a solution of *N,N*-diethyl-*p*-phenylene (6.61 g, 40 mmol) and aqueous hydrochloric acid (0.5 M, 132 mL) pre-cooled to 5°C was added aqueous sodium thiosulfate (10.91 g, 44 mmol in 15 mL of H_2O) in one portion. Sodium dichromate (4.96 g, 15.75 mmol in 10 mL of H_2O) was added drop-wise over a 10 minute period and the black solution was stirred for 1 hour at 5°C (pH = 8.22, Temp. = 1.8°C). Meanwhile a heterogeneous solution containing *N,N*-diethylaniline (5.96 g, 40 mmol), sulphuric acid (3.96 g), and water (6.60 mL) was prepared, cooled to 5°C, and then added to the stirring black suspension in one portion. A second sodium dichromate (12.52 g, 42 mmol in 40 mL of H_2O) solution was added drop-wise over a 15 minute period and the dark green suspension which formed was left stirring at 5°C for 2 hours (pH = 7.23, Temp. = 8.0°C). Sodium dithionite (1.48 g, 8.44 mmol) as an aqueous solution was added in one portion and the mixture was stirred for 15 minutes to warm to room temperature (22°C). The resulting reaction mixture was then used in one or the other of the following two alternatives.

In one alternative (Filtration of BG (two-pot)): The dark green solid which formed was filtered off, washed with water (2 x 50 mL) and sucked to reasonable dryness (30 minutes). The dark green solid was stirred in water acidified to pH 2 with hydrochloric acid and $CuSO_4.5H_2O$ (0.99 g, 4.00 mmol) was added. The solution was heated to 90°C and stirred for 1 hour at this temperature resulting in the formation of an intense blue solution. Heating was stopped and the reaction mixture was left to cool to room temperature. The blue solution was filtered to remove insolubles and the solid washed with water (2 x 50 mL). The filtrate was acidified to pH 2 with hydrochloric acid (5 M, 5 mL) and $NaNO_3$ (33 g, 388 mmol) was added in one portion. The solution was heated to 80°C and left to cool to room temperature with gentle stirring. The green needles which resulted were filtered from the solution and sucked to dryness to give the title compound (4.38g; 28%). Mp 178°C; ν_{max}(KBr)/cm^{-1} 3564 (CH), 3463 (CH), 3048 (CH), 2975 (CH), 1522 (C=C), 1489 (CH), 1370 (CH); δ_H (250 MHz, $CDCl_3$) 1.36 (12H, t, 7, CH_3), 3.72 (8H, q, 7 NCH_2), 7.23 (2H, d, 9.5, ArH), 7.39 (2H, s, ArH), 7.89 (2H, d 9.5, ArH); δ_C (62.9MHz, $CDCl_3$) 152.5, 138.8, 135.7, 135.6, 118.1, 106.4, 46.6, 12.9.

In another alternative (No-filtration of BG (one-pot)): Once the solution had warmed, $CuSO_4.5H_2O$ (0.99 g, 4.00 mmol) was added. The solution was then heated to 90°C and stirred at this temperature for 1 hour, after which the solution had turned deep blue. The blue solution was allowed to cool to room temperature and filtered to remove insolubles. The filtrate was adjusted to pH 2 with hydrochloric acid (5 M, 20 mL approx.) and $NaNO_3$ (33 g, 388 mmol) was added. The stirring solution was heated to 80°C and left to cool overnight with gentle stirring. The resulting green needles were filtered from solution and sucked to dryness to give the title compound (1g, 5%). Mp 178°C; ν_{max}(KBr)/cm^{-1} 3564 (CH), 3463 (CH), 3048 (CH), 2975 (CH), 1522 (C=C), 1489 (CH), 1370 (CH); δ_H (250 MHz, $CDCl_3$) 1.36 (12H, t, 7, CH_3), 3.72 (8H, q, 7 NCH_2), 7.23 (2H, d, 9.5, ArH), 7.39 (2H,

s, ArH), 7.89 (2H, d 9.5, ArH); δ_C (62.9MHz, $CDCl_3$) 152.5, 138.8, 135.7, 135.6, 118.1, 106.4, 46.6, 12.9.

Example 2 – Activity and Therapeutic Index

In vitro assay for establishing B50

This is described in detail in WO 96/30766. Briefly, a fragment of tau corresponding to the core repeat domain, which has been adsorbed to a solid phase substrate, is able to capture soluble full-length tau and bind tau with high affinity. This association confers stability against proteolytic digestion of the aggregated tau molecules. The process is self-propagating, and can be blocked selectively by prototype pharmaceutical agents.

More specifically, truncated tau (residues 297-390; dGA) diluted in carbonate buffer (pH 9.6) was bound to the assay plate, and full-length tau (T40) was added in the aqueous phase. The aqueous phase binding buffer contained 0.05% Tween-20 and 1% gelatine in phosphate-buffered saline (pH7.4). Bound tau was detected using mAb 499 that recognises an N-terminal epitope within the aqueous phase full-length tau but that fails to recognise the solid phase-bound truncated tau fragment.

The concentration of compound required to inhibit the tau-tau binding by 50% is referred to as the B50 value.

Cell-based assay for establishing EC50

The process is described in more detail in WO 02/055720. In essence, fibroblast cells (3T6) express full-length tau ("T40") under control of an inducible promotor, and low constitutive levels of the PHF-core tau fragment (12 kD fragment). When T40 expression is induced, it undergoes aggregation-dependent truncation within the cell, N-terminally at ~ αα 295 and C-terminally at ~ αα 390, thereby producing higher levels of the 12 kD PHF-core domain fragment. Production of the 12 kD fragment can be blocked in a dose-dependent manner by tau-aggregation inhibitors. Indeed the quantitation of inhibitory activity of compounds with respect to proteolytic generation of the 12 kD fragment within cells can be described entirely in terms of the same parameters which describe inhibition of tau-tau binding in vitro. That is, the extent of proteolytic generation of the 12 kD fragment within cells is determined entirely by the extent to tau-tau binding through the repeat domain. The availability of the relevant proteases within the cell is non-limiting.

Results are expressed as the concentration at which there is a 50% inhibition of generation of the 12 kD fragment. This is referred to as the EC50 value.

Toxicity in cells – LD50 and therapeutic index (RxI)

Toxicity of the compounds described herein was assessed in the cell based assay used to assess EC50. Toxicity was measured by cell numbers after 24 hrs exposure to the compound using a lactate dehydrogenase assay kit TOX-7(Sigma Biosciences) according to the manufacturer's instructions after lysis of remaining cells. Alternatively a kit from Promega UK (CytoTox 96) was used, again according to the manufacturer's instructions.

The therapeutic index (RxI) was calculated as follows: RxI = LD50 / EC50.

	Compound	B50 (μM)	EC50 (μM)	LD50 (μM)	RxI
	MTC	218 ± 20.1 (6)	0.59 ± 0.04 (69)	65.0 ± 5.0 (38)	110
	DMMTC	3.4 ± 0.2 (2)	0.04 ± 0.004 (22)	2.7 ± 1.2 (6)	67
A	ETC	49.0 ± 8.5 (10)	0.07 ± 0.007 (53)	32.0 ± 4.0 (26)	480
B	DEMTC	26.2 ± 5.3 (6)	0.0016 ± 0.0006 (13)	3.3 ± 0.6 (22)	2,173
C	DMETC	4.5 ± 0.3 (3)	0.004 ± 0.001 (6)	4.2 ± 2.2 (4)	1,048
D	DEETC	3.7 ± 0.5 (3)	0.0006 ± 0.0003 (3)	1.6 ± 0.4 (13)	2,667
F	ETZ	145.4 ± 5.7 (5)	0.06 ± 0.01 (6)	39.0 ± 7.0 (4)	670
G	MTI	382	0.72	120	168
H	MTI.HI	271	0.70	120	168
I	ETI	>500	0.06	-	-
J	ETI.HI	83	-	-	-
L	ETN	> 500 (2)	0.04 (1)	13.0 ± 0.5. (2)	325

CLAIMS

1. A compound for use in a method of treatment or prophylaxis of the human or animal body by therapy, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

2. A compound for use in a method of treatment or prophylaxis of a tauopathy condition in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

3. A compound for use in a method of treatment or prophylaxis of a disease of tau protein aggregation in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

4. A compound for use in a method of treatment or prophylaxis of Alzheimer's disease (AD), Pick's disease, Progressive Supranuclear Palsy (PSP), fronto-temporal dementia (FTD), parkinsonism linked to chromosome 17 (FTDP-17), disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC), pallido-ponto-nigral degeneration (PPND), Guam-ALS syndrome, pallido-nigro-luysian degeneration (PNLD), or cortico-basal degeneration (CBD) in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

5. A compound for use in a method of treatment or prophylaxis of Alzheimer's disease (AD) in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

6. A compound according to any one of claims 1 to 5, wherein the compound is ETC.

7. A compound according to any one of claims 1 to 5, wherein the compound is DEMTC.

8. A compound according to any one of claims 1 to 5, wherein the compound is DMETC.

9. A compound according to any one of claims 1 to 5, wherein the compound is DEETC.

10. A compound according to any one of claims 1 to 5, wherein the compound is MTZ.

11. A compound according to any one of claims 1 to 5, wherein the compound is ETZ.

12. A compound according to any one of claims 1 to 5, wherein the compound is MTI.

13. A compound according to any one of claims 1 to 5, wherein the compound is MTI.HI.

14. A compound according to any one of claims 1 to 5, wherein the compound is ETI.

15. A compound according to any one of claims 1 to 5, wherein the compound is ETI.HI.

16. A compound according to any one of claims 1 to 5, wherein the compound is MTN.

17. A compound according to any one of claims 1 to 5, wherein the compound is ETN.

18. A compound according to any one of claims 1 to 5, wherein the compound is ETC.

19. A compound according to any one of claims 1 to 18, wherein the compound is provided in the form of a dosage unit comprising the compound in an amount of from 20 to 300 mg and a pharmaceutically acceptable carrier, diluent, or excipient.

20. A compound according to any one of claims 1 to 19, wherein the treatment or prophylaxis comprises administration of the compound according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

21. A compound according to any one of claims 1 to 19, wherein the treatment or prophylaxis comprises administration of the compound according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

22. A compound according to any one of claims 1 to 21, wherein the treatment or prophylaxis comprises oral administration of the compound.

23. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with a cholinesterase inhibitor.

24. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with Donepezil (Aricept™), Rivastigmine (Exelon™), or Galantamine (Reminyl™).

25. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with an NMDA receptor antagonist.

26. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with Memantine (Ebixa™, Namenda™).

27. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with a muscarinic receptor agonist.

28. A compound according to any one of claims 1 to 22, wherein the treatment or prophylaxis further comprises treatment with an inhibitor of amyloid precursor protein processing to beta-amyloid.

* * *

29. Use of a compound in the manufacture of a medicament for use in a method of treatment or prophylaxis of a tauopathy condition in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

30. Use of a compound in the manufacture of a medicament for use in a method of treatment or prophylaxis of a disease of tau protein aggregation in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

31. Use of a compound in the manufacture of a medicament for use in a method of treatment or prophylaxis of Alzheimer's disease (AD), Pick's disease, Progressive Supranuclear Palsy (PSP), fronto-temporal dementia (FTD), parkinsonism linked to chromosome 17 (FTDP-17), disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC), pallido-ponto-nigral degeneration (PPND), Guam-ALS

syndrome, pallido-nigro-luysian degeneration (PNLD), or cortico-basal degeneration (CBD) in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

32. Use of a compound in the manufacture of a medicament for use in a method of treatment or prophylaxis of Alzheimer's disease (AD) in a patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

33. Use according to any one of claims 29 to 32, wherein the compound is ETC.

34. Use according to any one of claims 29 to 32, wherein the compound is DEMTC.

35. Use according to any one of claims 29 to 32, wherein the compound is DMETC.

36. Use according to any one of claims 29 to 32, wherein the compound is DEETC.

37. Use according to any one of claims 29 to 32, wherein the compound is MTZ.

38. Use according to any one of claims 29 to 32, wherein the compound is ETZ.

39. Use according to any one of claims 29 to 32, wherein the compound is MTI.

40. Use according to any one of claims 29 to 32, wherein the compound is MTI.HI.

41. Use according to any one of claims 29 to 32, wherein the compound is ETI.

42. Use according to any one of claims 29 to 32, wherein the compound is ETI.HI.

43. Use according to any one of claims 29 to 32, wherein the compound is MTN.

44. Use according to any one of claims 29 to 32, wherein the compound is ETN.

45. Use according to any one of claims 29 to 32, wherein the compound is ETC.

46. Use according to any one of claims 29 to 45, wherein the medicament is a dosage unit comprising the compound in an amount of from 20 to 300 mg and a pharmaceutically acceptable carrier, diluent, or excipient.

47. Use according to any one of claims 29 to 46, wherein the treatment or prophylaxis comprises administration of the compound according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

48. Use according to any one of claims 29 to 46, wherein the treatment or prophylaxis comprises administration of the compound according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

49. Use according to any one of claims 29 to 48, wherein the treatment or prophylaxis comprises oral administration of the compound.

50. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with a cholinesterase inhibitor.

51. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with Donepezil (Aricept™), Rivastigmine (Exelon™), or Galantamine (Reminyl™).

52. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with an NMDA receptor antagonist.

53. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with Memantine (Ebixa™, Namenda™).

54. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with a muscarinic receptor agonist.

55. Use according to any one of claims 29 to 49, wherein the treatment or prophylaxis further comprises treatment with an inhibitor of amyloid precursor protein processing to beta-amyloid.

* * *

56. A method of reversing or inhibiting the aggregation of tau protein comprising contacting the aggregate or protein with a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

57. A method of regulating the aggregation of a tau protein in the brain of a mammal, which aggregation is associated with a disease of tau protein aggregation, comprising the step of administering to said mammal a prophylactically or

therapeutically effective amount of a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

58. A method of inhibiting production of protein aggregates in the brain of a mammal, comprising the step of administering to said mammal a prophylactically or therapeutically effective amount of a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

59. A method of treatment or prophylaxis of a tauopathy condition in a patient comprising administering to said patient a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

60. A method of treatment or prophylaxis of a disease of tau protein aggregation in a patient comprising administering to said patient a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

61. A method of treatment or prophylaxis of Alzheimer's disease (AD), Pick's disease, Progressive Supranuclear Palsy (PSP), fronto-temporal dementia (FTD), parkinsonism linked to chromosome 17 (FTDP-17), disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC), pallido-ponto-nigral degeneration (PPND), Guam-ALS syndrome, pallido-nigro-luysian degeneration (PNLD), or cortico-basal degeneration (CBD) in a patient, comprising administering to said patient a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

62. A method of treatment or prophylaxis of Alzheimer's disease (AD) in a patient, comprising administering to said patient a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN.

* * *

63. A compound for use in a method of diagnosis or prognosis of a tau proteinopathy, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN; and wherein the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more detectable labels.

64. Use of a compound in the manufacture of a diagnostic or prognostic reagent for use in the diagnosis or prognosis of a tau proteinopathy of patient, wherein the compound is selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN; and wherein the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more detectable labels.

* * *

65. A method of labelling tau protein or aggregated tau protein comprising the step of:

contacting the tau protein or aggregated tau protein with a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN; wherein the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more detectable labels.

66. A method of detecting tau protein or aggregated tau protein comprising the steps of:

contacting the tau protein or aggregated tau protein with a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN; wherein the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more detectable labels; and

detecting the presence and/or amount of said compound bound to tau protein (or aggregated tau protein).

67. A method of diagnosis or prognosis of a tau proteinopathy in a subject believed to suffer from the disease, comprising the steps of:

(i) introducing into the subject a compound selected from the following compounds: ETC, DEMTC, DMETC, DEETC, MTZ, ETZ, MTI, MTI.HI, ETI, ETI.HI, MTN, and ETN; wherein the compound incorporates, is conjugated to, is chelated with, or is otherwise associated with, one or more detectable labels,

(ii) determining the presence and/or amount of said compound bound to tau protein or aggregated tau protein in the brain of the subject,

(iii) correlating the result of the determination made in (ii) with the disease state of the subject.