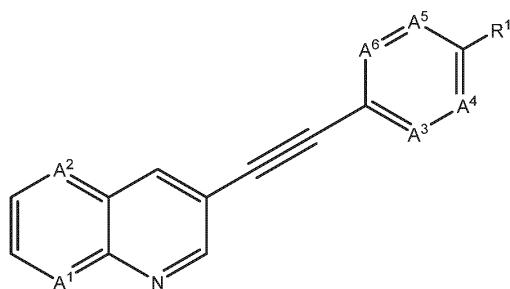


Synthetic Retinoids for Use in RAR Activation

The present invention relates to compounds of formula I:



5 in which A¹ to A⁶ and R¹ are as defined herein, as well as to their use in the treatment of conditions and diseases that are alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain. The invention also relates to pharmaceutical compositions comprising compounds of formula I, and to related methods of treatment.

10 Retinoids are a family of natural or synthetic compounds that are analogues of vitamin A and its derivatives. Retinoids act on a group of nuclear receptors known as retinoic acid receptors (RARs); inducible ligand-activated transcription factors which regulate multiple physiological mechanisms at a genomic level. Retinoic acid receptors are expressed in cells, tissues, and organs throughout the body.

15

Activation of RAR is a potential therapeutic mechanism for a multitude of diseases and conditions throughout the body owing to the ubiquitous expression of RAR in different tissues and organs, and the role that retinoic acid signalling plays in a variety of essential cellular processes. For example, retinoic acid signalling has been shown to induce neurite outgrowth and axonal regeneration, and therefore modulation of retinoic acid signalling has become a target for the development of therapeutics to treat diseases and conditions of the nervous system.

20 Various synthetic retinoids have been developed for different therapeutic applications, including some which exhibit exceptional potency and efficacy in nerve cells both *in vitro* and

in vivo. This makes synthetic retinoids promising candidates in the treatment of neurological conditions affecting the Central Nervous System (CNS) and Peripheral Nervous System (PNS). However, many known synthetic retinoids have a narrow therapeutic window. The exceptional potency of some of these drugs means that the dosage range between a minimum effective therapeutic concentration and minimum toxic concentration is small. Compounds with a narrow therapeutic window are generally considered to be less safe than those with a wide therapeutic window because it is more difficult for prescribers to arrive at a dosage which is optimised between efficacy and toxicity. As a result, the technical formulation of therapeutic compounds which have a narrow therapeutic window can prove challenging.

Furthermore, many of the known compounds are complicated, or expensive to synthesise, which can lead to challenges when scaling up manufacture.

A further drawback of some known synthetic retinoids is that they are also known to permeate the blood-brain barrier (BBB) and accumulate in the brain. Accumulation of these compounds raises the likelihood of modulating unintended biological targets, leading to “off-target” effects. In the brain, off-target effects can lead to serious symptoms such as drowsiness, impaired cognitive function, and behavioural changes. Therefore, while these drugs may be suitable for treating conditions of the CNS, when these compounds are used for the treatment of diseases and conditions which do not primarily affect the brain, but rather peripheral organs and tissues, such as the peripheral nervous system, off-target effects can sometimes lead to more debilitating effects than the initial condition or diseases the drug was intended to treat.

There is consequently an unmet need for compounds for use in the treatment of conditions and diseases affecting the peripheral nervous system (PNS).

The peripheral nervous system consists of all nerves which branch outward from the brain and spinal cord. Diseases and conditions affecting the PNS may impact upon sensory neurons, motor neurons or autonomic nerves (or any combination thereof) resulting in a range of

symptoms. These symptoms can include chronic pain, gastrointestinal dysfunction, loss of balance and co-ordination, muscular atrophy and fasciculations, paralysis, and incontinence.

5 Diseases of the PNS can be acquired as a result of damage to the peripheral nerves such as traumatic injury, autoimmune reaction, exposure to radiation, toxins or certain medicaments, vitamin deficiency, or as a result of systemic disease such as diabetes. PNS diseases can also arise as a result of a genetic mutation; for example, Charcot-Marie-Tooth disease is caused by mutations in genes that support or produce proteins involved in the structure and function of either the peripheral nerve axon or the myelin sheath.

10

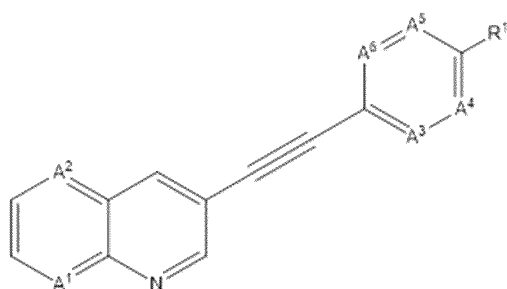
The current means of treating conditions and diseases of the PNS is to target the underlying cause of the nerve damage, for example in the case of diabetic neuropathy, by bringing the diabetes under control. In many instances, treatment of the underlying cause is not possible, for example in chemotherapy neuropathy where the causative agent, in this case the
15 chemotherapeutic, may be necessary in the treatment of a cancer. Even in cases where the underlying cause of the neuropathy is treatable, often there is lasting damage to the peripheral nerves which cannot be undone. Therefore, there is a great need for new therapeutics which can treat conditions and diseases which affect the peripheral nervous system by directly treating the peripheral nerves. Compounds which are efficacious in
20 activating RAR in the peripheral nerves without penetrating the BBB, would be particularly useful to eliminate the risk of off-target effects in the brain. Compounds which exhibit good pharmacodynamic properties, such as having a wide therapeutic window, would also be beneficial.

25 There is a need for new compounds for use in the treatment of diseases or conditions which are alleviated by the activation of retinoic acid receptors present in tissues or organs outside the brain. There is a need for new compounds to address or mitigate the problems associated with the prior art such as the potential to cause off target effects in the brain due to penetration of the BBB, and/or; complex synthesis processes, and/or; costly synthesis
30 processes, and/or; difficulties with manufacturing at scale, and/or poor pharmacokinetic

properties, and/or narrow therapeutic windows making formulation of therapeutics challenging.

Summary of the Invention

- 5 According to a first aspect of the present invention there is provided a compound of formula I:



in which:

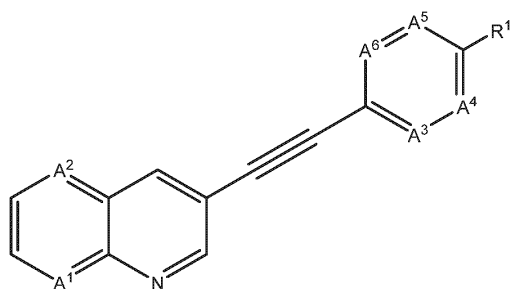
- A¹ and A² are each, independently, CR², in which R² is C₁-C₁₀ alkyl;
- 10 A³ is N or CR³;
- A⁴ is N or CR⁴;
- A⁵ is N or CR⁵;
- A⁶ is N or CR⁶;
- each of R³ to R⁶ is independently H, halogen or haloalkyl C₁-C₁₀; and
- 15 R¹ is C(=O)R⁷ or -C(=O)OR⁷ in which R⁷ is H or C₁₋₁₀ alkyl;
- and isomers thereof;
- in free or salt form.

- In aspects, the invention relates to pharmaceutical compounds comprising compounds of
- 20 formula I, and to the use of such compounds and compositions in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain.

- Conditions or diseases which are alleviated by the activation of retinoic acid receptors (RAR)
- 25 present in tissues or organs outside of the brain include those alleviated by RAR present in

the peripheral nervous system, such as diabetic neuropathy, and those alleviated by RAR present in other tissues or organs such as cardiac disease in Alzheimer's Disease (AD).

- Further aspects and embodiments of the invention are as defined in the claims, and described
 5 in more detail below. According to a first aspect of the present invention there is provided compound of formula I:



in which:

- 10 A¹ and A² are each, independently, CR², in which R² is C₁-C₁₀ alkyl;
 A³ is N or CR³;
 A⁴ is N or CR⁴;
 A⁵ is N or CR⁵;
 A⁶ is N or CR⁶;
 15 each of R³ to R⁶ is independently H, halogen or haloalkyl C₁-C₁₀; and
 R¹ is C(=O)R⁷ or -C(=O)OR⁷ in which R⁷ is H or C₁₋₁₀ alkyl;
 and isomers thereof;
 in free or salt form.
- 20 As used herein, the term "alkyl" refers to a fully saturated, branched, unbranched or cyclic hydrocarbon moiety, i.e. primary, secondary, or tertiary alkyl or, where appropriate, cycloalkyl or alkyl substituted by cycloalkyl. Where not otherwise indicated, an alkyl group comprises 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms, or more preferably 1 to 4 carbon atoms. Representative examples of alkyl groups include, but are not limited to,
 25 methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl, isopentyl,

neopentyl, *n*-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, *n*-heptyl, *n*-octyl, *n*-nonyl and *n*-decyl.

In an embodiment, R¹ is -C(=O)OR⁷ in which R⁷ is H or C₁₋₁₀ alkyl.

5

In an embodiment, R¹ is -C(=O)OR⁷ in which R⁷ is H or C₁₋₆ alkyl.

In an embodiment, R¹ is -C(=O)OR⁷ in which R⁷ is H or C₁₋₃ alkyl.

10 The term “halogen” or “halo” as used herein, means fluoro, chloro, bromo, or iodo.

As used herein the term “haloalkyl” refers to an alkyl group in which one or more hydrogen atoms are replaced by a halogen atom.

15 In an embodiment, in Formula I, A¹ and A² are each, independently, CR², in which R² is C_{1-C4} alkyl.

In an embodiment, in Formula I, A¹ and A² are each, independently, CR², in which R² is -(CH₃).

20 In an embodiment in Formula I, A¹ and A² are the same.

Compounds of formula I in which A¹ and A² are the same may be advantageous due to ease of synthesis.

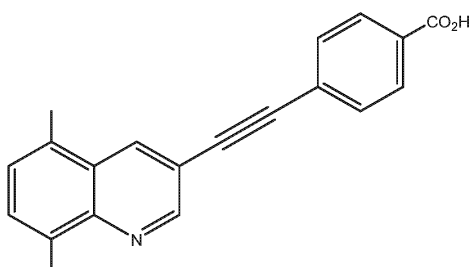
25 In an embodiment, in Formula I, A³ is CR³, A⁴ is CR⁴, A⁵ is CR⁵ and A⁶ is CR⁶.

In an embodiment, in Formula I, each of R³ to R⁶ is hydrogen.

In an embodiment, in formula I R¹ is -COOH.

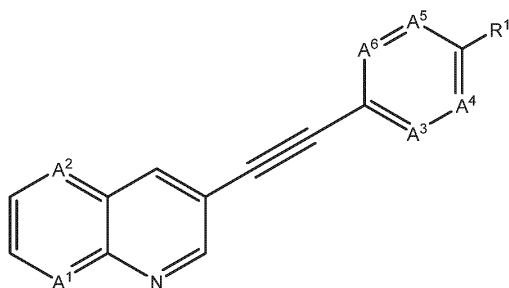
30

In an embodiment, the compound of formula I is a compound of formula I(a):



Formula I(a).

5 The invention relates to a compound of formula I:



Formula I

in which:

A¹ and A² are each, independently, CR², in which R² is C₁-C₁₀ alkyl;

A³ is N or CR³;

10 A⁴ is N or CR⁴;

A⁵ is N or CR⁵;

A⁶ is N or CR⁶;

each of R³ to R⁶ is independently H, halogen or haloalkyl C₁-C₁₀; and

R¹ is C(=O)R⁷ or -C(=O)OR⁷ in which R⁷ is H or C₁₋₁₀ alkyl;

15 and isomers thereof;

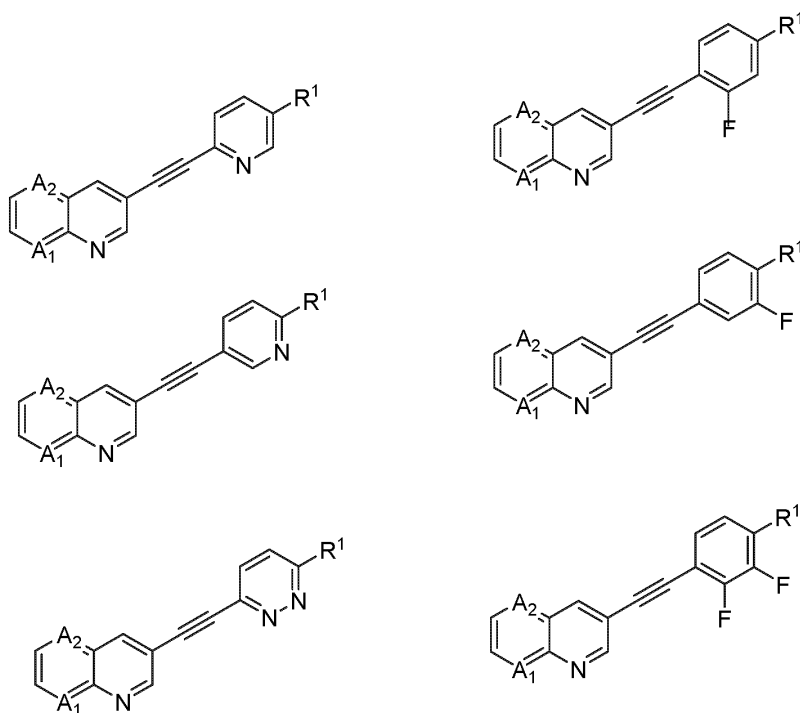
in free or salt form.

In an embodiment, at least one of A³ and A⁴ is N. In an embodiment A³ is N. In an embodiment, A⁴ is N. In an embodiment, both A³ and A⁴ are N.

20

In an embodiment, at least one of A^3 is CR^3 and A^4 is CR^4 , in which R^3 and/or R^4 is halogen. The halogen is preferably F. In an embodiment, A^3 is CF and A^4 is CH. In an embodiment, A^3 is CF and A^4 is CH. In an embodiment, A^3 and A^4 are both CF. Exemplary compounds are:

5



In these embodiments, A^1 and A^2 are preferably CR^2 , in which R^2 is $-(CH_3)$.

10

In these embodiments, R^1 is preferably $-C(=O)OR^7$ in which R^7 is H or C_{1-10} alkyl, or C_{1-6} alkyl, or more preferably, $-COOH$.

According to an aspect of the present invention there is provided a compound of formula I as described above for use in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside the brain.

15

The term "condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain" refers to any condition or disease in

which symptoms are alleviated by the activation of RAR located in cells and/or tissues and/or organs other than the brain. For example, spinal cord injury is a condition in which symptoms are alleviated by the activation of RAR present outside the brain.

- 5 A condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the CNS may be a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside the Central Nervous System (CNS). For example, diabetic neuropathy is a disease in which symptoms are alleviated by the activation of RAR present in the peripheral nervous
10 system (i.e., not in the Central Nervous System).

Conditions or diseases which are alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the CNS include, but are not limited to, cardiac disease in Alzheimer's Disease (AD), Scleroderma, Psoriasis, Psoriatic arthritis, autoimmune thyroid
15 and kidney diseases, long covid-autoimmune diseases, and a condition or disease affecting the peripheral nervous system (PNS).

The term "Central Nervous System (CNS)" refers collectively to the brain and spinal cord.

The term "condition or disease which is alleviated by the activation of retinoic acid receptors
20 (RAR) present in tissues or organs outside the central nervous system" refers to any condition or disease in which symptoms are alleviated by the activation of RAR located in cells and/or tissues and/or organs other than the CNS.

Conditions or diseases which are alleviated by the activation of retinoic acid receptors (RAR)
25 present in tissues or organs outside the central nervous system include cardiac disease in Alzheimer's Disease (AD), Scleroderma, Psoriasis, Psoriatic arthritis, autoimmune thyroid and kidney diseases, long covid-autoimmune diseases, and a condition or disease affecting the peripheral nervous system (PNS).

The term “peripheral nervous system (PNS)” refers to nerves that branch out from the brain and spinal cord and extend to all parts of the body. The PNS connects the central nervous system (CNS, i.e. the brain and spinal cord) to the different tissues and organs of the body.

- 5 The term “condition or disease affecting the peripheral nervous system” refers to any injury or disorder, acquired or inherited, which impacts the peripheral nervous system either directly, or indirectly.

In an embodiment, the disease or condition affecting the peripheral nervous system is
10 selected from peripheral nerve disorder, sciatic nerve injury, endometriosis, fibromyalgia, retinal disease, and multiple sclerosis.

In an embodiment, the peripheral nerve disorder is selected from peripheral neuropathy, neuropathic pain, and neuropathic itch.

15

The term “peripheral neuropathy” as used herein, means damage to the nerves of the peripheral nervous system (i.e. the nerves outside the brain and spinal cord). In an embodiment, the peripheral neuropathy is selected from diabetic neuropathy, chemotherapy neuropathy, Sjogren’s syndrome, lupus, Rheumatoid arthritis, Guillain-Barre syndrome,
20 postinfectious neuropathy, chronic inflammatory demyelinating neuropathy, myasthenia gravis, congenital myasthenic syndrome, vasculitis, Charcot-Marie Tooth disease, inherited peripheral neuropathies, sciatica, sciatic nerve injury and carpal tunnel syndrome.

The peripheral neuropathy may be a sensory neuropathy, motor neuropathy, autonomic
25 neuropathy, or a combination thereof. The term “sensory neuropathy” as used herein, means damage to the sensory nerves that carry messages of touch, temperature, pain, and other sensations to the brain. The term “motor neuropathy” as used herein, means damage to the motor nerves that control movement. The term “autonomic neuropathy” as used herein, means damage to the autonomic nerves that control involuntary bodily processes,
30 such as digestion, bladder function and control of blood pressure.

The peripheral neuropathy may be a mononeuropathy, mononeuritis multiplex or polyneuropathy. The term "mononeuropathy" as used herein, means damage to a single nerve outside of the central nervous system. The term "mononeuritis multiplex" (also referred to as "polyneuritis multiplex") as used herein, means damage to multiple nerves
5 outside the central nervous system. The term "polyneuropathy" means damage to all the peripheral nerves in the body.

According to an aspect of the present invention there is provided a pharmaceutical composition comprising a compound of formula I as described herein, optionally in
10 conjunction with one or more pharmaceutically acceptable excipients, diluents, or carriers.

The term "pharmaceutical composition" refers to a composition suitable for administration to a patient. Thus, the term "pharmaceutical composition" refers to compositions which
15 comprise the compound of the invention or mixtures thereof, or salts, solvates, prodrugs, isomers, or tautomers thereof, optionally in combination with one or more pharmaceutically acceptable excipients, carriers, or diluents. The term "pharmaceutical composition" is also intended to encompass both the bulk composition (i.e. in a form that has not yet been formed into individual dosage units) and individual dosage units. Such individual dosage units include
20 tablets, pills, caplets, ampoules, and the like.

Those skilled in the art will recognize those instances in which the compounds of the invention may be converted to prodrugs and/or solvates. The term "prodrug" refers to a compound (e.g., a drug precursor) that is transformed *in vivo* to yield a compound of the invention or a
25 pharmaceutically acceptable salt, hydrate or solvate of the compound. The transformation may occur by various mechanisms (e.g., by metabolic or chemical processes), such as, for example, through hydrolysis in blood.

The compounds of the invention may be unsolvated or may be solvated with
30 pharmaceutically acceptable solvents such as water, ethanol, and the like. For instance, it will be understood that a solvate may be capable of isolation, for example when one or more

solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolatable solvates. Suitable solvates include, but are not limited to, ethanolates, methanolates, hydrates, and the like.

- 5 Compounds for use in the invention include salts thereof, and reference to a compound of the invention is intended to include reference to salts thereof, unless otherwise stated. Suitable salts include for instance, acidic salts formed with inorganic and/or organic acids, basic salts formed with inorganic and/or organic bases, as well as zwitterions ("inner salts") which may be formed and are included within the term "salt(s)" as used herein.
- 10 Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are preferred, although other salts may be useful in certain circumstances. Exemplary acid addition salts which may be useful include acetates, ascorbates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, fumarates, hydrochlorides, hydrobromides, hydroiodides, lactates, maleates, methanesulfonates,
- 15 naphthalenesulfonates, nitrates, oxalates, phosphates, propionates, salicylates, succinates, sulfates, tartrates, thiocyanates, toluenesulfonates (also known as tosylates,) and the like. Exemplary basic salts which may be useful include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as
- 20 dicyclohexylamines, *t*-butyl amines, and salts with amino acids such as arginine, lysine, and the like. Basic nitrogen-containing groups may be quarternerized with agents such as lower alkyl halides (e.g. methyl, ethyl, and butyl chlorides, bromides, and iodides), dialkyl sulfates (e.g. dimethyl, diethyl, and dibutyl sulfates), long chain halides (e.g. decyl, lauryl, and stearyl chlorides, bromides, and iodides), arylalkyl halides (e.g. benzyl and phenethyl bromides), and
- 25 others.

- Compounds for use in the invention include pharmaceutically acceptable esters thereof, and may include carboxylic acid esters, obtained by esterification of the hydroxy groups, in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from
- 30 straight or branched chain alkyl (for example, acetyl, *n*-propyl, *t*-butyl, or *n*-butyl), alkoxyalkyl (for example, methoxymethyl), aralkyl (for example, benzyl), aryloxyalkyl (for example,

phenoxymethyl), aryl (for example, phenyl optionally substituted with, for example, halogen, C₁₋₄ alkyl, or C₁₋₄ alkoxy or amino); (2) sulfonate esters, such as alkyl- or aralkylsulfonyl (for example, methanesulfonyl); (3) amino acid esters (for example, L-valyl or L-isoleucyl); (4) phosphonate esters; and (5) mono-, di- or triphosphate esters.

5

Polymorphic forms of the compounds of the invention, and of the salts, solvates, esters and prodrugs of the compounds of the invention, are intended to be included in the present invention.

10 Suitable dosages for administering compounds of the invention to patients may be determined by those skilled in the art, e.g. by an attending physician, pharmacist, or other skilled worker and may vary according to factors such as patient weight, health, age, frequency of administration, mode of administration, the presence of any other active ingredients, and the condition for which the compounds are being administered.

15 Examples of excipients, diluents and carriers include buffers, as well as fillers and extenders such as starch, cellulose, sugars, mannitol, and silicic derivatives. Binding agents may also be included. Adjuvants may also be included.

Optionally the compound of formula I may be administered in combination with one or more
20 additional therapeutic agents. When used in combination with one or more additional therapeutic agents, the compounds of the invention may be administered together or sequentially.

The compositions may be administered by a variety of routes including oral, parenteral
25 (including subcutaneous, intravenous, intramuscular, and intraperitoneal), rectal, dermal, transdermal, intrathoracic, intrapulmonary, mucosal, intraocular, and intranasal routes.

Suitable dosage forms will be recognised by one skilled in the art and include, among others, tablets, capsules, solutions, suspensions, powders, aerosols, ampules, pre-filled syringes, small volume infusion containers or multi-dose containers, creams, milks, gels, dispersions,
30 microemulsions, lotions, impregnated pads, ointments, eye drops, nose drops, lozenges etc.

According to an aspect of the present invention there is provided a method of treatment of a patient with a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain, the method comprising administering to a patient a therapeutically effective amount of a compound formula I, as
5 described herein.

The term “therapeutically effective” amount, or "effective amount" refers to a quantity of the compound or composition of the present invention which is effective in producing the desired therapeutic, ameliorative, inhibitory, or preventative effect.

10

Aspects of the present invention relate to a compound of formula I as herein defined for use as a medicament.

Aspects of the present invention relate to a compound of formula I as herein defined for the
15 manufacture of a medicament for use in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain.

Various further features and aspects of the invention are defined in the claims.

20

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs.

Examples:

Embodiments of the present invention will now be described by way of example only with reference to the accompanying drawings where like parts are provided with corresponding reference numerals and in which:

- 5 Figure 1 shows synthetic reaction schemes to a comparative compound and to a compound of formula I(a) as outlined in Example 1 and Example 2;

Figure 2 shows mRNA expression levels of key retinoic acid pathway genes in rat brains after treatment with the compound of formula I(a);

- 10 Figure 3 shows mitochondrial viability in HDF cells after treatment with the compound of formula I(a) as measured using the MTT assay;

Figure 4 shows mitochondrial viability in HaCaT cells after treatment with the compound of formula I(a) as measured using the MTT assay;

Figure 5 shows mitochondrial viability in SH-SY5Y cells after treatment with the compound of formula I(a) as measured using the MTT assay;

- 15 Figure 6 shows cytotoxicity levels in C6 glia cells after treatment with the compound of formula I(a) as measured using the LDH assay;

Figure 7 shows cytotoxicity levels in SH-SY5Y cells after treatment with the compound of formula I(a) as measured using the LDH assay;

- 20 Figure 8 shows autophagy levels in C6 glia cells after treatment with the compound of formula I(a) as determined using the quantitative LC3 immunofluorescence assay;

Figure 9 shows autophagy levels in SH-SY5Y cells after treatment with the compound of formula I(a) as determined using the quantitative LC3 immunofluorescence assay;

Figure 10 shows the percentage of senescent C6 glia cells after treatment with the compound of formula I(a) as determined by senescence-associated β -galactosidase (SA- β -Gal) staining;

- 25 Figure 11 shows levels of pro-inflammatory cytokine IL-6 in HMC3 glia cells after treatment with the compound of formula I(a) as measured by ELISA assay;

Figure 12 shows levels of pro-inflammatory cytokine TNF α in SH-SY5Y cells after treatment with the compound of formula I(a);

Figure 13 shows levels of pro-inflammatory cytokine TNF α in HMC3 cells after treatment with the compound of formula I(a);

- 5 Figure 14 shows TEM images of amyloid β protein aggregation after treatment with the compound of formula I(a).

Synthesis Examples

Materials and Methods

- 10 All reactions were performed under an inert argon atmosphere, other than the ester hydrolyses which were open to air. All solvents and reagents were acquired from Merck™ Life Science UK Ltd and Fisher Scientific™, with the exception of methyl 4-iodobenzoate which had been previously synthesised in the laboratory. SiO₂ column chromatography was monitored via the use of silica TLC plates for all cases.
- 15
- ¹H and ¹³C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker-Avance™ 400 NMR spectrometer, with chemical shift values quoted in ppm. All NMR spectra were recorded in CDCl₃, with the exception of 4-(quinolin-3-ylethynyl)benzoic acid and 4-(3-ethynyl-5,8-dimethylquinoline)benzoic acid which were recorded in DMSO-d₆. For spectra
- 20 recorded in CDCl₃, the residual CHCl₃ peaks at 7.26 ppm in the ¹H NMR, and 77.4 ppm in the ¹³C NMR spectra, were used as references. For spectra recorded in DMSO-d₆, the residual DMSO quintets at 2.51 ppm in the ¹H NMR spectra and at 40.0 ppm in the ¹³C NMR spectra served as references. Melting points for all compounds were measured using an Eisco™ Melting Point Apparatus (230 V, 50-60 Hz). IR spectra were recorded on a Perkin Elmer™
- 25 1600 Series FTIR. Mass spectrometry via electrospray ionisation was carried out on a SQD mass spectrometer/Acquity™ UPLC (Waters Ltd, UK). Mass spectrometry via accurate mass analysis was carried out on a QtoF Premier mass spectrometer/Acquity™ UPLC (Waters Ltd, UK). Single crystal X-ray diffraction was performed on a Bruker™ D8 Venture diffractometer.

Example 1: Synthesis of comparative compoundsExample 1.1: Synthesis of 3-ethynylquinoline (2)

3-Ethynylquinoline (**2**) was synthesised from 3-quinolylcarboxaldehyde (**1**) via the Bestmann-Ohira modification of the Seyferth-Gilbert homologation according to reaction scheme 1 of Figure 1.

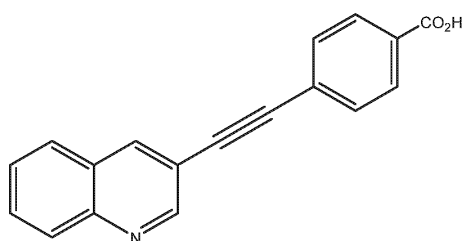
To a solution of 3-quinolylcarboxaldehyde (104 mg, 0.659 mmol) in anhydrous tetrahydrofuran (THF) (3.3 mL) and anhydrous MeOH (3.3 mL), was added K₂CO₃ (176 mg, 1.27 mmol) and the Bestmann-Ohira reagent (147 mg, 1.84 mL, 0.764 mmol), under an argon atmosphere. The reaction mixture was stirred for 19 h overnight, after which the solution was passed through a short celite plug, with DCM (4 x 40 mL) as the eluent. The solvent was removed under reduced pressure, and the resulting solid was redissolved in DCM (20 mL) before washing with 5% NaHCO₃ (3 x 20 mL) and NH₄Cl (3 x 20 mL). The organic layer was dried with MgSO₄ before removing the solvent under reduced pressure. The resulting pink-red solid was purified by SiO₂ column chromatography eluting in EtOAc, procuring 3-ethynylquinoline as a pale-pink solid (48.8 mg, 0.319 mmol, 50%). M.p. 80-81 °C. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 2101w (C≡C), 1351s (C–N_{aromatic}). ¹H NMR (400 MHz; CDCl₃) δ_{H} : 8.98 (1H, d, *J* = 2.08 Hz, H_F), 8.33 (1H, d, *J* = 2.0 Hz, H_A), 8.13 (1H, d, *J* = 8.5 Hz, H_E), 7.82 (1H, d, *J* = 8.2 Hz, H_B), 7.79-7.75 (1H, m, H_D), 7.63-7.59 (1H, m, H_C), 3.31 (1H, s, H_G). ¹³C NMR (101 MHz, CDCl₃) δ_{C} : 152.2 (C8), 147.1 (C7), 139.4 (C1), 130.5 (C5), 129.4 (C6), 127.7 (C2), 127.5 (C3), 127.1 (C4), 116.3 (C9), 80.9 (C10), 80.6 (C11). MS (ESI): *m/z* [M+H]⁺ 182.314. HRMS (ESI) for C₁₃H₁₁N for 182.0959 found [M+H]⁺ 182.0970. The experimental data acquired was in agreement with that stated in the literature (Smeyanov et al., Synthetic Communications, 2013, 43, 2809-2816).

Example 1.2: Synthesis of methyl 4-(quinolin-3-ylethynyl)benzoate (3)

Methyl 4-(quinolin-3-ylethynyl)benzoate (**3**) was synthesised from 3-ethynylquinoline (**2**) (Example 1.1) via the Sonogashira cross-coupling reaction with methyl 4-iodobenzoate according to reaction scheme 2 of Figure 1.

CuI (7.7 mg, 0.0404 mmol), PdCl₂(PPh₃)₂ (13.8 mg, 0.0197 mmol), DIPEA (168 µL, 0.964 mmol) and 3-ethynylquinoline (148 mg, 0.964 mmol) were successively added to a solution of methyl 4-iodobenzoate (101 mg, 0.386 mmol) in dry THF (2 mL), under an argon atmosphere. The mixture was stirred at room temperature for 22 h, after which water (4 mL) was added. The reaction mixture was passed through a short Celite™ plug, with NH₄Cl (2 x 4 mL) as the eluent, before separating the resulting organic and aqueous phases. The aqueous phase was extracted with EtOAc (3 x 10 mL), and the organic phases combined before being dried over MgSO₄. The solvent was then removed under reduced pressure. The resulting orange-brown solid was purified by SiO₂ column chromatography, eluting in hexanes followed by a slowly increasing ratio of hexane: EtOAc (up to 7:3) once the first product had been removed from the column, yielding methyl 4-(quinolin-3-ylethynyl)benzoate as a white solid (67.7 mg, 0.236 mmol, 61%). M.p. 134-135 °C. IR Spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 2944w (C—H), 2205w (C≡C), 1708s (C=O), 1272s (C—N_{aromatic}), 1098s (C—O). ¹H NMR (400 MHz; CDCl₃) δ_{H} : 9.04 (1H, d, *J* = 2.2 Hz, H_F), 8.37 (1H, d, *J* = 2.0 Hz, H_A), 8.15 (1H, d, *J* = 8.4 Hz, H_E), 8.10-8.07 (2H, m, H_H), 7.84 (1H, d, *J* = 8.2 Hz, H_B), 7.80-7.76 (1H, m, H_D), 7.69-7.66 (2H, m, H_G), 7.64-7.60 (1H, m, H_C), 3.96 (3H, s, H_I). ¹³C NMR (101 MHz, CDCl₃) δ_{C} : 166.5 (C16), 151.9 (C8), 146.9 (C7), 138.8 (C1), 131.7 (C13), 130.5 (C15), 130.0 (C5), 129.7 (C14), 129.4 (C6), 127.7 (C2), 127.5 (C3), 127.2 (C4), 116.9 (C9), 91.8 (C10), 89.4 (C11), 52.4 (C17). MS (ESI): *m/z* [M+H]⁺ 288.192. HRMS (ESI) for C₁₉H₁₃NO₂ for 288.1013 found [M+H]⁺ 288.1025. Crystal data C₁₉H₁₃NO₂, *M* = 287.30, triclinic, *a* = 6.0560(3) Å, *b* = 10.4400(5) Å, *c* = 11.4229(6) Å, *U* = 696.60(6) Å³, *T* = 120 K, space group P-1, *Z* = 2, 21280 reflections measured, 4059 unique (*R*_{int} = 0.0457), which were used in all calculations. The final *wR*(*F*²) was 0.1300 (all data).

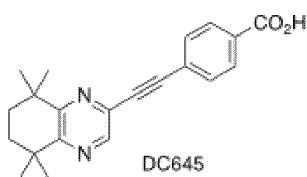
Example 1.3 Synthesis of 4-(quinolin-3-ylethynyl)benzoic acid (MH16, comparative compound)



4-(Quinolin-3-ylethynyl)benzoic acid (**4**) was synthesised by base-catalysed hydrolysis of methyl 4-(quinolin-3-ylethynyl)benzoate (**3**) according to reaction scheme 3 of Figure 1.

20% NaOH (2.2 mL) was added to a solution of methyl 4-(quinolin-3-ylethynyl)benzoate (**3**) (305 mg, 1.06 mmol) in THF (22 mL), and the mixture was refluxed for 19 h. The reaction mixture was then allowed to cool before being acidified to pH 4 with 5% HCl. The resulting aqueous and organic phases were separated, and the aqueous phase was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with water (2 x 40 mL) and brine (2 x 40 mL) before being dried over MgSO₄. The solvent was then removed under reduced pressure, yielding a white solid which was recrystallised from MeCN to afford 4-(quinolin-3-ylethynyl)benzoic acid (**4**) as a white solid (217 mg, 0.795 mmol, 75%). M.p. 252-253 °C. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 2975w (O—H), 1685w (C=O), 1285s (C—N_{aromatic}). ¹H NMR (400 MHz; DMSO-d₆) δ_{H} : 9.05 (1H, d, *J* = 2.1 Hz, H_F), 8.70 (1H, d, *J* = 2.1 Hz, H_A), 8.09-8.01 (4H, m, H_E, H_H, H_B), 7.87-7.83 (1H, m, H_D), 7.78-7.76 (2H, m, H_G), 7.72-7.68 (1H, m, H_C). ¹³C NMR (101 MHz, DMSO-d₆) δ_{C} : 167.1 (C16), 152.0 (C8), 147.0 (C7), 139.4 (C1), 132.2 (C13), 131.4 (C15), 131.3 (C5), 130.1 (C14), 129.4 (C6), 128.7 (C2), 128.1 (C3), 127.3 (C4), 126.5 (C12), 116.4 (C9), 92.0 (C10), 89.8 (C11). MS (ESI): *m/z* [M+H]⁺ 274.245. HRMS (ESI) for C₁₈H₁₁NO₂ for 274.0894 found [M+H]⁺ 274.0868.

20 Example 1.4 Synthesis of 4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydroquinoxalin-2-yl)ethynyl]benzoic acid, comparative compound DC645 (also called NVG0645 throughout this specification and figures).



25 Comparative compound DC645 was synthesised as outlined in PCT/GB2020/050607, published as WO2020/183173.

Example 2.1 Synthesis of 5,8-dimethyl-3-hydroxymethylquinoline (6)

5,8-Dimethyl-3-hydroxymethylquinoline (6) was synthesised from 5,8-dimethylquinoline-3-carboxylic acid (BLDPharm) (5) via the DIBAL-H reduction according to reaction scheme 4 of Figure 1.

- 5 5,8-Dimethylquinoline-3-carboxylic acid (**5**) (494 mg, 2.46 mmol) was dissolved in dry THF (30 mL) at -78°C under an atmosphere of argon. After 5 minutes of stirring, DIBAL-H in toluene (9.82 mL, 9.82 mmol, 1.0 M) was carefully added and the mixture was left stirring at room temperature for 4 days. 5% NaOH (25 mL) was added, upon which a yellow solution was formed. The resulting aqueous and organic phases were separated, and the aqueous phase
- 10 was extracted with EtOAc (3 x 25 mL). The combined organic phases were washed with brine (3 x 25 mL) before being dried over MgSO_4 . The solvent was removed under reduced pressure, leaving a yellow oil. The product was then purified by SiO_2 column chromatography eluting in hexanes followed by a slowly increasing ratio of hexane : EtOAc (up to 1:9), with the product eluting in 4:6 hexane/EtOAc. 5,8-Dimethyl-3-hydroxymethylquinoline (**6**) was
- 15 acquired as a pale-yellow solid (278 mg, 1.50 mmol, 61%). M.p. $58-59^{\circ}\text{C}$. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 3161br (O-H), 2917w (C-H), 1604w (C-H_{aromatic}), 1376s (C-N_{aromatic}). ^1H NMR (400 MHz; CDCl_3) δ_{H} : 8.90 (1H, s, H_F) 8.26 (1H, s, H_A) 7.45 (1H, d, $J = 7.3$ Hz, H_D) 7.27 (1H, d, $J = 7.4$ Hz, H_C) 4.93 (2H, s, H_G) 2.79 (3H, s, H_E) 2.64 (3H, s, H_B). ^{13}C NMR (101 MHz, CDCl_3) δ_{C} : 148.5 (C8), 147.0 (C7), 134.7 (C1), 132.6 (C5), 132.3 (C2), 130.7 (C6), 129.2 (C3), 127.2 (C4), 127.0
- 20 (C9), 63.3 (C12), 18.6 (C11), 18.2 (C10). MS (ESI): m/z $[\text{M}+\text{H}]^+$ 188.205. HRMS (ESI) for $\text{C}_{12}\text{H}_{13}\text{NO}$ for 188.107 found $[\text{M}+\text{H}]^+$ 188.1075.

Example 2.2 Synthesis of 5,8-dimethylquinoline-3-carbaldehyde (7)

- 5,8-Dimethylquinoline-3-carbaldehyde was synthesised from 5,8-dimethyl-3-hydroxymethylquinoline via manganese(IV) dioxide oxidation, according to reaction scheme
- 25 5 of Figure 1.

- To a solution of 5,8-dimethyl-3-hydroxymethylquinoline (**6**) (430 mg, 2.30 mmol) in dry THF (43 mL), was added activated MnO_2 (2.00 g, 23.0 mmol) under an atmosphere of argon. The
- 30 solution was left stirring at room temperature for 4 days, after which the reaction mixture

was passed through a CeliteTM/silica plug to remove any solid MnO₂. The plug was washed with EtOAc (30 mL), and the filtrate was concentrated under reduced pressure. The resulting orange-yellow solid was purified by SiO₂ column chromatography, eluting in 9:1 hexane/EtOAc, yielding 5,8-dimethylquinoline-3-carbaldehyde as a pale-yellow solid (279 mg, 1.51 mmol, 66%). M.p. 89-91 °C. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 2980w (C—H), 1677vs (C=O), 1584s (C—H_{aromatic}), 1194s (C—N_{aromatic}). ¹H NMR (400 MHz; CDCl₃) δ_{H} : 10.32 (1H, s, H_G), 9.40 (1H, d, J = 2.0 Hz, H_F), 8.83 (1H, d, J = 2.1 Hz, H_A), 7.64 (1H, d, J = 7.2 Hz, H_D), 7.40 (1H, d, J = 7.2 Hz, H_C), 2.83 (3H, s, H_E), 2.77 (3H, s, H_B). ¹³C NMR (101 MHz, CDCl₃) δ_{C} : 191.3 (C10), 150.1 (C8), 147.8 (C7), 136.5 (C9), 135.5 (C1), 134.3 (C2), 132.6 (C6), 128.0 (C3), 127.7 (C5), 126.6 (C4), 18.5 (C12), 18.2 (C11). MS (ESI): m/z [M+H]⁺ 186.191. HRMS (ESI) for C₁₂H₁₁NO for 186.0911 found [M+H]⁺ 186.0919.

Example 2.3 Synthesis of 3-ethynyl-5,8-dimethylquinoline (8)

3-Ethynyl-5,8-dimethylquinoline (8) was synthesised from 5,8-dimethylquinoline-3-carbaldehyde (7) via the Bestmann-Ohira modification of the Seyferth-Gilbert homologation according to reaction scheme 6 of Figure 1.

K₂CO₃ (417 mg, 3.02 mmol) and the Bestmann-Ohira reagent (348 mg, 4.1 mL, 1.81 mmol) were added to a solution of 5,8-dimethylmethylquinoline-3-carbaldehyde (7) (279 mg, 1.51 mmol) in dry THF (9 mL) and dry MeOH (9 mL) under an argon atmosphere. The reaction mixture was stirred for 21 h, after which the solution was passed through a short celite plug, with DCM (200 mL) as the eluent. The solvent was removed under reduced pressure, and the solid redissolved in DCM (50 mL). Saturated NaCl (40 mL) was added, ensuring the solution pH was slightly basic, and the resulting aqueous and organic phases were separated. The aqueous phase was extracted with DCM (3 x 50 mL) and the organic phases were combined, before being dried over MgSO₄. The solvent was then removed under reduced pressure, yielding 3-ethynyl-5,8-dimethylquinoline as a light-brown solid (245 mg, 1.35 mmol, 90%). TLC and NMR spectral analysis of the product indicated sufficient purity had been obtained from the workup procedure, therefore no purification was necessary. M.p. 54-55 °C. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 3179w (C—H), 2105w (C≡C), 1490w (C—H_{aromatic}), 1355s (C—N_{aromatic}). ¹H NMR (400 MHz; CDCl₃) δ_{H} : 8.99 (1H, d, J = 2.0 Hz, H_F), 8.46 (1H, d, J = 2.0 Hz, H_A), 7.48 (1H, d,

$J = 7.1$ Hz, H_D), 7.30 (1H, d, $J = 7.2$ Hz, H_C), 3.31 (1H, s, H_G), 2.77 (3H, s, H_E), 2.65 (3H, s, H_B). ^{13}C NMR (101 MHz, CDCl_3) δ_{C} : 150.5 (C8), 146.5 (C7), 136.3 (C1), 135.1 (C5), 132.2 (C2), 130.4 (C6), 127.6 (C3), 126.5 (C4), 115.4 (C9), 81.5 (C10), 80.2 (C11), 18.4 (C13), 18.0 (C12). MS (ESI): m/z $[\text{M}+\text{H}]^+$ 182.314. HRMS (ESI) for $\text{C}_{13}\text{H}_{11}\text{N}$ for 182.0959 found $[\text{M}+\text{H}]^+$ 182.0970.

5

Example 2.4 Synthesis of methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate

Methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate (**9**) was synthesised from 3-ethynyl-5,8-dimethylquinoline, via the Sonogashira cross-coupling reaction with methyl 4-iodobenzoate, as shown in reaction scheme 7 of Figure 1.

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CuI (14.2 mg, 0.0747 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (26.2 mg, 0.0373 mmol), DIPEA (195 μL , 1.12 mmol) and 3-ethynyl-5,8-dimethylquinoline (**8**) (203 mg, 1.12 mmol) were successively added to a solution of methyl 4-iodobenzoate (196 mg, 0.747 mmol) in dry THF (8.9 mL) under an argon atmosphere. The mixture was stirred at room temperature for 18 h before being treated with water (18 mL) and passed through a short celite plug, with saturated NH_4Cl (18 mL) as the eluent. The resulting aqueous and organic phases were separated, and the aqueous phase was extracted with EtOAc (3 x 45 mL). The organic phases were combined and dried over MgSO_4 , before removing the solvent under reduced pressure. The resulting orange solid was purified by SiO_2 column chromatography, eluting in hexanes followed by a slowly increasing ratio of hexane : EtOAc (up to 7:3) once the first product had been isolated from the column, yielding methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate as a pale-orange solid (221 mg, 0.699 mmol, 94%). However, NMR spectral analysis revealed 14% contamination of the product by the homo-coupled acetylene. The diyne was recrystallised from DCM/ Et_2O , followed by a second slow recrystallisation from DCM/hexane, yielding methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate (**9**) as a white solid (133 mg, 0.422 mmol, 57%). M.p. 124-125 $^\circ\text{C}$. IR spectrum $\nu_{\text{max}}/\text{cm}^{-1}$ 2949w (C—H), 2206w (C $\equiv$ C), 1720vs (C=O), 1432s (C—H_{aromatic}), 1272vs (C—O). ^1H NMR (400 MHz; CDCl_3) δ_{H} : 9.05 (1H, d, $J = 2.1$ Hz, H_F), 8.51 (1H, d, $J = 2.1$ Hz, H_A), 8.10-8.07 (2H, m, H_H), 7.71-7.68 (2H, m, H_G), 7.50 (1H, d, $J = 7.2$ Hz, H_D), 7.33 (1H, d, $J = 7.2$ Hz, H_C), 3.97 (3H, s, H_I), 2.79 (3H, s, H_E), 2.69 (3H, s, H_B). ^{13}C NMR (101 MHz, CDCl_3) δ_{C} : 166.5 (C16), 150.2 (C8), 146.4 (C7), 135.7 (C1), 135.1 (C5), 132.3 (C2), 131.7 (C13), 130.4 (C6), 129.9 (C3), 129.6 (C14) 127.6 (C15), 127.4 (C12), 126.6 (C4), 116.0 (C9), 91.5 (C10), 90.1 (C11),

30

52.3 (C17), 18.5 (C19), 18.1 (C18). MS (ESI): m/z $[M+H]^+$ 316.315. HRMS (ESI) for $C_{21}H_{17}NO_2$ for 316.1344 found $[M+H]^+$ 316.1338.

Example 2.5 Synthesis of 4-(3-ethynyl-5,8-dimethylquinoline)benzoic acid (MH21, Compound of Formula I(a) according to the invention)

Reaction scheme 8 shows the base-catalysed hydrolysis of methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate (9) to form 4-(3-ethynyl-5,8-dimethylquinoline)benzoic acid (10) of Figure 1.

20% NaOH (1.2 mL) was added to a solution of methyl 4-(3-ethynyl-5,8-dimethylquinoline)benzoate (**9**) (170 mg, 0.209 mmol) in THF, and the mixture was refluxed for 17 h. The reaction mixture was then allowed to cool before being acidified to pH 4 with 5% HCl. The resulting aqueous and organic phases were separated, and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with water (3 x 30 mL) and brine (3 x 30 mL) before being dried over $MgSO_4$. The solvent was then removed under reduced pressure, yielding a light-pink solid which was recrystallised from MeCN to afford 4-(3-ethynyl-5,8-dimethylquinoline)benzoic acid as a pale-pink solid (111 mg, 0.368 mmol, 68%). M.p. 236-238 °C. IR spectrum ν_{max}/cm^{-1} 2917br (O—H), 1673vs (C=O), 1424s (C—H_{aromatic}), 1276s (C—O). 1H NMR (400 MHz; DMSO- d_6) δ_H : 9.06 (1H, d, J = 2.1 Hz, H_F), 8.68 (1H, d, J = 2.2 Hz, H_A), 8.04-8.00 (2H, m, H_H), 7.78-7.76 (2H, m, H_G), 7.57 (1H, d, J = 7.1 Hz, H_D), 7.40 (1H, d, J = 7.0 Hz, H_C), 2.69 (3H, s, H_E), 2.66 (3H, s, H_B). ^{13}C NMR (101 MHz, DMSO- d_6) δ_C : 167.2 (C16), 150.5 (C8), 146.3 (C7), 136.5 (C1), 134.8 (C5), 133.3 (C2), 132.2 (C13), 131.4 (C6), 131.0 (C3), 130.1 (C14), 128.1 (C15), 126.6 (C12), 126.6 (C4), 115.8 (C9), 91.9 (C10), 90.3 (C11), 18.6 (C18), 18.1 (C17). MS (ESI): m/z 302.292. HRMS (ESI) for $C_{20}H_{15}NO_2$ for 302.1192 found $[M+H]^+$ 302.1181.

Biological Evaluation

Example 3: Evaluation of the compound of formula I(a) in the rat brain

In order to determine whether the compound of formula I(a) crossed the blood brain barrier (BBB), rats were dosed with the compound of formula I(a), and subsequent mRNA expression analysis was performed on the dosed rat brains. mRNA expression analysis evaluated

expression levels of key known retinoid pathway genes to determine whether there was any retinoic acid receptor activation in the brain of treated rats.

3.1 Materials & methods: animal husbandry

5 Wild-type Wistar rats (N= 12 per dose; 6 males and 6 females) were administered with the compound of formula I(a). The compound of formula I(a) was first dissolved in N-Methyl-2-pyrrolidone (NMP), then combined with Kolliphor-HS15. Dissolution was ensured and then 30% polyethylene glycol 400 (PEG-400) was added followed by 60% phosphate-buffered saline (PBS). Stock solution concentration was 1mg/mL and diluted to the appropriate dose
10 concentrations - 0.03 mg/mL, 0.1 mg/mL and 0.3 mg/mL. Rats (n=6 male and n=6 female per treatment) were injected intraperitoneally with vehicle or one of three doses of the compound of formula I(a) (blinded), and culled after 4 h. Each rat had one dose only. Animals were euthanised with 1.0 mL sodium pentobarbital. Gross dissection was performed on the brain collecting the striatum (for analysis) alongside other regions including substantia nigra,
15 cortex and hippocampus. These were snap frozen in liquid nitrogen then stored at -70 °C.

3.2 Materials and methods: mRNA expression

Striatum samples were weighed and homogenised using a 15 mL glass homogeniser and polytetrafluoroethylene (PTFE) plunger in TRIzol™ reagent (Invitrogen; 15596026) following
20 company recommendations.

Chloroform was added to the samples in TRIzol™, centrifuged, and the aqueous phase - containing RNA - was removed. Isopropanol was added and then centrifuged to form an RNA pellet which was subsequently washed with 75% ethanol and resuspended in 30µL nuclease-
25 free water for further processing. Samples had their absorbances measured at 230, 260 and 280nm, their relative ratios measured, and their concentration determined in µg/µl. The RNA was treated with DNase (Sigma-Aldrich; AMPD1) to remove DNA contamination followed by cDNA synthesis (iSCRIPT™, Bio-Rad; 1708891) as per the supplier's protocol. Samples were diluted according to primer requirements to give either 5 ng or 10 ng in a 10 µL reaction for
30 qPCR. This was achieved with nuclease-free water and Qiagen's template buffer diluent.

Master mixes of primer sets were made up with QuantiNova SYBR™ Green (Qiagen; 208056) as per supplier's recommendations with 0.7 μM primer concentration. A 384-well white plate was used with 9 μL master mix and 1 μL of sample and subsequently run on a Roche 480 LightCycler™ using the recommended settings. Namely, denaturation at 95 °C for 2 mins
5 followed by 40 two-step cycles of 5s denaturation at 95 °C then 10 s annealing at 60 °C. A melt curve was run at the end of the cycle and checked to ensure only one product was produced in each reaction. No reverse transcriptase controls were run on the same plate with each primer set to ensure amplification of cDNA only. CT values were analysed according to the Livak method (Livak et al., Analysis of relative gene expression data using real-time
10 quantitative PCR and the 2(-Delta Delta C(T)) Method; Methods. 2001, 25(4):402-8). The reference used, when calculating the $\Delta\Delta CT$, was the average ΔCT of the vehicle group (both males and females included).

One-way ANOVA with Dunnett's multiple comparisons to Vehicle group was conducted on
15 Cytochrome P450 Family 26 Subfamily B Member 1 (CYP26b1), Cytochrome P450 family 2 subfamily S member 1 (CYP2S1), Aldehyde dehydrogenase 1 family member A1 (ALDH1A1), and Retinoic Acid Receptor α (RAR α); Kruskal-Wallis with Dunn's multiple comparisons to Vehicle group was conducted for the remaining genes (Cellular retinoic acid-binding protein 1 (CRABP1), Cellular retinoic acid-binding protein 2 (CRABP2), Retinoic Acid Receptor β
20 (RAR β), Retinoic Acid Receptor γ (RAR γ)). Statistical analysis was conducted on $\log(2^{-\Delta\Delta CT})$ values normalised to β -actin and subsequently presented as the non-log transformed values ($2^{-\Delta\Delta CT}$). Bars are plotted as mean $\pm$ SEM and given numerical P values refer to the ANOVA as a whole.

25 3.3 Retinoid signalling in rat brain after treatment with the compound of formula I(a)

Figure 2 shows mRNA levels of key retinoid pathway genes in the rat brain after treatment with the compound of formula I(a). No increase in retinoid pathway gene expression level was seen after treatment with the compound of formula I(a) in comparison with vehicle-treated control. This data demonstrates that the compound of formula I(a) does not show
30 activity in the rat brain, indicating no penetration of the blood-brain barrier.

Example 4: General cell culture methods**4.1 Materials & methods: cell lines and culture**

Human dermal fibroblasts (HDF), human keratinocytes (HaCaT), C6 rat glioma, human microglial clone 3 (HMC3), and human neuroblastoma (SH-SY5Y) were obtained from Durham University and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% Penicillin Streptomycin Solution (Pen-Strep, Lonza) at 37 °C in a humidified 5% CO₂ incubator. The growth medium was changed every 2 days. When the culture reached 80% confluence, trypsin-EDTA was added and incubated for 3-5 min to detach adherent cells. Cells were seeded at a ratio of 1:2 into 24-well plates or T75 flasks for further growth.

SH-SY5Y cells were differentiated by adding retinoic acid (RA) to Dulbecco's modified Eagle's medium (DMEM, Gibco) with 1% Penicillin Streptomycin Solution (Pen-Strep, Lonza) to a final concentration of 10 µM, 24 h after subculturing. Cultures were differentiated for 6 days. The medium was changed every 2 days. After being differentiated, cells were cultured under normal conditions for two days to eliminate the effects of RA.

4.2 Induction of stress and Aβ plaque formation in cells

After trypsinization, cells were plated (40000 cells/mL) in 24-well plate chambers and left to grow for 24 hours at 37 °C and 5% CO₂ before being treated with 10% DMSO (control) or 10 nM the compound of formula I(a) for 4 hours before being stressed.

A final concentration of 100 µM H₂O₂ was used to induce oxidative stress. A final concentration of 10 µg/mL lipopolysaccharides (LPS) was used to induce inflammatory stress. A final concentration of 2 µM amyloid β-peptide (1-42) (Aβ1-42) was used to induce amyloid beta (Aβ) plaque formation.

4.3 Treatment of cells with the compound of formula I(a)

The compound of formula I(a) (1 mM in DMSO) was prepared as outlined in Example 2.5 and was stored at -20 °C. The drug was prepared to 1 µM stock solution using PBS and was stored

at 4 °C. 10 µl of the compound of formula I(a) stock solution (1 µM) was applied gently to media (1 ml/well) to yield a final test concentration of 10 nM.

Example 5: Mitochondrial viability

5 Mitochondria are subcellular organelles responsible for generating adenosine triphosphate (ATP) within a cell and are crucial to metabolic homeostasis. In nerves, mitochondria have additional key roles in calcium homeostasis, membrane excitability and neurotransmission and plasticity. Mitochondrial dysfunction is therefore linked to numerous neurological disorders (Norat *et al.*, *npj Regen Med*; 5(1):22; 2020). In order to determine whether the
10 compound of formula I(a) affected mitochondrial viability, the MTT assay was used to measure mitochondrial viability in skin and nerve cells after treatment with the compound of formula I(a). Mitochondrial viability was also measured in treated cells under stress conditions to determine whether the compound of formula I(a) had a protective effect on mitochondria. The MTT assay measures the conversion of water soluble MTT (3-(4,5-
15 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) compound to an insoluble formazan product.

5.1 Materials & methods: Methyl thiazolyl-diphenyl-tetrazolium bromide (MTT) assay

50 µl of 5 mg/ml MTT (M2128, Sigma) was added to each well and left to incubate for 4 hours
20 at 37 °C and 5% CO₂. Subsequently, the media was removed and 200 µl DMSO was added to each well to dissolve the formazan crystals. Finally, 100 µl from each well was transferred to a 96-well tissue culture plate and the absorbance was measured at 595 nm, using a microplate reader.

5.2 Mitochondrial viability in human dermal fibroblasts (HDF)

Figure 3A shows mitochondrial viability in HDF cells treated with the compound of formula I(a). Figure 3B shows mitochondrial viability in HDF cells treated with the compound of formula I(a) after induction of oxidative stress. No change is observed in mitochondrial viability after treatment with the compound of formula I(a) under normal conditions (figure
30 3A). After induction of oxidative stress, a small increase in mitochondrial viability is seen in

cells treated with the compound of formula I(a), indicating that the compound of formula I(a) may be protective against oxidative stress (figure 3B).

5.3 Mitochondrial viability in keratinocytes (HaCaT)

- 5 Figure 4A shows mitochondrial viability in HaCaT cells treated with the compound of formula I(a). Figure 4B shows mitochondrial viability in HaCaT cells treated with the compound of formula I(a) after induction of oxidative stress. An increase in mitochondrial viability under oxidative stress conditions is observed, suggesting the compound of formula I(a) has a protective effect on mitochondrial viability in the presence of oxidative stress (figure 4B).

10

5.4 Mitochondrial viability in SH-SY5Y neurons

- Figure 5 shows mitochondrial viability in SH-SY5Y cells treated with the compound of formula I(a), under normal and oxidative stress conditions (+H₂O₂). A significant increase in mitochondrial viability was observed after treatment with the compound of formula I(a) under low stress conditions (figure 5).

15

Example 6: Cytotoxicity

- In order to determine toxicity in cells, cytotoxicity was measured after treatment with the compound of formula I(a) using the LDH assay. Measurements were also taken under stress conditions to determine whether the compound of formula I(a) reduced toxicity associated with stress. The LDH assay measures lactate dehydrogenase which is a cytosolic enzyme that is released upon cell lysis and therefore acts as an indicator of cell death.

20

6.1 Materials & methods: Lactate Dehydrogenase (LDH) release assay

- 25 LDH release was measured using CytoTox 96 kit (ADG1781, Promega). 100 µl of the supernatant was taken out of each well and transferred to a 96-well tissue culture plate. 100 µl of the cytotoxicity detection kit LDH solution was added to each well and incubated for 30 minutes in the dark at room temperature. The reaction was stopped by adding 50 µl of stop solution. Subsequently, the optical density was measured at 490nm. This assay was
- 30 normalised by freezing the remaining plate and later thawing it, pipetting the contents of each well into Eppendorf tubes, centrifuging those for 10 minutes, and subsequently removing

100µl of the supernatant from each Eppendorf tube and following the same procedure as described above. This gave an indication of total LDH and allowed normalisation.

6.2 Cytotoxicity in C6 glia cells

- 5 Figure 6 shows a significant reduction in cytotoxicity in C6 glia cells under inflammatory stress conditions (+LPS) after treatment with the compound of formula I(a).

6.3 Cytotoxicity in SH-SY5Y neurons

- 10 Figure 7A shows a significant reduction in cytotoxicity in SH-SY5Y neurons after treatment with the compound of formula I(a) under low stress conditions. A small reduction in cytotoxicity was also observed under oxidative stress conditions (+H₂O₂), however this reduction did not reach statistical significance. Figure 7B shows a significant reduction in cytotoxicity in SH-SY5Y neurons after treatment with the compound of formula I(a) under inflammatory stress conditions (+LPS).

15

Example 7: Autophagy

- Autophagy is a cellular process whereby damaged and dysfunctional proteins and organelles are degraded. Autophagy is important to maintain cellular homeostasis and it is thought to play a role in nerve regeneration. To determine whether the compound of formula I(a) treatment of cells might impact upon autophagy, levels of autophagy were determined by quantitative LC3 immunofluorescence assay in low stress, oxidative stress, and serum starvation conditions.
- 20

7.1 Materials & methods: quantitative LC3 immunofluorescence assay

- 25 Cells were plated at 8000/mL in 6-well (35mm) chambers onto 15mm × 15mm coverslips. 24 hours after stressing the cells, immunocytochemistry staining was carried out using the VECTASTAIN™ Elite™ ABC Universal Kit (PK-6200) and ImmPACT™ DAB Substrate Kit, Peroxidase (SK-4105) with LC3B (PA1-46286, Invitrogen) according to the manufacturer's protocol.

30

7.2 Autophagy in C6 glia cells

Figure 8 shows autophagy in C6 glia cells after treatment with the compound of formula I(a). Notably, a significant increase in autophagy can be seen after treatment with the compound of formula I(a) under low stress conditions. No significant difference in autophagy was
5 observed after treatment with the compound of formula I(a) under oxidative stress (+H₂O₂) or under serum starvation (+SFM).

7.3 Autophagy in SH-SY5Y cells

Figure 9 shows autophagy in SH-SY5Y neurons after treatment with the compound of formula
10 I(a). A significant increase in autophagy was observed after treatment with MH21 under serum starvation (+SFM).

Example 8: Senescence

Cellular senescence is the cessation of cell division despite sufficient resources and stimuli.
15 Cellular senescence compromises tissue repair and regeneration and has been linked to a number of peripheral nervous system disorders such as chemotherapy neuropathy and neuropathic pain (Borgonetti and Galeotti, Pain, 164(5), 2023; Calls *et al.*, *Neuro-Oncology*, 23(1); 2021). Senescence was measured in glia cells after treatment with the compound of formula I(a) by senescence-associated β -galactosidase (SA- β -Gal) staining.

20

8.1 Materials & methods: senescence-associated β -galactosidase staining

Cells were plated 8000/mL in 6-well (35mm) chambers onto 15 mm $\times$ 15 mm coverslips. 24 hours after stressing the cells, Senescence-Associated β -Galactosidase (SA- β -Gal) Staining was carried out using Senescence Cells Histostaining Kit (Sigma-Aldrich, CS0030-1KT)
25 according to the manufacturer's protocol. Cell nuclei were then stained by DAPI. The stained cells were counted and compared to the total number of cells, evaluated by counting the DAPI-stained nuclei.

8.2 Senescence in C6 glia cells

30 Figure 10 shows a decrease in the percentage of senescent C6 glia cells after treatment with the compound of formula I(a). The decrease can be seen in low stress (the compound of

formula I(a) alone), oxidative stress (+H₂O₂) and inflammatory stress (+LPS) conditions. This result indicates that the compound of formula I(a) may be protective against cellular senescence both in normal (low stress) conditions and under stress conditions.

5 **Example 9: Inflammatory response**

Inflammation occurs in response to tissue damage and infection. The inflammatory response is a complex and coordinated system whereby cells detect inflammatory stimuli, such as tissue damage or infection, and secrete inflammatory mediators (e.g. cytokines) which go on to trigger inflammatory responses in different tissues.

10

In order to determine whether the compound of formula I(a) had an effect on the inflammatory response, levels of pro-inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF α) were measured in cells after treatment with the compound of formula I(a) by ELISA. Cytokine release was also measured in treated cells under stress

15 conditions.

9.1 Materials and methods: enzyme-linked immunosorbent assay (ELISA) - IL-6 and TNF α

24 hours after stressing the cells, 100 μ l of the supernatant was collected from each well and ELISA was carried out using the Human IL-6 ELISA kit (ab178013, Abcam) and Human TNF- α

20 ELISA kit (ab46087) according to the manufacturer's protocol. The standard curve generated was used to calculate concentrations from the absorbance measurements.

9.2 IL-6 ELISA in HMC3 microglia cells

Figure 11A shows HMC3 cells after treatment with the compound of formula I(a) under

25 oxidative stress conditions. As can be seen in the figure, treatment with the compound of formula I(a) significantly reduces the IL-6 levels under oxidative stress when compared to vehicle control under oxidative stress (DMSO + H₂O₂). Figure 11B shows IL-6 release in HMC3 cells after treatment with the compound of formula I(a) under inflammatory stress conditions. As can be seen in the figure, treatment with the compound of formula I(a)

30 significantly reduces the IL-6 levels under inflammatory stress when compared to treatment vehicle control under inflammatory stress (DMSO+ LPS).

9.3 TNF α ELISA in SH-SY5Y cells

Figure 12 shows SH-SY5Y cells after treatment with the compound of formula I(a) under inflammatory stress conditions. Treatment with the compound of formula I(a) significantly reduced TNF α levels in SH-SY5Y cells under inflammatory stress.

5

9.4 TNF α ELISA in HMC3 microglia cells

Figure 13 shows HMC3 cells after treatment with the compound of formula I(a) under inflammatory stress conditions. Treatment with the compound of formula I(a) significantly reduced TNF α levels in HMC3 cells under inflammatory stress.

10

Example 10: Neurite outgrowth

Neurite outgrowth is the growth of new projections from neurons in response to growth factors or neurotrophins. The PNS is capable of regeneration, therefore stimulating regeneration of the PNS is a promising therapeutic target for many diseases and conditions affecting the PNS. In order to determine whether the compound of formula I(a) might stimulate regeneration of nerves, SH-SY5Y neurons were treated with the compound of formula I(a) and neurite outgrowth was measured.

15

10.1 Materials & methods: measurement of neurite outgrowth

16 mm glass coverslips were acid treated in a mixture of 69% nitric acid and 37% hydrochloric acid in a 2:1 ratio for 2 h. The coverslips were then washed extensively with MilliQ deionised water until the pH of the water reached 5.5-6. The coverslips were stored in 70% ethanol until needed. The coverslips were put on a 70% ethanol-soaked tissue paper under the cell culture hood to dry. The coverslips were then placed into 12-well plates. After that they were coated by addition of 1 ml of 0.002% poly-L-lysine (PLL) solution onto each coverslip and incubated at 37 °C for 2 hours. Two washes with sterile PBS followed and afterwards the coverslips were air dried under sterile conditions.

20

25

SH-SY5Y cells were removed from stock flasks by trypsinization, counted and plated at 10,000 cells/well in 12-well plates containing acid-treated/PLL-coated coverslips. The plates were maintained at 37 °C in a humid atmosphere containing 5% CO₂ for 24 h. Each retinoid was

added to the medium at two different concentrations, 10 μ M and 10 nM, with a final DMSO concentration of 0.01% or 0.0001%, respectively. The cells were incubated for 5 days at 5% CO₂ and 37 °C. All conditions were tested in triplicate.

- 5 After retinoid treatment, SH-SY5Y cells on coverslips were washed twice in PBS and fixed in 4% paraformaldehyde (PFA) for 20 min at room temperature. Coverslips were then washed twice with PBS and stored at 4 °C in PBS until stained. For immunocytochemical staining of neurites, cells on coverslips were washed three times in PBS, and incubated in blocking solution (10% donkey serum and 0.1% Triton X-100 in PBS) for 1 h at room temperature. Cells
10 were then labelled by incubation overnight at 4 °C with β -III tubulin primary antibody (Sigma-Aldrich) diluted 1:1000 in blocking buffer, washed three times with PBS containing 0.1% Triton X-100 solution (PBST) before incubation with anti-mouse monoclonal secondary antibody (1:300 in PBST; Jackson ImmunoResearch) for 2 h at room temperature. Finally, after three washes in PBST and a final wash in PBS, the coverslips were mounted on slides and stored at
15 4 °C.

- ImageJ software with the NeuronJ plugin was used to quantify neurite outgrowth on stained cells. For each experiment, 10 different randomly selected images were taken from each cover slip using a Nikon Eclipse E400 fluorescence microscope. Each image was converted
20 into an 8-bit image (as necessary for the NeuronJ plugin) and optimised with the brightness and contrast tool in GIMP (GNU Image Manipulation Program). For each image, individual traces were drawn for each clearly-identifiable neurite using the tracing tool in the NeuronJ plugin. Neurite length was measured in pixels and transformed into the corresponding length in μ m depending on the magnification used. The average neurite length for each image was
25 calculated by dividing total neurite length by the total number of neurites per image. Ten images per cover slip were measured and the mean calculated for the coverslip overall. Coverslips were in triplicate for each retinoid and concentration.

10.2 Neurite outgrowth in SH-SY5Y cells

- 30 The results of the neurite outgrowth assay are provided in the table below. Table 1 demonstrates that the compound of formula I(a) has the ability to induce neurite outgrowth

- in SH-SY5Y cells. Furthermore, the neurite outgrowth seen in the cells treated with the compound of formula I(a) is comparable to that seen in cells treated with the endogenous retinoid ATRA. This suggests that the compound of formula I(a) may be useful for the repair and regrowth of neuronal tissues and peripheral nerves. Considering the structural similarity
- 5 between MH16 and the compound of formula I(a), the difference in the neurite outgrowth demonstrated in this Example is surprising.

	Fold increase
	10 nM
ATRA	Did not induce neurite outgrowth
MH16	Did not induce neurite outgrowth
The compound of formula I(a)	1.8

Table 1. Neurite outgrowth measurements in SH-SY5Y cells

Example 11: Amyloid beta aggregation

- 10 Amyloid β ($A\beta$) plaques are a characteristic pathophysiological mechanism in Alzheimer's disease (AD). The build-up of $A\beta$ plaques in the brains of AD patients is known to contribute to the cerebral pathology of the disease. Patients with AD have also been found to have build-up of $A\beta$ plaques within the myocardium which may be causative of cardiovascular disease within the AD patient population (Tini *et al.*, Cardiol. Res. Pract. 2020). To determine
- 15 whether the compound of formula I(a) might be a candidate for treatment of cardiovascular disease in AD patients, $A\beta$ aggregation was measured with and without treatment with the compound of formula I(a).

11.1 Materials & methods: measurement of extracellular $A\beta$ 42 fibril formation

- 20 $A\beta$ 42 is the 42 amino acid form of the $A\beta$ peptide which is found as the predominant form of the peptide present in the brains of AD patients.

- TEM was used to observe the effect of the compound of formula I(a) (10 nM) on $A\beta$ 42 fibril formation. $A\beta$ 42 monomers were incubated in for 24 h at 37 °C for aggregation, and
- 25 incubated with or without the compound of formula I(a) (10nM) for another 24 h. A 10 μ L

sample was then spotted onto a 200 mesh Formvar-coated copper grid and left for 20 min. The sample was removed from the grid, before the grid was washed with water for 30 s and incubated with 10 μ L 2.5% (v/v) glutaraldehyde in water for a further 5 min. After another wash with water for 30 s, the grid was stained with 10 μ L of 2% (v/v) filtered (0.2 mm) uranyl acetate in water for 30 s, and allowed to dry at room temperature. The samples were
5 examined using a Hitachi TEM at 80 kV with a 5000 $\times$ magnification.

11.2 Amyloid beta aggregation

Figure 14 shows the TEM images of A β aggregation without treatment (panel A) and with
10 treatment with the compound of formula I(a) (panel C). As can be clearly seen in the images, a significant reduction in A β aggregation is seen after treatment with the compound of formula I(a).

Example 12: Genomic activity

15 Activation of RAR can result in genomic activity, i.e. transcriptional activity. In order to determine the extent of transcription induction as a result of RAR activation by the compound of formula I(a), an X-gal based retinoic acid reporter assay was used. As a comparison, cells were also treated with All-trans retinoic acid (ATRA) which is the most abundant endogenous retinoid and is used as a model compound in the study of retinoids.

20

12.1 Materials & methods: X-gal Based Retinoic Acid (RA) Reporter Assay

The X-Gal Assay utilizes Sil-15 reporter cells in which the transcription of the LacZ gene is under control of a promoter linked to a retinoic acid response element (RARE). Sil-15 cells were used to detect and quantify the transcriptional activity of retinoids added to the medium
25 by monitoring β -galactosidase activity produced by the reporter cells. 96-well plates were coated with 0.1% gelatin and incubated for at least 2 h at 37 $^{\circ}$ C, washed twice with PBS, wrapped with parafilm, and stored at 4 $^{\circ}$ C until use. Sil-15 cells were removed from stock culture flasks by trypsinization, counted and plated at 100,000 cells per well in the pre-coated 96-well plates. After attachment overnight in DMEM containing 10% FCS, the medium was
30 replaced with fresh DMEM/10% FCS and serial dilutions of retinoid ligands, prepared in DMEM containing 10% FCS were added at concentrations from 10^{-6} M to 10^{-14} M. The plates

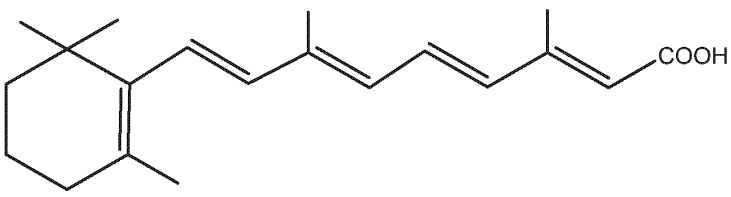
were incubated overnight at 37 °C / 5% CO₂. All concentrations for the ATRA standard curve and the other retinoid ligands were tested in triplicate. The next day, the assay plates were washed twice with PBS, fixed with 100 µl per well of 1% glutaraldehyde and 1 mM MgCl₂ in PBS for 15 min, washed twice with PBS and β-galactosidase activity detected by adding to

5 each well 100 µl of freshly-prepared 0.2% X-Gal in 1 mM MgCl₂, 3.3 mM potassium ferricyanide and 3.3 mM potassium ferrocyanide in PBS. Plates were incubated for 6 h at 37 °C in 5% CO₂ and colour change at 650 nm measured on an Emax™ Precision Microplate Reader (Molecular Devices).

10 12.2 Genomic activity of the compound of formula I(a) in RA reporter cells

Table 2 shows that the compound of formula I(a) demonstrates genomic activity *in vitro*. ATRA is highly potent and is known to have a very narrow therapeutic window, which causes difficulties in achieving an effective therapeutic dose while avoiding severe side-effects. As can be seen in the table, the compound of formula I(a) is far less potent than ATRA indicating

15 its potential for therapeutic development. It is also far less potent than DC645.

	Potency (EC ₅₀) *nM*	Efficacy (E _{max})
	95% CI	
ATRA 	1.04	170.1

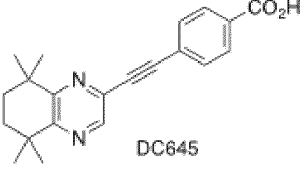
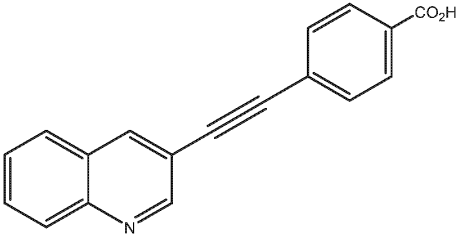
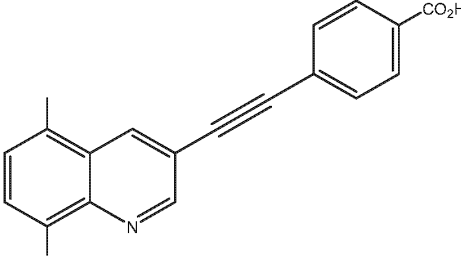
DC645  DC645	0.07	185.9
Comparative compound MH16 	No genomic response	
Compound of formula I(a) 	224	83.8

Table 2. Genomic activity of the compound of formula I(a) and other retinoids in RA reporter cells

Example 13: Non genomic activity

5 13.1 Materials & methods: ERK1/2 Phosphorylation Screening using the AlphaLISA™ SureFire™ Ultra Assay

The non-genomic activity of the compound of formula I(a) and other retinoids was evaluated by measurement of their ability to phosphorylate ERK1/2 in SH-SY5Y cells using the

AlphaLISA™ SureFire™ Ultra ERK1/2 kit (PerkinElmer). In this assay, SH-SY5Y cells (100,000 cells/well) were plated in 96-well plates and serum-starved in DMEM for 24 h. Retinoids were tested at concentrations from 10^{-5} M to 10^{-11} M and at a final concentration of 0.1% DMSO in the medium. SH-SY5Y cells were assayed in serum-free DMEM and stimulated for 30 min
5 (determined from a time course experiment) at 37 °C.

At the end of the assay, the medium was removed, and cells were lysed with 50 µl of freshly prepared 1X lysis buffer supplied in the kit. The 96-well plate was agitated on an orbital shaker SO1 (Stuart Scientific) at approximately 350 rpm for 10 min at room temperature.

10 In the meantime, the activation buffer was diluted 25-fold in the reaction buffers. Under green light in a dark room the acceptor beads were diluted 50-fold in the freshly prepared reaction mix while the donor beads were diluted 50-fold in dilution buffer to obtain two final reaction mixtures.

15 10 µl of cell lysate was then transferred to the wells of a 384-well white Proxiplates™ plate (PerkinElmer) and 5 µl of each prepared acceptor and donor reaction mixtures was added above the wells while still under green light in the dark room. Plates were next wrapped with aluminium foil and incubated at room temperature for at least 3 h and read with the Envision system (PerkinElmer Life Sciences) using AlphaScreen™ settings.

20

13.2 Non-genomic activity of the compound of formula I(a) in SH-SY5Y cells

Table 3 demonstrates the ability of the compound of formula I(a) to phosphorylate ERK in SH-SY5Y cells. The compound of formula I(a) is more potent than endogenous retinoid ATRA and demonstrates a similar efficacy.

25

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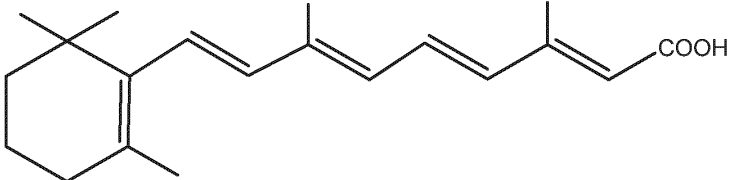
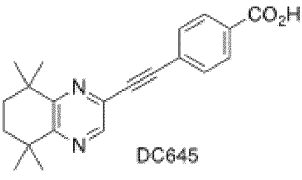
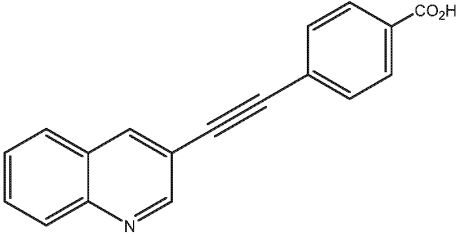
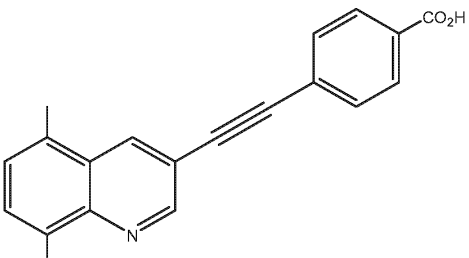
	Potency (EC ₅₀) *nM*	Efficacy (E _{max})
	95% CI	
ATRA 	33.1	48.6
DC645 	8.54	91.6
Comparative Compound MH16 	922.2	19.4
Compound of formula I(a) 	11.0	41.7

Table 3. Non-genomic activity of the compound of formula I(a) and other retinoids in SH-SY5Y cells

Summary of Biological Evaluation of the Compound of Formula I(a)

As detailed above, the compound of formula I(a) shows no RAR activation in the rat brain *in vivo*. Evaluation of the mechanisms of the compound of formula I(a) *in vitro* demonstrate a protective effect on mitochondrial viability in skin cells and neurons; a reduction in toxicity and increase in autophagy in both glia cells and neurons; a decrease in senescence in glia cells; a decrease in pro-inflammatory cytokine release in microglia and neurons; induction of neurite outgrowth; reduction in A β aggregation and both genomic and non-genomic activity *in vitro*. These results taken together with the pharmacodynamic properties of the compound of formula I(a) make it an excellent candidate for the treatment of conditions or diseases which are alleviated by the activation of retinoic acid receptors (RAR) and affect tissues or organs outside of the central nervous system (CNS). Advantageously, MH21 appears to exhibit strong binding affinity, allowing it to influence gene regulation effects related to reduced neural inflammation and improved neuronal repair. Beneficially, the compound of formula I(a) can be prepared as outlined in Example 2, from commercially available, and relatively inexpensive starting materials. The synthesis is short compared with the methods to the comparative compounds, has no low yielding steps, and avoids the use of diazophosphonate reagents.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in this specification (including any accompanying claims, abstract and drawings) may be replaced by alternative features serving the same, equivalent, or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features. The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

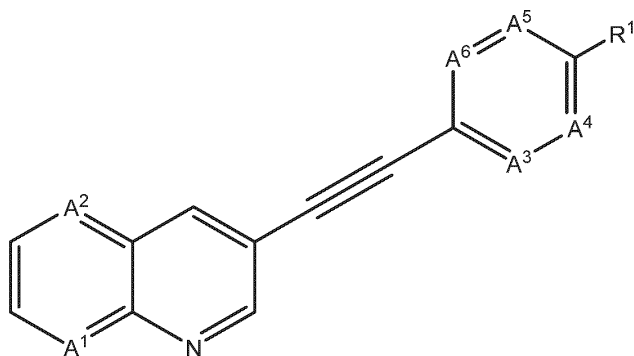
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It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims are generally intended as “open” terms (e.g., the term “including” or “comprising” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as
10 “includes but is not limited to,” etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases “at least one” and “one or more” to introduce claim recitations.
15 However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or “at least one” and indefinite articles such as “a” or “an” (e.g., “a” and/or “an” should be interpreted to mean “at
20 least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of “two recitations,” without other modifiers, means at least two recitations, or two or more
25 recitations).

It will be appreciated that various embodiments of the present disclosure have been described herein for purposes of illustration, and that various modifications may be made without departing from the scope of the present disclosure. Accordingly, the various
30 embodiments disclosed herein are not intended to be limiting, with the true scope being indicated by the following claims.

CLAIMS

1. A compound of formula I:



- 5 in which:

A^1 and A^2 are each, independently, CR^2 in which R^2 is C_1 - C_{10} alkyl;

A^3 is N or CR^3 ;

A^4 is N or CR^4 ;

A^5 is N or CR^5 ;

- 10 A^6 is N or CR^6 ;

each of R^3 to R^6 is independently H, halogen or haloalkyl C_1 - C_{10} ; and

R^1 is $C(=O)R^7$ or $-C(=O)OR^7$ in which R^7 is H or C_1 - C_{10} alkyl;

and isomers thereof;

in free or salt form.

15

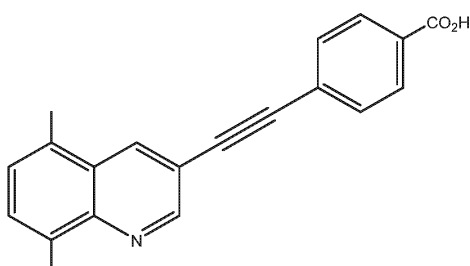
2. A compound of formula I as claimed in claim 1, wherein R^2 is C_1 - C_4 alkyl.
3. A compound of formula I as claimed in claim 2, wherein R^2 is $-CH_3$.
- 20 4. A compound of formula I as claimed in any preceding claim, wherein A^1 and A^2 are the same.
5. A compound of formula I as claimed in any preceding claim, wherein A^3 is CR^3 , A^4 is CR^4 , A^5 is CR^5 and A^6 is CR^6 .

6. A compound of formula I as claimed in claim 5, wherein each of R³ to R⁶ is hydrogen.

7. A compound of formula I as claimed in any preceding claim, wherein R¹ is -COOH.

5

8. A compound of formula I as claimed in any preceding claim, wherein the compound is a compound of Formula I(a):



Formula I(a).

10

9. A compound as claimed in any of claims 1 to 8 for use in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside the brain.

15

10. A compound as claimed in claim 9, wherein the condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs of outside the brain.

20 11. A compound as claimed in claim 10, wherein the condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain is a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside the Central Nervous System (CNS).

25

12. A compound as claimed in claim 11, wherein the condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs

outside Central Nervous System (CNS) is selected from cardiac disease in Alzheimer's Disease (AD), Scleroderma, Psoriasis, Psoriatic arthritis, autoimmune thyroid and kidney diseases, long covid-autoimmune diseases, and a condition or disease affecting the peripheral nervous system (PNS).

5

13. A compound as claimed in claim 12, wherein the condition or disease affecting the peripheral nervous system is selected from peripheral nerve disorder, sciatic nerve injury, endometriosis, fibromyalgia, retinal disease, and multiple sclerosis.

10 14. A compound as claimed in claim 13, wherein the peripheral nerve disorder is selected from peripheral neuropathy, neuropathic pain, and neuropathic itch.

15. A compound as claimed in claim 14, wherein the peripheral neuropathy is selected from: diabetic neuropathy, chemotherapy neuropathy, Sjogren's syndrome, lupus, 15 Rheumatoid arthritis, Guillain-Barre syndrome, postinfectious neuropathy, chronic inflammatory demyelinating neuropathy, myasthenia gravis, congenital myasthenic syndrome, vasculitis, Charcot-Marie Tooth disease, inherited peripheral neuropathies, sciatica, sciatic nerve injury and carpal tunnel syndrome.

20 16. A pharmaceutical composition comprising a compound of formula I as claimed in any of claims 1 to 8, optionally in conjunction with one or more pharmaceutically acceptable excipients, diluents, or carriers, for use in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain.

25

17. A method of treatment of a patient with a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain, the method comprising administering to a patient a therapeutically effective amount of a compound formula I, wherein formula I is as defined in any of 30 claims 1 to 8.

18. A compound of formula I as defined in any of claims 1 to 8 for use as a medicament.
19. A compound of formula I as defined in any of claims 1 to 8 for the manufacture of a medicament for use in the treatment of a condition or disease which is alleviated by the activation of retinoic acid receptors (RAR) present in tissues or organs outside of the brain.
- 5

10