PYRIDONE DERIVATIVES FOR THE TREATMENT OF VIRAL INFECTIONS AND FURTHER DISEASES.

This invention relates to pyridone derivatives, processes for their preparation, pharmaceutical compositions, and their use in therapy.

The present invention relates to the use of pyridone derivatives in the treatment of viral infections, immune or inflammatory disorders, whereby the modulation, or agonism, of toll-like-receptors (TLRs) is involved. Toll-Like Receptors are primary transmembrane proteins characterized by an extracellular leucine rich domain and a cytoplasmic extension that contains a conserved region. The innate immune system can recognize pathogen-associated molecular patterns via these TLRs expressed on the cell surface of certain types of immune cells. Recognition of foreign pathogens activates the production of cytokines and upregulation of co-stimulatory molecules on phagocytes. This leads to the modulation of T cell behaviour.

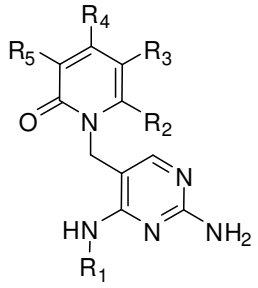
It has been estimated that most mammalian species have between ten and fifteen types of Toll-like receptors. Thirteen TLRs (named simply TLR1 to TLR13) have been identified in humans and mice together, and equivalent forms of many of these have been found in other mammalian species. However, equivalents of certain TLR found in humans are not present in all mammals. For example, a gene coding for a protein analogous to TLR10 in humans is present in mice, but appears to have been damaged at some point in the past by a retrovirus. On the other hand, mice express TLRs 11, 12, and 13, none of which are represented in humans. Other mammals may express TLRs which are not found in humans. Other non-mammalian species may have TLRs distinct from mammals, as demonstrated by TLR14, which is found in the Takifugu pufferfish. This may complicate the process of using experimental animals as models of human innate immunity.

For detailed reviews on toll-like receptors see the following journal articles. Hoffmann, J.A., Nature, 426, p33-38, 2003; Akira, S., Takeda, K., and Kaisho, T., Annual Rev. Immunology, 21, p335-376, 2003; Ulevitch, R. J., Nature Reviews: Immunology, 4, p512-520, 2004.

Compounds indicating activity on Toll-Like receptors have been previously described such as purine derivatives in WO 2006 117670, adenine derivatives in WO 98/01448 and WO 99/28321, and pyrimidines in WO 2009/067081.

However, there exists a strong need for novel Toll-Like receptor modulators having preferred selectivity, and an improved safety profile compared to the compounds of the prior art.

In accordance with the present disclosure a compound of formula (I) is provided



(I)

or a pharmaceutically acceptable salt, solvate or polymorph thereof, wherein

R_1 is C_{1-6} alkyl, optionally substituted by one or more substituents independently selected from aryl, halogen, hydroxyl, amino, carboxylic acid, carboxylic ester, carboxylic amide, acyl sulfonamide, C_{1-3} alkyl, C_{3-6} cycloalkyl, sulfone, sulfoxide, sulfonamide, heterocycle or nitrile;

R_2, R_3, R_4, and R_5 are independently selected from hydrogen, halogen, C_{1-3} alkyl, C_{1-3} alkoxy, C_{3-6} cycloalkyl, aryl, $-CF_3$ or heterocycle;

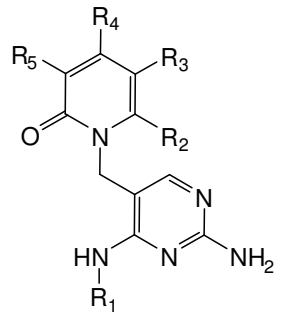
or wherein

R_2 is fused with R_3 to form a ring structure,

R_3 is fused with R_4 to form a ring structure or

R_4 is fused with R_5 to form a ring structure.

In one aspect the present disclosure provides a compound of formula (I)



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein

R_1 is C_{1-6} alkyl, optionally substituted by one or more substituents independently selected from aryl, with aryl being an aromatic ring structure having 5, 6 or 7 ring atoms, halogen, hydroxyl, amino, carboxylic acid, carboxylic ester, carboxylic amide, acyl sulfonamide, C_{1-3} alkyl, C_{3-6} cycloalkyl, sulfone, sulfoxide, sulfonamide, saturated or partially saturated heterocycle or nitrile;

R_2, R_3, R_4, and R_5 are independently selected from hydrogen, halogen, C_{1-3} alkyl, C_{1-3} alkoxy, C_{3-6} cycloalkyl, aryl, with aryl being an aromatic ring structure having 5, 6 or 7 ring atoms, $-CF_3$ or saturated or partially saturated heterocycle;

or wherein

R_2 is fused with R_3 to form a 5-7 membered saturated or partially saturated monocyclic ring structure,

R_3 is fused with R_4 to form a 5-7 membered saturated or partially saturated monocyclic ring structure or

R_4 is fused with R_5 to form a 5-7 membered saturated or partially saturated monocyclic ring structure.

In a first embodiment the present disclosure provides compounds of formula (I) wherein R_1 is *n*-butyl and wherein R_2, R_3, R_4, and R_5 are hydrogen.

In a further embodiment the current disclosure relates to compounds of formula (I) wherein R_1 is *n*-butyl and wherein R_2 is fused with R_3 to form a ring structure, R_3 is fused with R_4 to form a ring structure or R_4 is fused with R_5 to form a ring structure.

The compounds of formula (I) and their pharmaceutically acceptable salt, solvate or polymorph thereof have activity as pharmaceuticals, in particular as modulators of Toll-Like Receptor TLR7 and/or TLR8 activity especially TLR7 activity.

The present disclosure provides a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof together with one or more pharmaceutically acceptable excipients, diluents or carriers. In another aspect the present disclosure provides a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof as defined herein together with one or more pharmaceutically acceptable excipients, diluents or carriers.

Furthermore a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof according to the current disclosure, or a pharmaceutical composition

comprising said compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof can be used as a medicament. In another aspect the present disclosure provides a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof as defined herein or a pharmaceutical composition as defined herein for use as a medicament.

Another embodiment of the disclosure is that a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof, or said pharmaceutical composition comprising said compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof can be used accordingly in the treatment of any disorder in which the modulation of TLR7 and /or TLR8 is involved.

The term “alkyl” refers to a straight-chain or branched-chain saturated aliphatic hydrocarbon containing the specified number of carbon atoms.

The term “halogen” refers to fluorine, chlorine, bromine or iodine.

The term “cycloalkyl” refers to a carbocyclic ring containing the specified number of carbon atoms.

The term “alkoxy” refers to an alkyl (carbon and hydrogen chain) group singular bonded to oxygen like for instance a methoxy group or ethoxy group.

The term “aryl” means an aromatic ring structure optionally comprising one or two heteroatoms selected from N, O and S, in particular from N and O. Said aromatic ring structure may have 5, 6 or 7 ring atoms. In particular, said aromatic ring structure may have 5 or 6 ring atoms.

The term “heterocycle” refers to molecules that are saturated or partially saturated and include ethyloxide, tetrahydrofuran, dioxane or other cyclic ethers. Heterocycles containing nitrogen include, for example azetidine, morpholine, piperidine, piperazine, pyrrolidine, and the like. Other heterocycles include, for example, thiomorpholine, dioxolinyl, and cyclic sulfones.

The term “ring structure” means a 5-7 membered, preferably 6-membered, saturated or partially saturated monocyclic moiety optionally comprising one or more heteroatoms selected from nitrogen, oxygen or sulfur.

Pharmaceutically acceptable salts of the compounds of formula (I) include the acid addition and base salts thereof. Suitable acid addition salts are formed from acids which form non-toxic salts. Suitable base salts are formed from bases which form non-toxic salts.

The compounds of the invention may also exist in unsolvated and solvated forms. The term “solvate” is used herein to describe a molecular complex comprising the compound of the invention and one or more pharmaceutically acceptable solvent molecules, for example, ethanol.

The term “polymorph” refers to the ability of the compound of the invention to exist in more than one form or crystal structure.

The compounds of the present invention may be administered as crystalline or amorphous products. They may be obtained for example as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze drying, spray drying, or evaporative drying. They may be administered alone or in combination with one or more other compounds of the invention or in combination with one or more other drugs. Generally, they will be administered as a formulation in association with one or more pharmaceutically acceptable excipients. The term “excipient” is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient depends largely on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

The compounds of the present invention or any subgroup thereof may be formulated into various pharmaceutical forms for administration purposes. As appropriate compositions there may be cited all compositions usually employed for systemically administering drugs. To prepare the pharmaceutical compositions of this invention, an effective amount of the particular compound, optionally in addition salt form, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirably in unitary dosage form suitable, for example, for oral, rectal, or percutaneous administration. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs, emulsions, and solutions; or solid carriers such as starches, sugars, kaolin, diluents, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are obviously employed. Also included are solid form preparations that can be converted, shortly before use, to liquid forms. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on, as an ointment. The compounds of the present invention may also be administered via inhalation or insufflation by means of methods and formulations employed in the art for administration via this way. Thus, in general the compounds of the present invention may be administered to the lungs in the form of a solution, a suspension or a dry powder.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such unit dosage forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, suppositories, injectable solutions or suspensions and the like, and segregated multiples thereof.

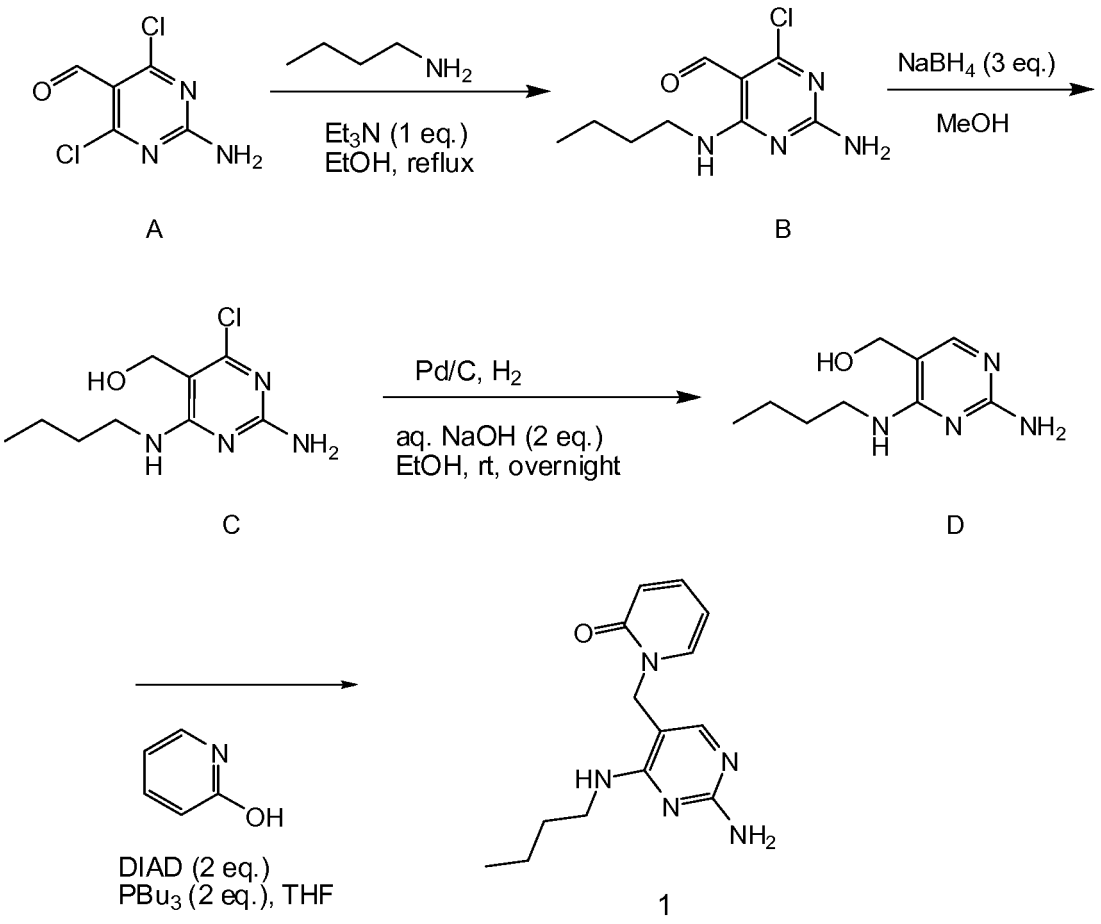
Those of skill in the treatment of infectious diseases will be able to determine the effective amount from the test results presented hereinafter. In general it is contemplated that an effective daily amount would be from 0.01 mg/kg to 50 mg/kg body weight, more preferably from 0.1 mg/kg to 10 mg/kg body weight. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses may be formulated as unit dosage forms, for example, containing 1 to 1000 mg, and in particular 5 to 200 mg of active ingredient per unit dosage form.

The exact dosage and frequency of administration depends on the particular compound of formula (I) used, the particular condition being treated, the severity of the condition being treated, the age, weight and general physical condition of the particular patient as well as other medication the individual may be taking, as is well known to those skilled in the art. Furthermore, it is evident that the effective amount may be lowered or increased depending on the response of the treated subject and/or depending on the evaluation of the physician prescribing the compounds of the instant invention. The effective amount ranges mentioned above are therefore only guidelines and are not intended to limit the scope or use of the invention to any extent.

Preparation of compounds

Compounds of formula (I) are prepared according to **scheme 1.**

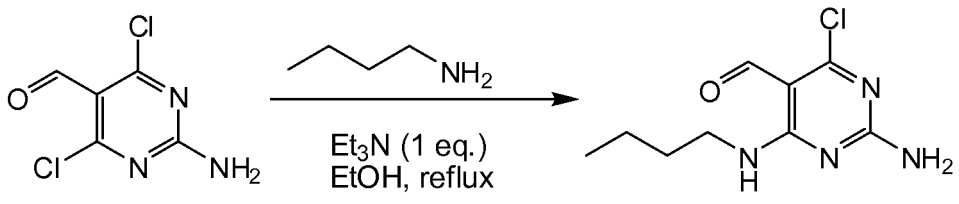
Scheme 1 (where preparation of **1** is shown as an example):



Compounds of type **A** in scheme 1 can be functionalized with amines under thermal conditions in a polar solvent, for example ethanol, with or without a base (e.g. triethylamine). The aldehyde group of **B** can be converted to the alcohol via a reducing agent like NaBH4 in a polar solvent (e.g. methanol). The chlorine in compounds of type **C** can be removed using Pd/C under a hydrogen atmosphere and basic conditions. The alcohol group is then functionalized under standard mitsunobu conditions to afford the pyridone final products.

The preparation of intermediates **A** and **B** is described in the literature (Bioorganic and Medicinal Chemistry 11, 2003, p4161; J. Heterocyclic Chem., 20, 41 (1983); Bioorganic and Medicinal Chemistry Letters 13 (2003) p217).

Preparation of B

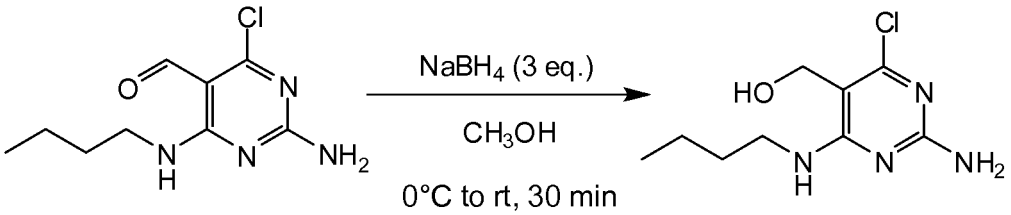


A (10.0 g, 52.08 mmol) was added in ethanol (100 mL) then *n*-butylamine (3.81 g, 52.08 mmol) and triethylamine (5.27 g, 52.08 mmol) were added to the solution. The mixture was heated to reflux for 8 hours. The solution was allowed to reach to 0°C and the solid was isolated via filtration and washed with ethanol then dried under vacuum to afford **B** (10 g).

LC-MS m/z = 229 (M+H); 1.20 min

^{1}H NMR (400 MHz, *d*-Chloroform) δ ppm 0.95 (t, 3 H), 1.31 - 1.42 (m, 2 H), 1.53 - 1.65 (m, 2 H), 3.40 - 3.50 (m, 2 H), 5.11 (br. s., 2 H), 9.22 (br. s., 1 H), 10.07 (s, 1 H)

Preparation of C



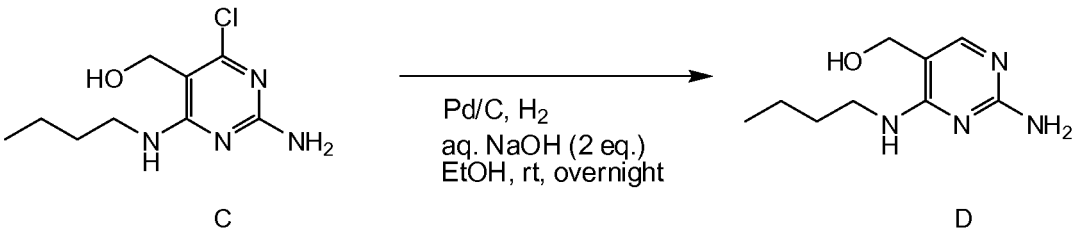
B C

$NaBH_4$ (4 g, 105.73 mmol) was added in small portions to a mixture of **B** (8.0 g, 35 mmol) in methanol at 0°C. The mixture was stirred at room temperature for 30 minutes. The mixture was treated with saturated $NaHCO_3$ (100 mL) and H_2O (100 mL) slowly at 0°C, then stirred at room temperature for 10 min. The precipitate was isolated by filtration and washed with water (50 mL) and ethyl acetate: methyl *t*-butyl ether (1:5). The filtrate was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), the solids were removed by filtration and the solvent of the filtrate was removed under reduced pressure. The residue was treated with ethyl acetate: methyl *t*-butyl ether (1:5). The precipitate was isolated by filtration and washed with methyl *t*-butyl ether. The precipitates were combined and dried (vacuum, 50°C, 30 minutes) to afford **C**.

LC-MS m/z = 231 (M+H), 0.90 min

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 (t, 3 H), 1.31 (m, 2 H), 1.50 (m, 2 H), 3.28 (m, 2 H), 4.4 (m, 2 H), 4.92 (m, 1 H), 6.29 (br. s., 2 H), 6.55 (m, 1 H)

Preparation of D



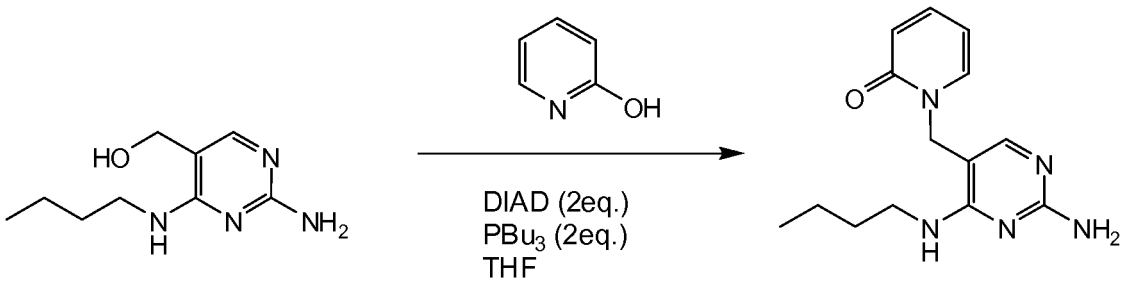
C D

A solution of NaOH (1.6 g, 40 mmol) in H_2O (5 mL) was added to a solution of **C** (5.8 g, 21.37 mmol, purity 85%) in ethanol (150 mL) at room temperature. To this was added 10% Pd/C (0.6 g). The flask was sealed and exposed to hydrogen gas for 15 hours. The hydrogen gas was removed and replaced with nitrogen, the catalyst was removed by filtration, and the solvent of the filtrate was removed under reduced pressure. Ethyl acetate was added and the mixture was washed with H_2O, brine, dried (Na_2SO_4). The solids were removed via filtration and the solvent of the filtrate was removed under reduced presssure. The residue was washed with methyl *t*-butyl ether. The precipitate was isolated by filtration and washed with methyl *t*-butyl ether. The solid was collected and dried (vacuum, 50°C, 30 minutes) to afford **D**.

LC-MS m/z = 197 (M+H); 3.21 min

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 (t, *J*=7.4 Hz, 3 H), 1.24 - 1.41 (m, 2 H), 1.41 - 1.59 (m, 2 H), 3.23 - 3.34 (m, 2 H), 4.21 (br. s., 2 H), 4.85 (br. s., 1 H), 5.87 (s, 2 H), 6.19 (t, *J*=5.3 Hz, 1 H), 7.50 (s, 1 H)

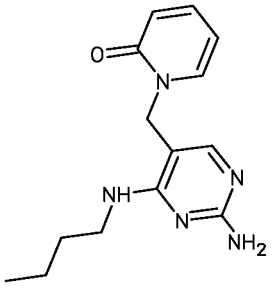
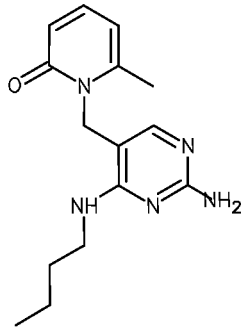
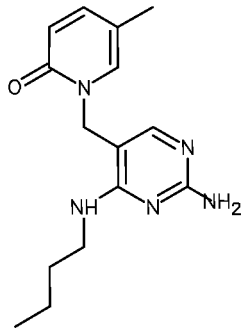
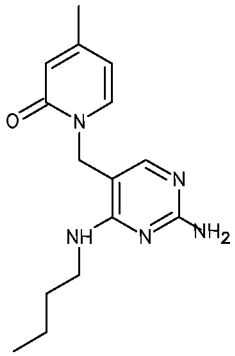
Preparation of compound 1

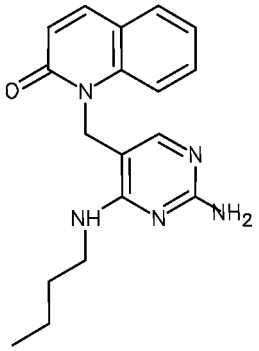
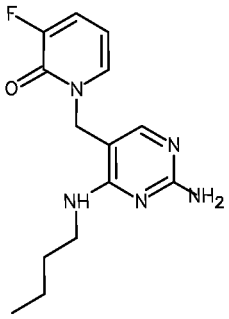
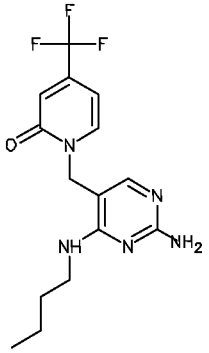
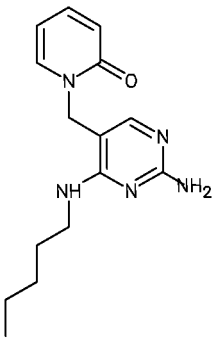


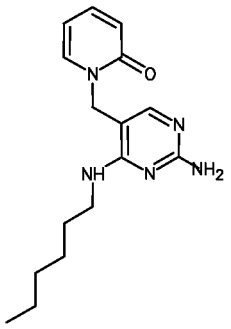
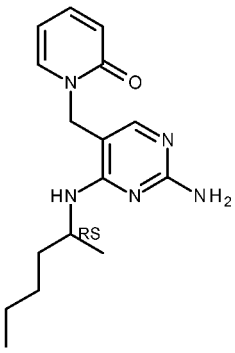
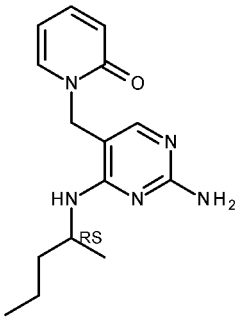
D compound 1

DIAD (1.3 g, 6.429 mmol) was added to a mixture of **D** (0.5 g, 2.29 mmol, purity 90%), 2-hydroxypyridine (0.326 g, 3.43 mmol) and tributylphosphine (1.34 g, 6.62 mmol) in anhydrous THF (10 mL) at 0°C under N_2 atmosphere. The mixture was stirred at room temperature overnight and then refluxed for 3h. The mixture was evaporated under vacuum. The residue was treated with petroleum ether: methyl *t*-butyl ether (1:1). The mixture was evaporated under reduced pressure. Methyl *t*-butyl ether was added. The mixture was stirred at 0°C for 20 min. The precipitate was isolated by filtration and washed with methyl *t*-butyl ether. The solid was collected and dried (vacuum, 50°C, 30 minutes) to afford **1**.

Table 1. Compounds of formula (I).

Co. No.	STRUCTURE	H NMR	LC Method, Rt (minutes)
1		^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.85 (t, *J*=7.4 Hz, 3 H), 1.26 (dq, *J*=15.0, 7.3 Hz, 2 H), 1.44 (quin, *J*=7.2 Hz, 2 H), 3.19 - 3.27 (m, 2 H), 4.79 (s, 2 H), 6.02 (s, 2 H), 6.33 (td, *J*=6.7, 1.3 Hz, 1 H), 6.48 (d, *J*=8.8 Hz, 1 H), 7.30 (t, *J*=5.0 Hz, 1 H), 7.45 (ddd, *J*=9.0, 6.8, 2.0 Hz, 1 H), 7.74 (dd, *J*=6.8, 1.5 Hz, 1 H), 7.85 (s, 1 H)	A, 3.77
2		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.94 (t, *J*=7.3 Hz, 3 H), 1.29 - 1.42 (m, 2 H), 1.62 (quin, *J*=7.3 Hz, 2 H), 2.51 (s, 3 H), 3.52 (t, *J*=6.9 Hz, 2 H), 5.18 (s, 2 H), 6.43 (d, *J*=6.8 Hz, 1 H), 6.57 (d, *J*=9.0 Hz, 1 H), 7.46 - 7.57 (m, 2 H)	A, 3.82
3		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.94 (t, *J*=7.4 Hz, 3 H), 1.27 - 1.40 (m, 2 H), 1.55 - 1.65 (m, 2 H), 2.14 (s, 3 H), 3.49 (t, *J*=7.0 Hz, 2 H), 4.97 (s, 2 H), 6.60 (d, *J*=9.3 Hz, 1 H), 7.50 (dd, *J*=9.2, 2.4 Hz, 1 H), 7.58 (s, 1 H), 7.91 (s, 1 H)	A, 3.84
4		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.95 (t, *J*=7.4 Hz, 3 H), 1.35 (dd, *J*=14.9, 7.4 Hz, 2 H), 1.52 - 1.62 (m, 2 H), 2.25 (s, 3 H), 3.36 (t, *J*=7.0 Hz, 2 H), 4.91 (s, 2 H), 6.37 (dd, *J*=7.0, 2.0 Hz, 1 H), 6.45 (s, 1 H), 7.57 (d, *J*=7.0 Hz, 1 H), 7.82 (s, 1 H)	A, 3.98

Co. No.	STRUCTURE	H NMR	LC Method, Rt (minutes)
5		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.98 (t, *J*=7.4 Hz, 3 H), 1.34 - 1.47 (m, 2 H), 1.67 (quin, *J*=7.4 Hz, 2 H), 3.53 - 3.60 (m, 2 H), 5.34 (s, 2 H), 6.78 (d, *J*=9.5 Hz, 1 H), 7.34 - 7.42 (m, 1 H), 7.51 (s, 1 H), 7.58 (d, *J*=8.5 Hz, 1 H), 7.65 - 7.73 (m, 1 H), 7.79 (d, *J*=7.8 Hz, 1 H), 8.05 (d, *J*=9.3 Hz, 1 H)	A, 4.29
6		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.96 (t, *J*=7.3 Hz, 3 H), 1.37 (sxt, *J*=7.5 Hz, 2 H), 1.58 - 1.69 (m, 2 H), 3.53 (t, *J*=7.0 Hz, 2 H), 5.09 (s, 2 H), 6.45 - 6.53 (m, 1 H), 7.46 (t, *J*=8.3 Hz, 1 H), 7.66 (br. s., 1 H), 7.95 (br. s., 1 H)	A, 3.26
7		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.92 (t, *J*=7.4 Hz, 3 H), 1.33 (dq, *J*=15.0, 7.4 Hz, 2 H), 1.55 (quin, *J*=7.2 Hz, 2 H), 3.36 (t, *J*=6.9 Hz, 2 H), 4.97 (s, 2 H), 6.61 (dd, *J*=7.3, 2.0 Hz, 1 H), 6.88 (s, 1 H), 7.84 (s, 1 H), 7.89 (d, *J*=7.3 Hz, 1 H)	A, 4.33
8		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.91 (t, *J*=7.0 Hz, 3 H), 1.18 - 1.40 (m, 4 H), 1.59 (quin, *J*=7.2 Hz, 2 H), 3.35 - 3.39 (m, 2 H), 4.96 (s, 2 H), 6.48 (td, *J*=6.8, 1.3 Hz, 1 H), 6.63 (d, *J*=9.0 Hz, 1 H), 7.57 (ddd, *J*=9.0, 6.8, 2.0 Hz, 1 H), 7.71 (dd, *J*=6.8, 1.8 Hz, 1 H), 7.85 (s, 1 H), exchangable protons not seen.	A, 4.12

Co. No.	STRUCTURE	H NMR	LC Method, Rt (minutes)
9		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.87 - 0.95 (m, 3 H), 1.28 - 1.37 (m, 6 H), 1.53 - 1.64 (m, 2 H), 3.37 (t, *J*=7.0 Hz, 2 H), 4.96 (s, 2 H), 6.45 - 6.54 (m, 1 H), 6.63 (d, *J*=9.0 Hz, 1 H), 7.57 (ddd, *J*=9.0, 6.8, 2.0 Hz, 1 H), 7.71 (dd, *J*=6.8, 1.5 Hz, 1 H), 7.85 (s, 1 H)	A, 4.47
10		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.86 (t, *J*=7.2 Hz, 3 H), 1.09 - 1.17 (m, 3 H), 1.17 - 1.35 (m, 4 H), 1.46 - 1.57 (m, 2 H), 4.11 - 4.22 (m, 1 H), 4.79 - 4.87 (m, 1 H), 5.03 - 5.11 (m, 1 H), 6.49 (t, *J*=6.8 Hz, 1 H), 6.63 (d, *J*=9.0 Hz, 1 H), 7.57 (ddd, *J*=9.0, 6.8, 2.0 Hz, 1 H), 7.66 - 7.73 (m, 1 H), 7.85 (s, 1 H)	A, 4.31
11		^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.88 (t, *J*=7.3 Hz, 3 H), 1.13 (d, *J*=6.5 Hz, 3 H), 1.16 - 1.33 (m, 2 H), 1.39 - 1.63 (m, 2 H), 4.09 - 4.25 (m, 1 H), 4.86 (d, *J*=14.8 Hz, 1 H), 5.04 (d, *J*=14.8 Hz, 1 H), 6.47 (td, *J*=6.8, 1.3 Hz, 1 H), 6.62 (d, *J*=9.0 Hz, 1 H), 7.56 (ddd, *J*=9.0, 6.8, 2.0 Hz, 1 H), 7.69 (dd, *J*=6.9, 1.6 Hz, 1 H), 7.84 (s, 1 H)	A, 4.05

Analytical Methods. All compounds were characterized by LC-MS using the following method:

Method A.

Column	YMC-PACK ODS-AQ, 50×2.0mm 5μm		
Mobile Phase	A :H_2O（0.1%TFA）		
	B:CH_3CN（0.05%TFA）		
Gradient	Stop Time :10 min		
	TIME(min)	A%	B%
	0	100	0
	1	100	0
	5	40	60
	7.5	40	60
	8	100	0
Flow Rate	0.8 mL/min		
Wavelength	UV 220 nm		
Oven Tem.	50□		
MS polarity	positive		
LCMS	Agilent 1100		

Biological Activity of compounds of formula (I)

Description of Biological Assays

Assessment of TLR7 and TLR8 activity

The ability of compounds to activate human TLR7 and/or TLR8 was assessed in a cellular reporter assay using HEK293 cells transiently transfected with a TLR7 or TLR8 expression vector and NFκB-luc reporter construct.

Briefly, HEK293 cells were grown in culture medium (DMEM supplemented with 10% FCS and 2 mM Glutamine). For transfection of cells in 15 cm dishes, cells were detached with Trypsin-EDTA, transfected with a mix of CMV-TLR7 or TLR8 plasmid (1700 ng), NFκB-luc plasmid (850

ng) and a transfection reagent and incubated for 48 h at 37°C in a humidified 5% CO_2 atmosphere. Transfected cells were then washed in PBS, detached with Trypsin-EDTA and resuspended in medium to a density of 1.25 x 10^5 cells/mL. Forty microliters of cells were then dispensed into each well in 384-well plates, where 200 nL of compound in 100% DMSO was already present. Following 6 hours incubation at 37°C, 5% CO_2, the luciferase activity was determined by adding 15 µL of Steady Lite Plus substrate (Perkin Elmer) to each well and readout performed on a ViewLux ultraHTS microplate imager (Perkin Elmer). Dose response curves were generated from measurements performed in quadruplicates. Lowest effective concentrations (LEC) values, defined as the concentration that induces an effect which is at least two fold above the standard deviation of the assay, were determined for each compound.

Compound toxicity was determined in parallel using a similar dilution series of compound with 40 µL per well of cells transfected with the CMV-TLR7 construct alone (1.25 x 10^5 cells/mL), in 384-well plates. Cell viability was measured after 6 hours incubation at 37°C, 5% CO_2 by adding 15 µL of ATP lite (Perkin Elmer) per well and reading on a ViewLux ultraHTS microplate imager (Perkin Elmer). Data was reported as CC_{50}.

In parallel, a similar dilution series of compound was used (200 nL of compound in 100% DMSO) with 40 µL per well of cells transfected with NFκB-luc reporter construct alone (1.25 x 10^5 cells/mL). Six hours after incubation at 37°C, 5% CO_2, the luciferase activity was determined by adding 15 µl of Steady Lite Plus substrate (Perkin Elmer) to each well and readout performed on a ViewLux ultraHTS microplate imager (Perkin Elmer). Counterscreen data is reported as LEC.

Activation of ISRE promoter elements

The potential of compounds to induce IFN-I was also evaluated by measuring the activation of interferon-stimulated responsive elements (ISRE) by conditioned media from PBMC. The ISRE element of sequence GAAACTGAAACT is highly responsive to the STAT1-STAT2-IRF9 transcription factor, activated upon binding of IFN-I to their receptor IFNAR (Clontech, PT3372-5W). The plasmid pISRE-Luc from Clontech (ref. 631913) contains 5 copies of this ISRE element, followed by the firefly luciferase ORF. A HEK293 cell line stably transfected with pISRE-Luc (HEK-ISREluc) was established to profile the conditioned PBMC cell culture media.

Briefly, PBMCs were prepared from buffy coats of at least two donors using a standard Ficoll centrifugation protocol. Isolated PBMCs were resuspended in RPMI medium supplemented with 10% human AB serum and 2 x 10^5 cells/well were dispensed into 384-well plates containing compounds (70 µL total volume). After overnight incubation, 10 µL of supernatant was transferred to 384-well plates containing 5 x 10^3 HEK-ISREluc cells/well in 30 µL (plated the day before). Following 24 hours of incubation, activation of the ISRE elements was measured by assaying luciferase activity using 40 µL/well Steady Lite Plus substrate (Perkin Elmer) and

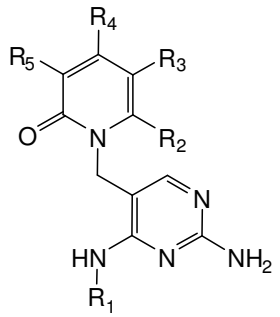
measured with ViewLux ultraHTS microplate imager (Perkin Elmer). The stimulating activity of each compound on the HEK-ISREluc cells was reported as LEC value, defined as the compound concentration applied to the PBMCs resulting in a luciferase activity at least two fold above the standard deviation of the assay. The LEC in turn indicates the degree of ISRE activation on transfer of a defined amount of PBMC culture medium. Recombinant interferon α-2a (Roferon-A) was used as a standard control compound.

Biological activity of compounds of formula (I). All compounds showed CC50 of >24uM.

#	Human TLR 7 (LEC) μM	Human TLR 8 (LEC) μM	HEK-ISRE luc (LEC) μM
1	1.8	7.3	0.7
2	1.1	2.1	0.8
3	0.8	10.3	0.3
4	0.5	2.2	0.5
5	0.9	2.7	0.04
6	1.2	6.9	0.5
7	0.5	6.8	0.5
8	1.5	>25	0.6
9	7.3	>25	3
10	1.0	16	0.3
11	1.3	14.6	0.3

Claims

1. A compound of formula (I)



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein

R_1 is C_{1-6} alkyl, optionally substituted by one or more substituents independently selected from aryl, with aryl being an aromatic ring structure having 5, 6 or 7 ring atoms, halogen, hydroxyl, amino, carboxylic acid, carboxylic ester, carboxylic amide, acyl sulfonamide, C_{1-3} alkyl, C_{3-6} cycloalkyl, sulfone, sulfoxide, sulfonamide, saturated or partially saturated heterocycle or nitrile;

R_2, R_3, R_4, and R_5 are independently selected from hydrogen, halogen, C_{1-3} alkyl, C_{1-3} alkoxy, C_{3-6} cycloalkyl, aryl, with aryl being an aromatic ring structure having 5, 6 or 7 ring atoms, $-CF_3$ or saturated or partially saturated heterocycle;

or wherein

R_2 is fused with R_3 to form a 5-7 membered saturated or partially saturated monocyclic ring structure,

R_3 is fused with R_4 to form a 5-7 membered saturated or partially saturated monocyclic ring structure or

R_4 is fused with R_5 to form a 5-7 membered saturated or partially saturated monocyclic ring structure.

2. A compound of formula (I) according to claim 1 wherein R_1 is *n*-butyl and wherein R_2, R_3, R_4, and R_5 are hydrogen.

3. A compound of formula (I) according to claim 1 wherein R_1 is *n*-butyl and wherein R_2 is fused with R_3 to form a ring structure, R_3 is fused with R_4 to form a ring structure or R_4 is fused with R_5 to form a ring structure.

4. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof according to one of claims 1-3 together with one or more pharmaceutically acceptable excipients, diluents or carriers.

5. A compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof according to one of claims 1-3 or a pharmaceutical composition according to claim 4

for use as a medicament.