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(54) Title: SUBSTITUTED 1-ARYLETHYL-4-ACYLAMINOPIPERIDINE DERIVATIVES AS OPIOID/ALPHA-ADRENORECEPTOR MODULATORS AND METHOD OF THEIR PREPARATION

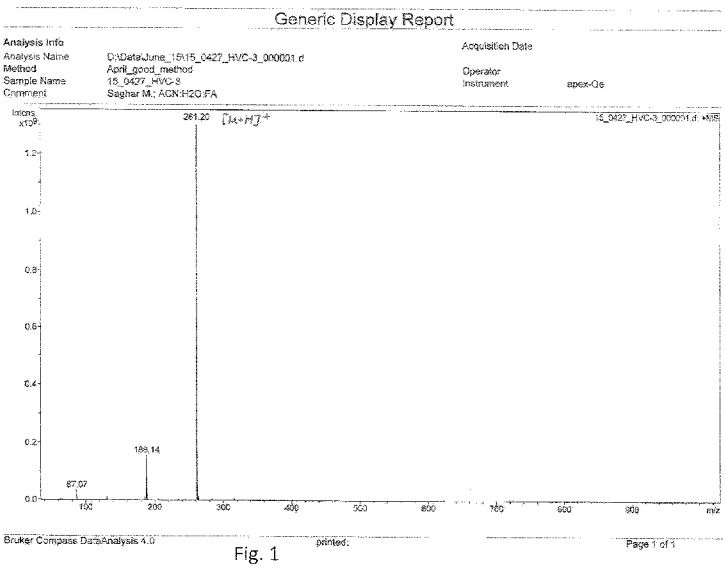


Fig. 1

(57) Abstract: The invention provides compounds that bind with high affinities to the μ-, δ- and κ- opioid receptors and α_2 - adrenoreceptor. In addition to providing these compounds with novel pharmacological binding properties, the invention also describes detailed novel methods for the preparation of representative compounds and a scheme for the synthesis of related compounds that bind to the opioid receptors and/or α_2 - adrenoreceptor.

Substituted 1-arylethyl-4-acylaminopiperidine derivatives as opioid/alpha-adrenoreceptor modulators and method of their preparation.

The invention relates to novel pharmacological compounds, and more specifically to the creation of a new class of small molecules which simultaneously exhibit high binding affinities to the μ–, δ–, and κ–opioid receptors and the α_2-adrenoreceptor. The binding activity is believed to be antagonistic at least with respect to the μ–opioid receptors. In addition to providing these compounds with novel pharmacological binding properties, the invention also describes detailed novel methods for the preparation of representative compounds and a scheme for the synthesis of related compounds that bind to the opioid receptors and/or α_2–adrenoreceptor.

Opioid antagonists are drugs which bind to the opioid receptors with higher affinity than opioid agonists but do not activate the opioid receptors. Commonly known opioid antagonists include drugs such as, for example, naltrexone, naloxone, nelmefene, nalorphine, and nalbuphine. Opioid antagonists effectively block the receptor from the action of both naturally occurring agonists (e.g., morphine, codeine, thebaine) and synthetic agonists (e.g., fentanyl, pethidine, levorphanol, methadone, tramadol, dextropropoxyphene) and uses include counteracting life-threatening depression of the central nervous and respiratory systems and thus are used for emergency overdose and dependence treatment (e.g., naloxone). There are many excellent reviews dedicated to different aspects of opioid antagonists [28-46].

Opioid receptor antagonists are known to modulate numerous central and peripheral effects including those associated with opioid abuse, the development of opioid tolerance and dependence, opioid-induced constipation, alcohol and cocaine abuse, depression, and immune responses [1]. The diverse therapeutic applications of μ-opioid antagonists include opioid-overdose-induced respiratory depression, opioid and cocaine abuse, alcohol dependence, smoking cessation, obesity, psychosis[1-19] and for the treatment of dyskinesia associated with Parkinson's disease [20-27].

The few opioid antagonists currently on the market are represented by very few drugs (e.g., naloxone, naltrexone, and nalorphine (a partial agonist)) that have been shown to have therapeutic utility in a variety of indications. During last two decades only Alvimopan [13,14]—a peripherally acting μ-opioid antagonist for the treatment of postoperative ileus—has received approval as new drug. In addition, some

azabicyclohexane derivatives and series of bi(hetero)aryl ethers as biological tools have been proposed as new chemical entities in this class of compounds [15].

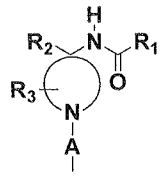
Every chemical class of compounds with opioid-agonist activity has a structurally similar opioid-antagonist pair. Agonist-antagonist transformation in any of these cases takes place as a result of a small change in the structure of the agonist. The only exceptions, where the corresponding change for agonist-antagonist transformations has not been found, are the compounds of the fentanyl series.

Since the discovery of the "army" of opioid agonists of the fentanyl series (sufentanyl, alfentanyl, carfentanyl, remifentanyl, etc.) beginning in the 1960s, a structurally corresponding antagonist has not been found for any of these compounds. Thus, for decades there has been an evident gap in the art with respect to a possible specific structural change that could make possible the transformation of powerful opioid agonist properties of compounds of fentanyl series into powerful antagonists.

Similar to the general action of the opioid antagonists, antagonists of the adrenoreceptors (adrenergic receptors) bind to the adrenoreceptors and act to inhibit the action of those receptors. Alpha antagonists, or alpha-blockers, may selectively act at the α_1-adrenoreceptors or at the α_2-adrenoreceptors, or they may non-selectively act at both receptors. Commonly known α-blockers include, for example, phenoxybenzamine and phentolamine (non-selective); alfuzosin and prazosin (α_1-blockers); and atipamezole, idazoxan, mirtazapine and yohimbine (α_2-blockers). Generally, α-blockers have shown to be effective in the treatment of various medical conditions, including Raynaud's disease, hypertension, scleroderma, anxiety and panic disorders, and in the treatment of dyskinesia associated with Parkinson's disease.

The present invention is based on the discovery of certain compounds exhibiting high binding affinity for the μ–, δ–, and κ- opioid receptors and the α_2-adrenoreceptor. The compounds are believed to exhibit antagonistic activity at least with respect to μ–opioid receptors. The compounds are structurally related to the fentanyl series of opioid receptor agonists. Processes for preparing these compounds are also included in this disclosure.

In one embodiment of the disclosure, a compound having the formula



Ar , or (HetAr), or $COOR_5$, or $CON(R_6)_2$ (III)

is disclosed, wherein R_1 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or hetaryl; R_2 is H, -CH_2O-C_{1-4} alkyl; COO-C_{1-4} alkyl; -$CONR_4$; R_3 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or unsubstituted aryl or hetaryl; A is substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl; Ar or HetAr is substituted or unsubstituted monocyclic or polycyclic aromatic or heteroaromatic moiety; $COOR_5$, or $CON(R_6)_2$, where R_5 and R_6 are H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or unsubstituted aryl or hetaryl; the central nitrogen-containing ring is a substituted or unsubstituted 5- to 7-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound.

In a preferred embodiment, the prepared compound may belong to the series of N-(1-arylethylpiperidin-4-yl)acylamides. In another embodiment, the compound may be N-(1-phenethylpiperidin-4-yl)propionamide (Compound I, below). In yet another embodiment the compound may be the oxalate salt, or other pharmaceutically acceptable salt, of N-(1-phenethylpiperidin-4-yl)propionamide.

In another embodiment, a process for preparing a compound of formula III is provided. The process comprising the following steps: (a) reacting a cyclic ketone having a protecting group in a Grignard or Reformatsky reaction to obtain a first product; (b) reacting the product of step (a) in a Ritter reaction to obtain a second product; and (c) deprotecting the product of step (b) with acylation or alkylation to obtain a compound of formula III.

In yet another embodiment, a process for preparing a compound of formula III is provided, comprising the following steps: (a) reacting a cyclic ketone having a protecting group in a Strecker reaction to obtain a first product; (b) reacting the product of step (a) in a selective carbalkoxy group transformation to obtain a second product; and (c) deprotecting the product of step (b) with acylation or alkylation to obtain a compound of formula III.

In another embodiment, a process for preparing N-(1-phenethylpiperidin-4-yl) propionamide is provided, comprising the following steps: (a) reacting

phenethylpiperidin-4-one with hydroxylamine hydrochloride in ethanol in the presence of a base, to produce 1-phenethylpiperidin-4-one oxime; (b) reducing the oxime obtained in step (a) with iso-amyl alcohol and sodium metal to produce 1-phenethylpiperidin-4-amine; and (c) acylating the product of step (b) with propionic acid chloride in chloroform in the presence of triethylamine to produce N-(1-phenethylpiperidin-4-yl)propionamide. The N-(1-phenethylpiperidin-4-yl)propionamide may optionally be further treated with oxalic acid to obtain N-(1-phenethylpiperidin-4-yl)propionamide oxalate.

These and other embodiments, features and advantages of the present invention will become more fully apparent when read in conjunction with the following detailed description taken in conjunction with the accompanying drawings, wherein

Fig. 1 is a (LC/MS) plot of a preferred compound designated HVC-3.

For the purposes of this disclosure, a "salt" is any acid addition salt, preferably a pharmaceutically acceptable acid addition salt, including but not limited to, halogenic acid salts such as hydrobromic, hydrochloric, hydrofluoric and hydroiodic acid salt; an inorganic acid salt such as, for example, nitric, perchloric, sulfuric and phosphoric acid salt; an organic acid salt such as, for example, sulfonic acid salts (methanesulfonic, trifluoromethan sulfonic, ethanesulfonic, benzenesulfonic or *p*-toluenesulfonic), acetic, malic, fumaric, succinic, citric, benzoic, gluconic, lactic, mandelic, mucic, pamoic, pantothenic, oxalic and maleic acid salts; and an amino acid salt such as aspartic or glutamic acid salt. The acid addition salt may be a mono- or di-acid addition salt, such as a di-hydrohalogenic, di-sulfuric, di-phosphoric or di-organic acid salt. In all cases, the acid addition salt is used as an achiral reagent which is not selected on the basis of any expected or known preference for interaction with or precipitation of a specific optical isomer of the products of this disclosure.

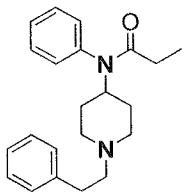
"Pharmaceutically acceptable salt" is meant to indicate those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of a patient without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. (1977) J. Pharm. Sciences, vol. 6. 1-19, which is hereby incorporated by reference in its entirety, describes pharmaceutically acceptable salts in detail.

"Modulation" is meant to refer to the binding activity of a compound with respect to a particular receptor. The binding activity of the compound, or "modulator," may be

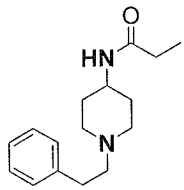
that of an agonist, inverse agonist, antagonist, allosteric regulator, positive allosteric modulator, negative allosteric modulator, or any other type of ligand-receptor interaction that is known in the art.

In this invention we disclose a new class of molecules that simultaneously bind with high affinity to opioid μ–, δ–, κ– receptors and also to α-adrenoreceptors, thereby exhibiting modulation-type interactions with those receptors. The interaction of the molecules with μ–receptors is believed to have the character of antagonist action, based at least in part on the observed high affinity binding of the molecules with respect to the μ–receptors.

Although not wishing to be bound by theory, it appears that the principal structural change for agonist-antagonist transformation is the removal of a phenyl group from an N-phenylpropionamide fragment of fentanyl. This transformation is depicted below, wherein N-(1-phenethylpiperidin-4-yl)-N-phenylpropionamide (II) is transformed to N-(1-phenethylpiperidin-4-yl)-N-propionamide (I), causing a transformation of μ–agonist properties to μ–antagonist with simultaneous modulation of delta-, kappa- and alpha-receptors:

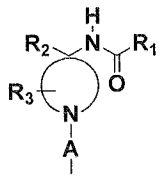


Fentanil (II)



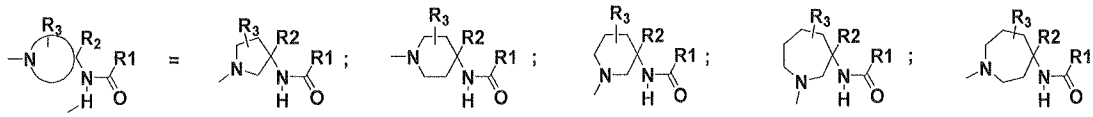
μ-Opioid Antagonist/α-agonist (I)

The invention also relates to processes for preparing compounds of general formula III, which are pharmacologically active compounds:



Ar , or (HetAr), or $COOR_5$, or $COON(R_6)_2$ (III)

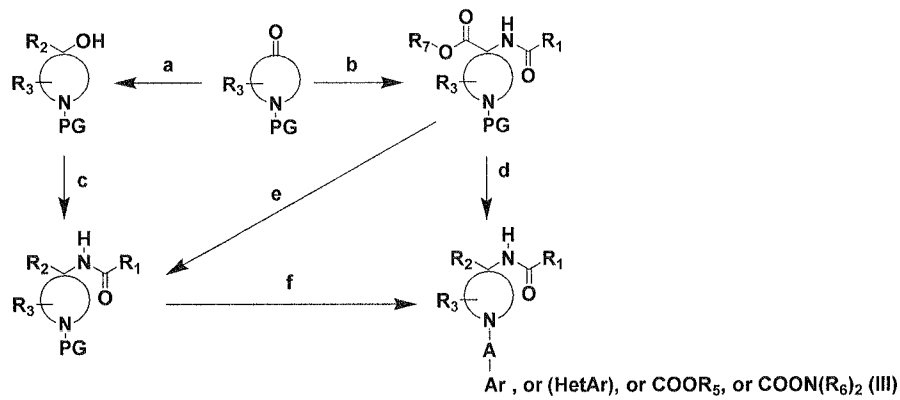
Representative compounds of the present invention also include the following compounds, wherein the central nitrogen containing ring is a substituted or unsubstituted 5- to 7-membered heterocyclic ring:



These compounds may include the following structural and functional groups:

- R_1 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkenyl, alkynyl, or substituted or unsubstituted aryl or hetaryl;
- R_2 is H, -CH_2O-C_{1-4} alkyl, etc.; COO-C_{1-4} alkyl, etc.; -$CONR_4$ etc.;
- R_3 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or unsubstituted aryl or hetaryl;
- A is substituted or un-substituted C_1-C_{10} alkyl, alkylene, alkynyl;
- Ar or HetAr is substituted or un-substituted monocyclic or polycyclic aromatic or heteroaromatic moiety; and
- $COOR_5$, or $CON(R_6)_2$, where R_5 and R_6 are H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or un-substituted aryl or hetaryl.

In certain embodiments of the invention, compounds of formula (III) can be prepared according to the following general schemes (wherein PG is a protecting group):



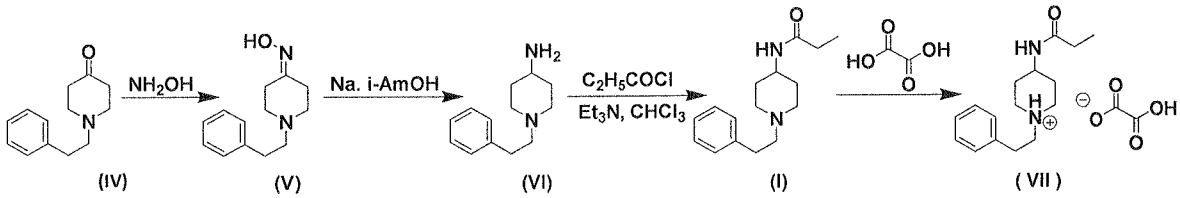
As shown above, these schemes generally include transformations of different starting cyclic ketones (e.g., a variety of piperidin-4-ones, pyrrolidin-3-ones and azepan-4-one) to desired compounds of formula (III) via, for example:

a) Grignard or Reformatsky type reactions;
b) a Strecker type reaction;
c) a Ritter type reaction;
d) selective carbalkoxy group transformations, deprotection, andfurther appropriate acylation or alkylation;

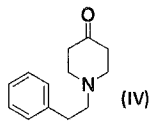
e) selective carbalkoxy group transformations; or

f) deprotection with further appropriate acylation or alkylation.

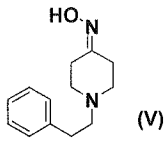
The process for the preparation of the first example of μ-opioid modulator/α-modulator N-(1-phenethylpiperidin-4-yl)propionamide and its salt is described in the present invention in detail below (Scheme 1):



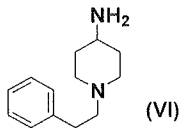
The synthetic procedure starts with the commercially available 1-phenethylpiperidin-4-one (IV) of formula:



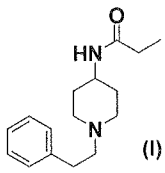
which may be reacted with hydroxylamine hydrochloride in ethanol in the presence of base, to give 1-phenethylpiperidin-4-one oxime (V) of formula:



Reducing the C=N double bond of obtained oxime (V) by the Bouveault-Blanc protocol using iso-amyl alcohol and sodium metal as a hydrogen source, 1-phenethylpiperidin-4-amine (VI) may be obtained:

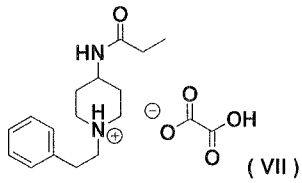


By treating of compound (VI) with propionic acid chloride in chloroform in the presence of triethylamine the desired N-(1-phenethylpiperidin-4-yl)propionamide (I) may be prepared:



(I)

The obtained N-(1-phenethylpiperidin-4-yl)propionamide (I) may be transformed to oxalate by treating with a molar equivalent of oxalic acid in ethanol:



(VII)

The tables (Tables 1-3) in the attached Appendix 1 incorporated herein by reference include data from binding assays performed with the N-(1-phenethylpiperidin-4-yl)propionamide compound (I). This data demonstrates the high binding affinities of the compounds of the invention for μ–, δ–, and κ- opioid receptors and for α2-adreno-receptors. Table 1 illustrates the results of binding assays performed with the N-(1-phenethylpiperidin-4-yl)propionamide (compounds R1 and R2) and various receptors. As can be seen in the table, at a test concentration of 1.0E-05 M, N-(1-phenethylpiperidin-4-yl)propionamide demonstrated the highest percentage binding inhibition of control specific binding with respect to the α2B adrenoreceptor (74% and 55%); the δ–opioid receptor (44% and 69%); the κ-opioid receptor (107% and 104%); the μ–opioid receptor (98% and 99%). Table 2 contains reference compound data for the various receptors used in the binding assays. Finally, Table 3 provides summary results of the binding assays, showing the receptors from Table 1 for which the test compound demonstrated the highest percentage binding inhibition of control specific binding.

Appendix 2 incorporated herein by reference includes x-ray crystallography data for a representative compound of the invention, N-(1-phenethylpiperidin-4-yl)propionamide oxalate.

Appendix 3 incorporated herein by reference includes data showing that a representative compound of the invention, N-(1-phenethylpiperidin-4-yl)propionamide, conforms to "Lipinski's rule of five," which provides a general rule of thumb for evaluating the activity of an orally administered drug.

The compounds of the present invention may be utilized in various pharmaceutical and medical applications in which the use of a compound exhibiting high binding affinities for μ–, δ– or κ– opioid receptors and/or α2-adreno-receptors is

indicated. The compounds may be of particular use in applications in which the use of a μ–, δ– or κ– opioid receptor modulator, including particularly a μ–opioid receptor antagonist, and/or an α2-adrenoreceptor modulator is indicated. Accordingly, in certain embodiments, the present invention also includes salts, and particularly pharmaceutically acceptable salts, of the disclosed compounds, as well as processes for preparing the salt forms of the disclosed compounds.

Examples

The following examples illustrate the preparation of N-(1-phenethylpiperidin-4-yl)propionamide and its oxalate salt form, N-(1-phenethylpiperidin-4-yl)propionamide oxalate, in accordance with the present disclosure.

Example 1.

1-Phenethylpiperidin-4-one oxime (Compound V)

1-Phenethylpiperidin-4-one (10.15 g (0.05 mol) dissolved in 60 mL of ethanol) was added drop-wise at 0°C to a solution of hydroxylamine in water. The water solution of hydroxylamine was preliminarily prepared by adding at 0°C in portions 13.8 g (0.1 mol) of K_2CO_3 to the solution of 6.95 g (0.1 mol) hydroxylamine hydrochloride in 50 mL of water.

The mixture was set aside for a night. Ethanol was evaporated under slight vacuum. Water (~100 mL) was added, and the mixture was stirred on ice bath for an hour. The separated solid product was filtered, washed with water and allowed to air-dry. The crude oxime (10.71 g (98.25%), m.p. 132-134°C) was reserved for use in the next reaction without further purification. Analysis with electrospray ionization mass spectrometry (MS (ESI)) resulted in a peak at 219.1 (MH+).

Example 2

1-Phenethylpiperidin-4-amine (Compound VI)

1-Phenethylpiperidin-4-one oxime (6.54 g (0.03 mol)) was dissolved in 100 mL of dry i-AmOH on heating. A ten-fold excess of sodium (6.9 g (0.3 mol)) was slowly (1 hour) added to the stirred solution in small pieces, while the temperature was maintained around 110°. The solution was stirred on heating at 110° for two hours and left to cool to room temperature. 150 mL of ether, followed by 75 mL of water, was then added to the solution. The organic layer was separated and dried on $MgSO_4$. After evaporation of solvents under slight vacuum, the product was distilled to give 4.3 g (70%) of 1-

phenethylpiperidin-4-amine (VI) with a boiling point of 138-142°/1.5mm. MS (ESI): 205.0 (MH+).

Example 3

N-(1-Phenethylpiperidin-4-yl)propionamide (Compound I)

Propionyl chloride (2.775 g (0.03 mol)) in 5.55 mL of $CHCl_3$ was added drop-wise on stirring to the cooled (0°C) solution of 4.08 g (0.02 mol) 1-phenethylpiperidin-4-amine and 3.03 g (0.03 mol) of Et_3N in 30 mL of $CHCl_3$. The mixture was left to come to room temperature and stirred overnight. After working up with 5% solution of $NaHCO_3$ (2.52 g (0.03 mol)) in 47.88 H_20, the organic layer was separated, washed with water and dried on $MgSO_4$. After evaporation of solvents under slight vacuum, the residue was crystallized from hexane to give 4.9 g (94%) of N-(1-phenethylpiperidin-4-yl)propionamide (I) with m.p.134-135°. MS (ESI): 261.2 (MH+).

- The results of proton NMR spectroscopy were as follows:
  ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27 (t, J = 7.4 Hz, 2H), 7.19 (m, 3H), 5.32 (d, J = 7.4 Hz, 1H), 3.82 (qt, J = 7.8, 4.2 Hz, 1H), 2.92 (dt, J = 11.8, 3.4 Hz, 2H), 2.79 (m*, 2H), 2.59 (m*, 2H), 2.19 (q, J = 7.5 Hz, 2H), 2.18 (m, 2H), 1.95 (dtd, J = 12.4, 4.4, 1.7 Hz, 2H), 1.46 (qd, J = 11.7, 3.8 Hz, 2H), 1.15 (t, J = 7.5 Hz, 3H).
  * these two multiplets arise from two methylene groups that have magnetically inequivalent protons AA'BB' with $^2J_{AA'} = {}^2J_{BB'} = 12$ Hz, $^3J_{AB} = 3J_{A'B'} = 11$ Hz, $^3J_{AB'} = {}^3J_{A'B} = 4$ Hz, consistent with a preferred *anti* conformation for the phenyl and piperidine rings
- The results of carbon-13 NMR spectroscopy were as follows:
  ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.0, 140.2, 128.6, 128.4, 126.0, 60.4, 52.3, 46.3, 33.7, 32.3, 29.8, 9.9.

Example 4

N-(1-Phenethylpiperidin-4-yl)propionamide oxalate (Compound VII)

Oxalic acid (1 g (0.011 mol)) in 10 mL of ethanol was added drop-wise to the solution of 2.93 g (0.011 mol) of N-(1-phenethylpiperidin-4-yl)propionamide (I) in 29.3 mL of ethanol. The mixture was set aside overnight. The obtained crystals were then separated and dried in a desiccator over P_2O_5 to give 3.5 g of N-(1-phenethylpiperidin-4-

yl)propionamide oxalate (VII) with m.p. 216-218 (MS (ESI): 261.2 ([M + H]) – see Fig. 1

The compound designated as HCV-3 was then subject to cellular functional assay and results reported below:

Cellular functional assays

Experiment Assay	Catalog Ref	Client Com	Batch	Compound Test Conce	% of Contr	% of Control Agonist Response			Reference	EC50 Ref (M)
						1st	2nd	Mean		
27/07/201 alpha 2B (h	1813	HCV-3	1	10002332	21.0E-05	7.1	9.2	5.1	7.1	dexmedetc 1.3E-08
27/07/201 kappa (KO	2071	HCV-3	1	10002332	21.0E-05	-4.2	-10.3	1.8	-4.2	U 50488 1.6E-09
27/07/201 mu (MOP)	1392	HCV-3	1	10002332	21.0E-05	33.5	28.1	38.9	33.5	DAMGO 4.2E-09

Cellular functional assays

Experiment Assay	Catalog Ref	Client Com	Batch	Compound Test Conce	% Inhibitio	Agonist Response (% of Control)			Reference	IC50 Ref (M	Kb Ref (M)
						1st	2nd	Mean			
27/07/201 alpha 2B (h	1814	HCV-3	1	10002332	21.0E-05	-28	116.8	138.7	127.8	yohimbine	3.7E-07 4.8E-08
27/07/201 kappa (KO	2072	HCV-3	1	10002332	21.0E-05	6	112.4	76.5	94.4	nor-BNI	4.3E-10 7.2E-11
27/07/201 mu (MOP)	1393	HCV-3	1	10002332	21.0E-05	-1	100.1	101.8	101.0	CTOP	2.1E-07 2.3E-08

Compound HCV-3 also was tested for hERG inhibition. Over the concentration range tested (up to 25 micromolar) no dose-response was obtained. Therefore the inhibition IC_{50} was considered as >25 micromolar. There was a hint of some inhibition at the top concentration of 25 micromolar, with 32.5% inhibition observed (insufficient to generate an IC_{50} value). As such, this compound is categorised as having weak or no hERG inhibition. The control compounds behaved as expected in the assay.

Compound HCV-3 also was tested for CYP inhibition, and was found to inhibit CYP2D6, and to weakly inhibit CYP2C19. However, with CYP2C19 the inhibition was too weak to generate an IC_{50} value, and we observed just 36.4% inhibition at the top concentration of 25 micromolar. With CYP2D6, an IC_{50} of 4.2 micromolar was observed. Thus, this compound was considered to be a moderate CYP2D6 inhibitor, and a weak CYP2C19 inhibitor. No inhibition was observed at CYP2B6, CYP2C9, CYP3A4 (with either substrate), CYP2C8 or CYP1A2. The significance of this CYP2D6 inhibition will depend on the levels of the compound *in vivo*.

Compound HCV-3 also was tested in cellular and nuclear receptor functional assays, and the results reported in Appendix 4, incorporated herein by reference.

Compound HCV-3 was also subjected to AMES testing, and the results reported in Appendix 5, incorporated herein by reference.

In summary, the compound HCV-3 was negative for genotoxicity against both strains used in this assay (TA98 and TA 100) up to a maximum tested concentration of 1mg/mL, in both the absence and presence of S9 metabolic activation. The assay controls behaved as expected.

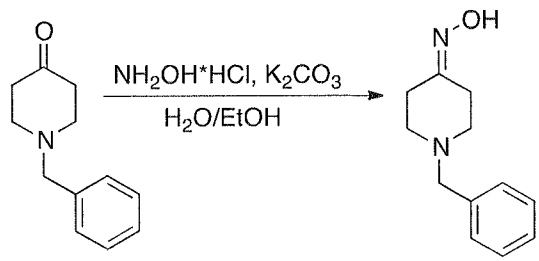
Compound HCV-3 also was subjected to in vitro metabolic disposition in mouse, rat, monkey and human microsomes. The test compound was incubated with pooled liver microsomes, since drip stability in liver microsomes can be predictive of drug stability *in vivo*. Aliquots were taken at 0, 5, 15, 30 and 45 minutes and quenched immediately. The samples were extracted and analyzed by LC-MS/MS. Compound HCV-3 was observed to have low clearance in human, monkey and mouse microsomes, and moderate clearance in rat.

Compound HCV-3 also was subjected to MDCK permeability assay. The compound was observed to be highly permeable in the MDCK assay. There was a slight difference between the plus and minus inhibitor data in terms of the efflux ratio obtained (1.48 minus inhibitor, versus 0.929 plus inhibitor). A ratio of greater than 2 generally indicates that efflux, i.e., blood brain barrier permeability, is occurring. The control compounds behaved as expected, with prazosin (a P-gp substrate) showing efflux in the absence of Cyclosporin A, which was inhibited in its presence.

Various derivatives of the above compounds with potential opiod and alpha antagonist activity were prepared as follows, and characterized by NMR and mass-spec as reported below.

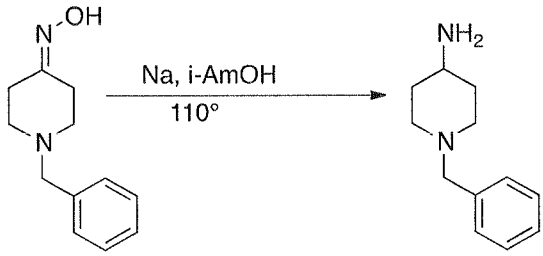
Protocols for the synthesis of substituted 1-arylethyl-4-acylaminopiperidine derivatives

Synthesis of 1-benzylpiperidin-4-one oxime



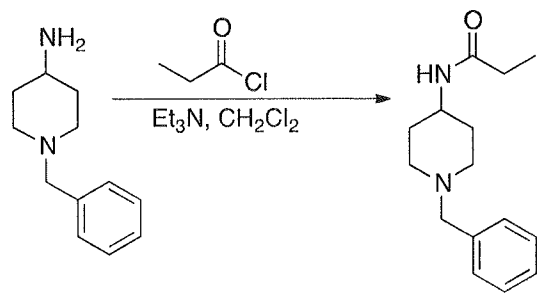
28.35 g (1 equiv., 0.15 mol) of 1-benzylpiperidin-4-one was dissolved in 60 mL of EtOH and then cooled to 0 °C using an ice bath. A solution containing 20.85 g (2 equiv., 0.30 mol) of hydroxylamine hydrochloride dissolved in 75 mL of H_2O was prepared and then added dropwise to the reaction mixture followed by dropwise addition of a solution containing 20.7 g (1 equiv., 0.15 mol) of K_2CO_3 dissolved in 75 mL of H_2O. The reaction mixture was then brought to room temperature and stirred overnight. The EtOH was then removed via rotary evaporation and the reaction mixture was then cooled in an ice bath to allow the product to crystallize out of solution. The product was filtered and washed several times with H_2O and recrystallized in EtOH. Yield: **27.78 g** (70.17%).

Synthesis of 1-benzylpiperidin-4-amine



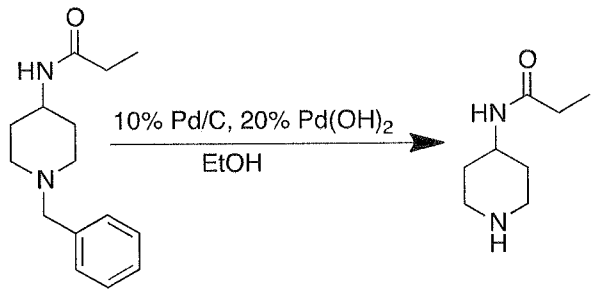
A solution containing 6.12 g (1 equiv., 0.03 mol) of 1-benzylpiperidin-4-one oxime dissolved in 90 mL of iso-amyl alcohol was prepared and heated to approximately 110 °C. 6.9 g (10 equiv., 0.3 mol) of Na metal was then added slowly to the reaction mixture. After addition of Na, the reaction mixture was allowed to cool to room temperature and stirred until the reaction mixture turned into a thick slurry. The slurry was dissolved in 50 mL of ethyl acetate and 25 mL of H_2O. The organic layer was separated and washed with H_2O (2 × 20 mL) followed by drying over anhydrous magnesium sulfate. The solvent was removed via rotary evaporation, resulting in a yellow oil. The crude product was purified via column chromatography utilizing silica gel and a DCM:MeOH solvent system in a ratio of 4:1 with an additional 1% of Et_3N. Yield: **3.7 g** (64%).

***Synthesis of N*-(1-benzylpiperidin-4-yl)propionamide**



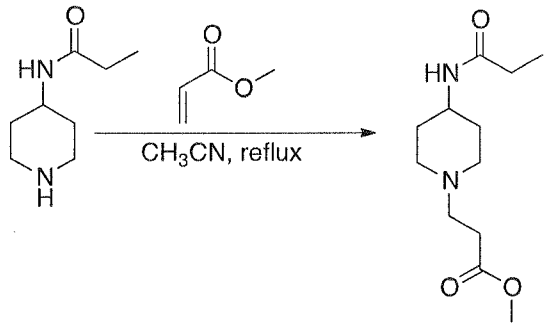
A solution of 3.7 g of 1-benzylpiperidin-4-amine (1 equiv., 0.019 mol) dissolved in 45 mL of dry dichloromethane was prepared followed by the addition of 5 mL of Et_3N (2.6 equiv., 0.05 mol). The reaction mixture was then cooled to 0 °C using an ice bath, and then 2.17 mL (1.3 equiv., 0.025 mol) of propionyl chloride dissolved in 10 mL of dry dichloromethane was added dropwise to the reaction mixture. The reaction mixture was then warmed to room temperature and stirred overnight. Once the reaction was complete, 4 mL of NH_4OH and 45 mL of H_2O were added to the reaction mixture. The organic layer was separated, and the aqueous layer was washed with dichloromethane (3 × 20 mL) followed by $NaHCO_3$ solution and brine. The organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed via rotary evaporation, resulting in a white solid. The product was washed with hexanes to obtain an analytically pure sample. Yield: **2.8 g** (72%)

***Synthesis of N*-(piperidin-4-yl)propionamide**



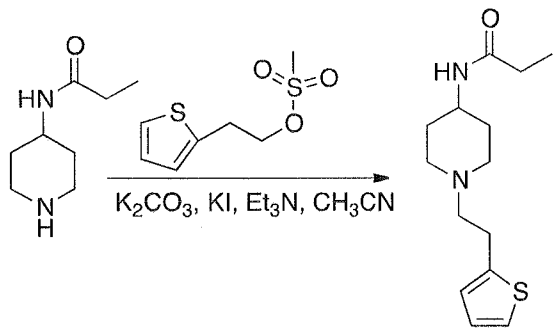
0.7 g of *N*-(1-benzylpiperidin-4-yl)propionamide (1 equivalent, 0.003 moles) were added to a parr hydrogenation flask and dissolved in 30 mL of EtOH. The solution was then degassed with argon for 30 min followed by the addition of 0.07 g of 10% Pd/C (0.2 equiv., 6.58×10^{-4} mol) and 0.07 g of 20% $Pd(OH)_2$ (0.17 equiv., 4.98×10^{-4} mol). The black solution was then degassed with argon for an additional 15 min. The reaction mixture was then charged with 50 psi of H_2 gas and shaken for 24 h. The product was filtered through celite and the solvent was removed via rotary evaporation. No further purification was required. Yield: **0.467 g** (99%)

Synthesis of methyl 3-(4-propionamidopiperidin-1-yl)propanoate (CRA5)



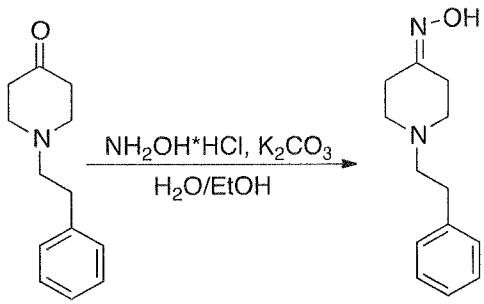
0.1 g of *N*-(piperidin-4-yl)propionamide (1 equiv., 5.26×10^{-4} moles) was dissolved in 2 mL of dry acetonitrile followed by the addition of 0.071 mL of methyl acrylate (1.5 equiv., 7.89×10^{-4} mol). The reaction mixture was refluxed overnight. The solvent was removed via rotary evaporation. The crude product was purified by washing with hexanes followed by drying under high vacuum. Yield: **0.90 g** (71%)

Synthesis of N-(1-(2-(thiophen-2-yl)ethyl)piperidin-4-yl)propionamide (CRAS1)



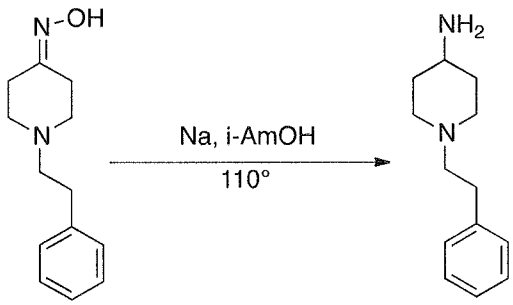
0.1 g of *N*-(piperidin-4-yl)propionamide (1 equiv., 6.40×10^{-4} mol), 0.145 g of 2-(thiophen-2-yl)ethyl methanesulfonate (1.1 equiv., 7.04×10^{-4} moles), 0.097 g of K_2CO_3 (1.1 equiv., 7.04×10^{-4} mol), 0.032 g of KI (1.92×10^{-4} mol), and 0.178 mL of Et_3N (2 equiv., 1.28×10^{-3} mol) were added to a round bottom flask and dissolved in 5 mL of dry acetonitrile. The reaction mixture was stirred and refluxed overnight. The solvent was then removed via rotary evaporation followed by the addition of H_2O. The mixture was extracted with ethyl acetate (3 × 5 mL), and the organic extracts were combined and dried over anhydrous magnesium sulfate. The solvent was removed via rotary evaporation. The crude product was washed with hexanes to obtain an analytically pure sample. Yield: **0.101 g** (60%).

Synthesis of 1-phenethylpiperidin-4-one oxime



28.35 g (1 equivalent, 0.14 mol) of 1-benzylpiperidin-4-one were dissolved in 60 mL of EtOH and then cooled to 0° C using an ice bath. A solution containing 19.46 g (2 equivalents, 0.28 mol) of hydroxylamine hydrochloride dissolved in 75 mL of H_2O was prepared and then added dropwise to the reaction mixture followed by dropwise addition of a solution containing 19.35 g (1 equivalent, 0.14 mol) of K_2CO_3 dissolved in 75 mL of H_2O. The reaction mixture was then brought to room temperature and stirred overnight. The EtOH was then removed via rotary evaporation and the reaction mixture was then cooled in an ice bath to allow the product to crystallize out of solution. The product was filtered and washed several times with H_2O and recrystallized in EtOH. Yield: **25.60 g** (83.77%).

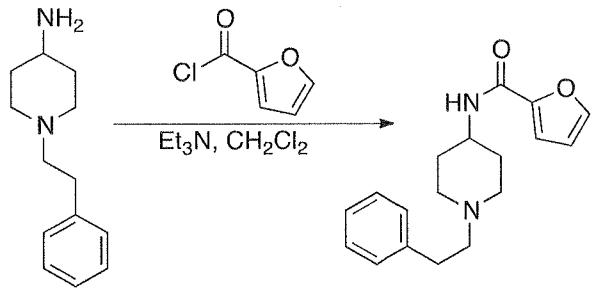
Synthesis of 1-phenethylpiperidin-4-amine



A solution containing 6.00 g (1 equiv., 0.027 mol) of 1-phenethylpiperidin-4-one oxime dissolved in 90 mL of iso-amyl alcohol was prepared and heated to approximately 110 °C. 6.21 g (10 equiv., 0.27 mol) of Na metal was then added slowly to the reaction mixture. After addition of Na, the reaction mixture was allowed to cool to room temperature and stirred until the reaction mixture turned into a thick slurry. The slurry was dissolved in 50 mL of ethyl acetate and 25 mL of H_2O. The organic layer was separated and washed with H_2O (2 × 20 mL) followed by drying over anhydrous magnesium sulfate. The solvent was removed via rotary evaporation, resulting in a yellow oil. The crude product was purified via column chromatography utilizing silica

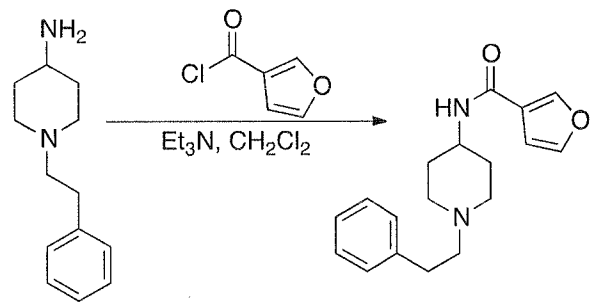
gel and a DCM:MeOH solvent system in a ratio of 4:1 containing an additional 1% of Et_3N.

Synthesis of N-(1-phenethylpiperidin-4-yl)furan-2-carboxamide (CRA8)



A solution of 0.1 g of 1-phenethylpiperidin-4-amine (1 equiv., 4.89×10^{-4} mol) dissolved in 2 mL of dry dichloromethane was prepared followed by the addition of 0.178 mL (2.6 equiv., 1.27×10^{-3} moles) of Et_3N. The reaction mixture was then cooled to 0 °C using an ice bath, and then 0.063 mL (1.3 equiv., 6.36×10^{-4} mol) of 2-furoyl chloride dissolved in 0.25 mL of dry dichloromethane was added dropwise to the reaction mixture. The reaction mixture was then warmed to room temperature and stirred overnight. Once the reaction was complete, 4 mL of NH_4OH and 45 mL of H_2O were added to the reaction mixture. The organic layer was separated, and the aqueous layer was washed with dichloromethane (3 × 5 mL) followed by $NaHCO_3$ solution and brine. The organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed via rotary evaporation, resulting in a white solid. The product was washed with hexanes to obtain an analytically pure sample. Yield: **0.0845 g** (58.3 %).

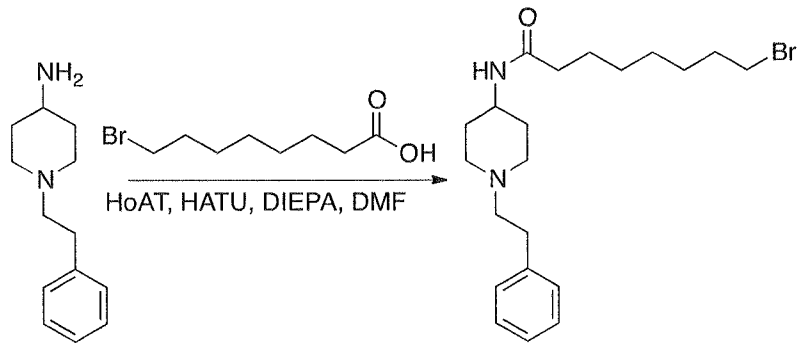
Synthesis of N-(1-phenethylpiperidin-4-yl)furan-3-carboxamide (CRA9)



A solution of 0.1 g of 1-phenethylpiperidin-4-amine (1 equiv., 4.89×10^{-4} mol) dissolved in 2 mL of dry dichloromethane was prepared followed by the addition of 0.178 mL (2.6 equiv., 1.27×10^{-3} mol) of Et_3N. The reaction mixture was then cooled to 0 °C using an ice bath, and then 0.063 mL (1.3 equiv., 6.36×10^{-4} mol) of 3-furoyl chloride dissolved in 0.25 mL of dry dichloromethane was added dropwise to the reaction mixture. The

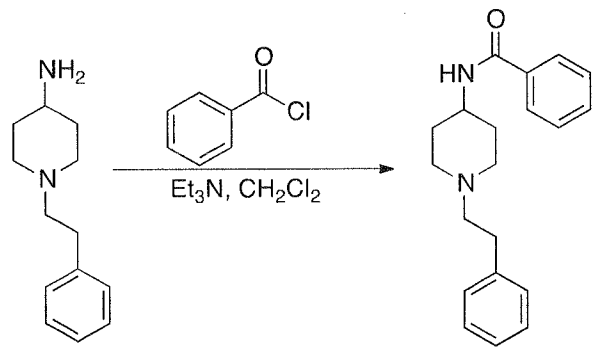
reaction mixture was then warmed to room temperature and stirred overnight. Once the reaction was complete, 4 mL of NH_4OH and 45 mL of H_2O were added to the reaction mixture. The organic layer was separated, and the aqueous layer was washed with dichloromethane (3 × 5 mL) followed by $NaHCO_3$ solution and brine. The organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed via rotary evaporation, resulting in a white solid. The product was washed with hexanes to obtain an analytically pure sample. Yield: **0.104 g** (72%).

Synthesis of 8-bromo-N-(1-phenethylpiperidin-4-yl)octanamide (CRA10)



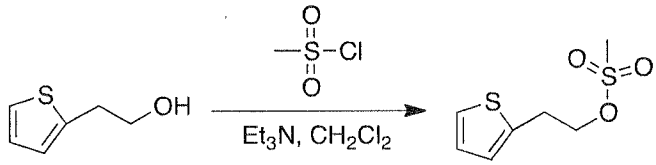
A solution of 0.101 g of 1-phenethylpiperidin-4-amine (1.1 equiv., 4.93×10^{-4} mol), 0.1 g of 8-bromooctanoic acid (1.0 equiv., 4.48×10^{-4} mol), 0.170 g of HATU (1.0 equiv., 4.48×10^{-4} mol), 0.061 g of HOAt (1.0 equiv., 4.48×10^{-4} mol), and 0.314 mL of DIEPA (4.0 equiv., 0.0018 mol) in dry DMF was prepared. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then quenched with 0.5 M $KHSO_4$ solution followed by the addition of dichloromethane. The organic and aqueous layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 5 mL) followed by washing with $NaHCO_3$ solution and Brine. The organic extracts were then dried over anhydrous magnesium sulfate.

Synthesis of N-(1-phenethylpiperidin-4-yl)benzamide (CRA11)



A solution of 0.1 g of 1-phenethylpiperidin-4-amine (1 equiv., 4.89×10^{-4} mol) dissolved in 2 mL of dry dichloromethane was prepared followed by the addition of 0.178 mL (2.6 equiv., 1.27×10^{-3} mol) of Et_3N. The reaction mixture was then cooled to 0 °C using an ice bath, and then 0.074 mL (1.3 equiv., 6.36×10^{-4} mol) of 3-furoyl chloride dissolved in 0.25 mL of dry dichloromethane was added dropwise to the reaction mixture. The reaction mixture was then warmed to room temperature and stirred overnight. Once the reaction was complete, 4 mL of NH_4OH and 45 mL of H_2O were added to the reaction mixture. The organic layer was separated, and the aqueous layer was washed with dichloromethane (3 × 5 mL) followed by $NaHCO_3$ solution and brine. The organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed via rotary evaporation, resulting in a white solid. The product was washed with hexanes to obtain an analytically pure sample.

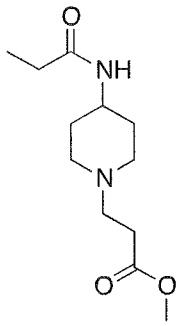
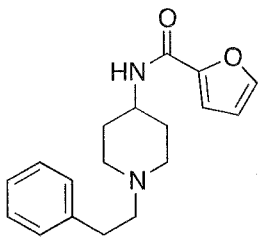
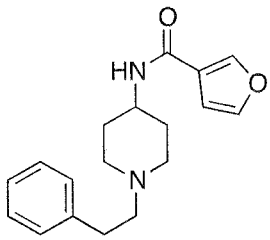
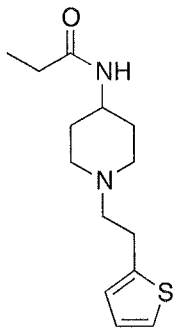
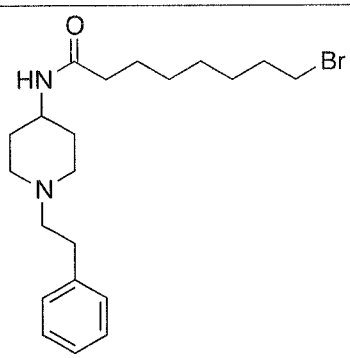
Synthesis of 2-(thiophen-2-yl)ethyl methanesulfonate

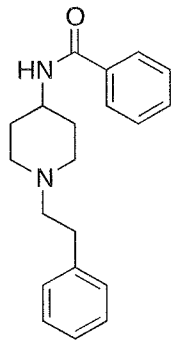


2.6 mL of 2-(thiophen-2-yl)ethanol (1 equiv., 0.023 mol) was dissolved in 45 mL of dry dichloromethane followed by the addition of 3.63 mL of Et_3N (1.13 equiv., 0.026 mol). The reaction mixture was stirred at room temperature for 1 h. It was then cooled to -5 °C using an ice bath and solid NaCl. Once cooled, 1.92 mL of methanesulfonyl chloride was added dropwise over the course of 10 min. The reaction mixture was then warmed to room temperature and stirred for 1 h. Once the reaction was complete, 30 mL of $NaHCO_3$ solution was added followed by separation of the organic and aqueous layers. The aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed via rotary evaporation, resulting in a brown oil. No further purification was required.

NMR (^1H & ^{13}C) & mass-spec data of novel substituted 1-arylethyl-4-acylaminopiperidine derivatives (total six compounds)

Compound	Molecular Formula	Structure	R_f	Exact Mass	Observed Mass $[M+H]^+$	Yield

CRA5	$C_{12}H_{22}N_2O_3$		0.72 (20% MeOH in DCM)	242.16	243.3	90 mg (70.7%)
CRA8	$C_{18}H_{22}N_2O_2$		0.825 (4:1 MeOH DCM)	298.17	299.3	84.5 mg (58.3%)
CRA9	$C_{18}H_{22}N_2O_2$		0.757 (4:1 MeOH DCM	298.17	299.3	104.3 mg (71.9 %)
CRAS1	$C_{14}H_{22}N_2OS$			266.15	267.73	101.4 mg (60%)
CRA10	$C_{21}H_{33}BrN_2O$		0.90	408.18	409.19 411.19	262 mg (130%)

CRA11	$C_{20}H_{24}N_2O$		0.875	308.19	309.2	132 mg (87%)

CRA5 NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.14 (t, J= 7.59, 7.59 Hz, 3H), 1.41 (dtd, J=3.70, 11.10, 11.13, 12.61 Hz, 2H), 1.91 (m, 2H), 2.17 (m, 4 H), 2.49 (m, 2H), 2.68 (m, 2H), 2.81 (m, 2H), 3.67 (s, 3H), 3.78 (dddd, J=4.29, 4.36, 11.92, 15.26, 1H), 5.25 (d, J= 7.96 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 10.02, 29.99, 32.41, 46.40, 51.76, 52.25, 55.63, 173.14, 174.05.

CRA8 NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.63 (d, J=11.93 Hz, 2H), 2.05 (d, J=12.33 Hz, 2H), 2.26 (t, J=11.35, 11.35 Hz, 2H), 2.64 (dd, J=6.16, 10.21 Hz, 2H), 2.83 (dd, J=6.12, 10.24 Hz, 2H), 2.99 (d, J=12.01 Hz, 2H), 3.98 (m, 1H), 6.21 (d, J=8.20 Hz, 1H), 6.49 (dd, J=1.79, 3.47 Hz, 1H), 7.10 (dd, J=0.84, 3.47 Hz, 1H), 7.24 (m, 5H), 7.43 (dd, 0.84, 1.77 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 32.67, 34.17, 46.27, 46.61, 52.76, 60.88, 112.60, 114.57, 126.56, 128.87, 129.13, 140.58, 144.16, 148.48, 158.13.

CRA9 NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.63 (m, 2H), 2.06 (d, J=11.35 Hz, 2H), 2.26 (m, J=11.35, 2H), 2.65 (m, 2H), 2.84 (m, 2H), 3.02 (d, J=11.86 Hz, 2H), 3.99 (m, 1H), 5.65 (d, J=7.99 Hz, 1H), 6.59 (dd, J=0.91, 1.93 Hz, 1H), 7.24 (m, 5H), 7.42 (dd, 1.58, 1.91 Hz, 1H), 7.91 (dd, J=0.90, 1.59 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 32.04, 33.49, 46.43, 52.35, 60.26, 108.25, 122.66, 126.19, 128.46, 128.68, 139.88, 143.72, 144.69, 161.95

CRA1S NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.15 (t, J=7.58, 7.58 Hz, 3H), 1.47 (m, 2H), 1.95 (m, 2H), 2.18 (m, 4H), 2.64 (dd, J=6.87, 8.62 Hz, 2H), 2.90 (m, 2H), 3.00 (m, 2H), 3.82 (dddd, J=4.20, 8.31, 10.86, 15.17 Hz, 1H), 5.32 (d, J=7.95 Hz, 1H), 6.81 (dq, J=1.02, 1.02, 1.02, 3.20 Hz, 1H), 6.91 (dd, J=3.39, 5.14 Hz, 1H), 7.11 (dd, J=1.21, 5.13 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 10.04, 28.06, 30.03, 32.15, 46.52, 52.42, 59.98, 123.62, 124.71, 126.69, 142.87, 173.15.

CRA10 NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.32-2.34 (m, 14H), 2.82-3.01 (m, 11H), 3.16 (dd, J=6.53, 10.91 Hz, 1H), 3.49 (d, J=11.91 Hz, 1H), 4.63 (dt, J=5.61, 5.61, 8.76 Hz, 1H), 7.24 (m, 4H), 7.42 (dd, J=4.46, 8.37, 1H), 8.02 (m, 1H). **^{13}C NMR** (100

MHz, $CDCl_3$) δ 25.17, 25.45, 27.85, 28.70, 28.75, 31.46, 36.55, 38.61, 81.47, 120.73, 128.66, 128.86, 129.19, 151.29, 162.71, 173.62.

CRA11 NMR data: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.35 (t, J=7.29, 7.29 Hz, 2H), 1.64 (qd, J=3.80, 11.29, 11.29, 11.35 Hz, 2H), 2.08 (m, 2H), 2.27 (td, J=2.57, 11.61, 11.65 Hz, 2H), 2.64 (m, 2H), 2.83 (m, 2H), 3.00 (m, 3H), 4.04 (dddd, J=4.28, 8.29, 10.85, 15.24 Hz, 1H), 6.04 (d, J=7.94 Hz, 1H), 7.25 (m, 5H), 7.44 (m, 3H), 7.75 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 32.22, 33.71, 45.83, 46.97, 52.38, 60.42, 114.25, 126.12, 126.86, 128.42, 128.56, 128.68, 131.42, 134.75, 140.11, 166.88

Many other variations and modifications may be made to the above-described embodiments of the disclosure without departing substantially from the spirit and principles of the disclosure. All such modifications and variations are intended to be included herein within the scope of the present disclosure and protected by the following claims.

Appendix 1

Docket No. UA 15-023

Table 1

Binding Assays

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
α_2 (non-selective)			
8255-1	RSA101c	1.0E-05	4
8255-2	R1	1.0E-05	33
8255-3	R2	1.0E-05	24
α_{2B}			
8255-1	RSA101c	1.0E-05	-21
8255-2	R1	1.0E-05	74
8255-3	R2	1.0E-05	55
BZD (central)			
8255-1	RSA101c	1.0E-05	6
8255-2	R1	1.0E-05	17
8255-3	R2	1.0E-05	12
CGRP *(h)*			
8255-1	RSA101c	1.0E-05	-7
8255-2	R1	1.0E-05	-8
8255-3	R2	1.0E-05	3
CB_1 *(h)*			
8255-1	RSA101c	1.0E-05	-3
8255-2	R1	1.0E-05	-3
8255-3	R2	1.0E-05	12
CB_2 *(h)*			
8255-1	RSA101c	1.0E-05	5
8255-2	R1	1.0E-05	15
8255-3	R2	1.0E-05	25
$GABA_B$			
8255-1	RSA101c	1.0E-05	6
8255-2	R1	1.0E-05	-1
8255-3	R2	1.0E-05	-11
Galanin (non-selective)			
8255-1	RSA101c	1.0E-05	-191
8255-2	R1	1.0E-05	-30
8255-3	R2	1.0E-05	-30
MC_1			
8255-1	RSA101c	1.0E-05	6
8255-2	R1	1.0E-05	7
8255-3	R2	1.0E-05	13
MC_4 *(h)*			
8255-1	RSA101c	1.0E-05	46
8255-2	R1	1.0E-05	1
8255-3	R2	1.0E-05	2
NK_1 *(h)*			
8255-1	RSA101c	1.0E-05	-7
8255-2	R1	1.0E-05	11
8255-3	R2	1.0E-05	6
δ (DOP)			

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
8255-1	RSA101c	1.0E-05	98
8255-2	R1	1.0E-05	44
8255-3	R2	1.0E-05	69
κ (KOP)			
8255-1	RSA101c	1.0E-05	79
8255-2	R1	1.0E-05	107
8255-3	R2	1.0E-05	104
μ *(h)* (MOP)			
8255-1	RSA101c	1.0E-05	100
8255-2	R1	1.0E-05	98
8255-3	R2	1.0E-05	99
ORL1 *(h)* (NOP)			
8255-1	RSA101c	1.0E-05	4
8255-2	R1	1.0E-05	22
8255-3	R2	1.0E-05	32
TXA_2/PGH_2 *(h)* (TP)			
8255-1	RSA101c	1.0E-05	13
8255-2	R1	1.0E-05	14
8255-3	R2	1.0E-05	18
P2Y			
8255-1	RSA101c	1.0E-05	-3
8255-2	R1	1.0E-05	-1
8255-3	R2	1.0E-05	-1
σ (non-selective)			
8255-1	RSA101c	1.0E-05	33
8255-2	R1	1.0E-05	104
8255-3	R2	1.0E-05	101
K^+_V channel			
8255-1	RSA101c	1.0E-05	-1
8255-2	R1	1.0E-05	-1
8255-3	R2	1.0E-05	0
NE transporter *(h)*			
8255-1	RSA101c	1.0E-05	-7
8255-2	R1	1.0E-05	-16
8255-3	R2	1.0E-05	29
GABA transporter			
8255-1	RSA101c	1.0E-05	5
8255-2	R1	1.0E-05	-1
8255-3	R2	1.0E-05	17

Table 2

Binding Assays

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
α_2 (non-selective) yohimbine	4.7E-08	2.0E-08	*1.1*
α_{2B} yohimbine	9.1E-09	3.6E-09	*1.3*
BZD (central) diazepam	2.1E-08	1.8E-08	*1.1*
CGRP *(h)* hCGRPα	4.0E-11	2.0E-11	*1.0*
CB_1 *(h)* WIN 55212-2	2.4E-08	1.8E-08	*1.2*
CB_2 *(h)* WIN 55212-2	2.9E-09	1.0E-09	*0.9*
$GABA_B$ baclofen	1.5E-07	8.1E-08	*1.2*
Galanin (non-selective) galanin galanin	5.1E-10 4.5E-10	3.4E-10 3.0E-10	*1.1* *2.1*
MC_1 NDP-α-MSH	2.6E-10	1.3E-10	*1.1*
MC_4 *(h)* NDP-α-MSH	3.5E-10	2.9E-10	*1.0*
NK_1 *(h)* [Sar^9,$Met(O_2)^{11}$]-SP	5.8E-10	2.6E 10	*0.6*
δ (DOP) DPDPE	3.2E-09	1.2E-09	*1.0*
κ (KOP) U 50488	1.5E-09	4.9E-10	*2.0*
μ *(h)* (MOP) DAMGO	2.1E-09	7.6E-10	*0.7*
ORL1 *(h)* (NOP) nociceptin	7.6E-09	3.4E-09	*1.5*
TXA_2/PGH_2 *(h)* (TP) U 44069 U 44069	1.4E-07 3.9E-07	9.0E-08 2.5E-07	*0.7* *0.6*
P2Y dATPαS	2.4E-08	1.2E-08	*0.8*
σ (non-selective) haloperidol	7.9E-08	6.2E-08	*0.9*
K^+_V channel α–dendrotoxin	7.7E-10	6.1E-10	*2.8*
NE transporter *(h)* protriptyline	1.0E-08	7.9E-09	*1.1*
GABA transporter nipecotic acid	2.8E-06	2.7E-06	*0.9*

Table 3

Binding Assays

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
μ *(h)* (MOP)			
8255-2	R1	1.0E-05	98
8255-3	R2	1.0E-05	99
κ (KOP)			
8255-2	R1	1.0E-05	107
8255-3	R2	1.0E-05	104
σ (non-selective)			
8255-2	R1	1.0E-05	104
8255-3	R2	1.0E-05	101
α_{2B}			
8255-2	R1	1.0E-05	74
8255-3	R2	1.0E-05	55

Appendix 2

Docket No. UA 15-023

X-ray Diffraction Facility
Department of Chemistry and Biochemistry
The University of Arizona

Your code: OxEtOH **Our code**: rv101

Comment

The crystals submitted were small needles which diffracted poorly. Reflections were streaky, indicating that the crystal was not single but comprised of more than one not-exactly-oriented component. Diffraction was observed only to about 1 Å resolution. Because of the poor resolution, A and B level checkcif alerts are generated. The quality of the structure is such that the identity of the molecule and its conformation is confirmed, but derived parameters (bond distances, angles, thermal motion) are not reliable.

Nevertheless, the structure could be determined and refined, and the molecule is shown in Figure 1. Figure 2 shows the contents of the unit cell, which also includes an oxalate molecule.

Figure 3 shows the unit cell viewed down the crystallographic *a* axis. The hydrogen bonding network in the unit cell connects the organic molecule to oxalate along the crystallographic b axis (ydrogen bonds between the N1 and O2 of the oxalate and between N2 and O4 of the oxalate). The oxalate molecule are connected by hydrogen bonds O4 and O5 of the oxalate, along the crystallographic a axis.

C8 has been modeled with disorder in two positions. There is unmodeled (probably rotational) disorder in the aromatic ring (C11-C16). Both distance and planarity restraints were applied to this ring during refinement. Not all hydrogen atoms were visible in the electron density map. Because of the low resolution and streaky diffraction pattern, the structure could not be refined without constraints. Constraints used in the refinement are included at the end of the report.

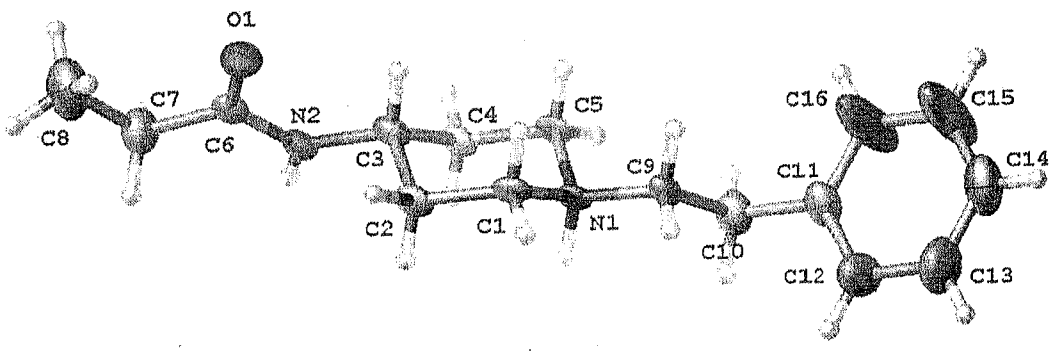


Figure 1. The molecule with displacement ellipsoids at the 50% probability level. C8 is disordered, the minor component has been removed for clarity. An oxalate molecule also in the unit cell is not shown in this figure.

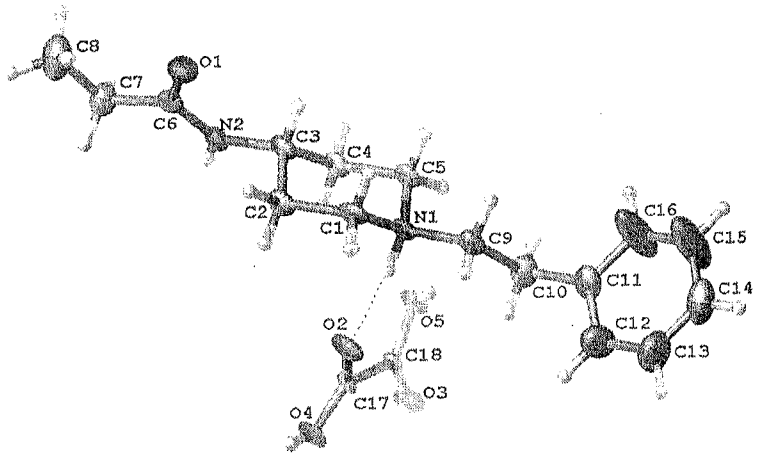


Figure 2. The contents of the asymmetric unit with displacement ellipsoids at the 50% probability level. B8 is disordered, and the minor compont is not shown. Fog has been added to show depth.

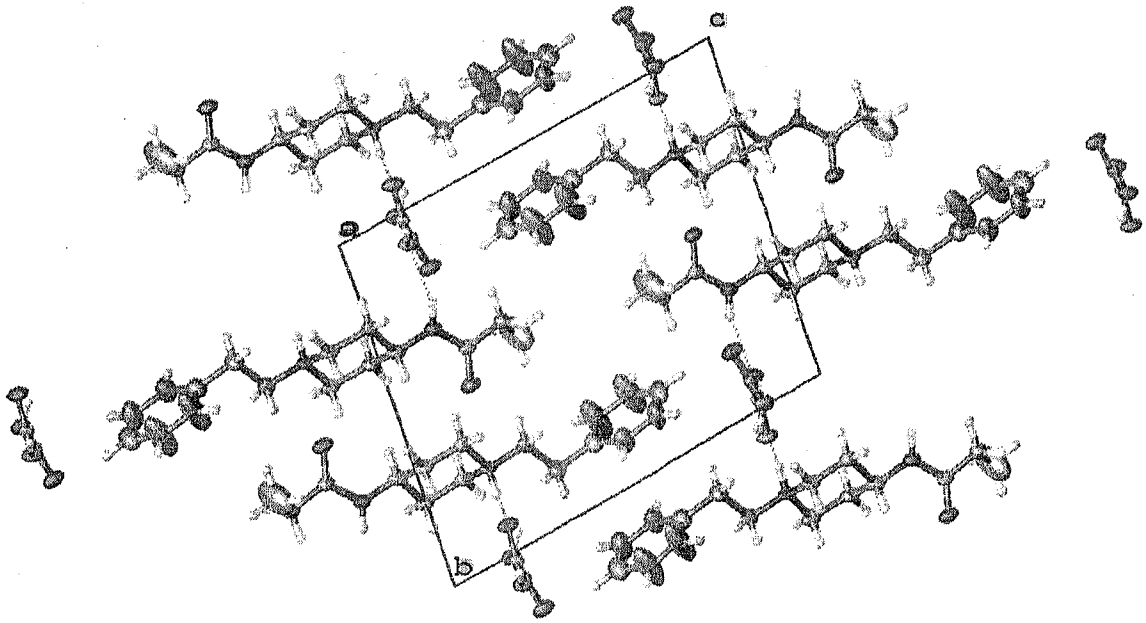


Figure 3. The contents of the unit cell viewed down the short *a* axis. Hydrogen bonds are shown between the N1 and O2 of the oxalate. Hydrogen bonds also exist between N2 and O4 of the oxalate and between O4 and O5 of the oxalate, connecting the oxalate molecules along the crystallographic a axis (looking down into the page).

Acknowledgement:

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References:

APEX2 (data collection)
Bruker (2007). *APEX2*. Bruker AXS Inc., Madison, Wisconsin, USA.

SAINT (integration and reduction)
Bruker (2007). *SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.

SADABS (absorption correction)
Sheldrick, G. M. (1996). SADABS. University of Göttingen, Germany.

SHELXTL (structure solution and refinement)
Sheldrick, G. M. (2008). *Acta Cryst.* **A64**, 112–122.

MERCURY (molecular graphics – hydrogen bonding and packing)
Macrae, C. F., Bruno, I. J., Chisholm, J. A., Edgington, P. R., McCabe, P. Pidcock, E., Rodriguez-Monge, L. Taylor, R., van de Streek, J. & Wood, P. A. (2008). *J. Appl. Cryst.* **41**, 466-470.

PLATON
Spek, A. L. (2003). *J. Appl. Cryst.* **36**, 7–13.

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Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K., Puschmann H. (2008), *J. Appl. Cryst.* **42**, 339-341.

Table 1 Crystal data and structure refinement

Identification code	rv101
Empirical formula	$C_{18}H_{27}N_2O_5$
Formula weight	351.41
Temperature/K	150.0
Crystal system	triclinic
Space group	P-1
a/Å	5.701(3)
b/Å	12.024(6)
c/Å	14.105(7)
α/°	100.473(14)
β/°	93.997(14)
γ/°	95.571(15)
Volume/Å^3	942.5(8)
Z	2
ρ_{calc}g/cm^3	1.238
μ/mm^{-1}	0.090
F(000)	378.0
Crystal size/mm^3	0.3 × 0.08 × 0.05
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	2.948 to 41.622
Index ranges	$-5 \leq h \leq 5$, $-11 \leq k \leq 11$, $-14 \leq l \leq 14$
Reflections collected	7408
Independent reflections	1965 [R_{int} = 0.0625, R_{sigma} = 0.0684]
Data/restraints/parameters	1965/237/222
Goodness-of-fit on F^2	1.592
Final R indexes [I>=2σ (I)]	R_1 = 0.0970, wR_2 = 0.2383
Final R indexes [all data]	R_1 = 0.1319, wR_2 = 0.2545
Largest diff. peak/hole / e Å^{-3}	0.57/-0.46

Table 2 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$)
U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O4	2499(7)	898(4)	1556(3)	26.4(12)
O5	-3506(7)	78(4)	1467(3)	28.1(12)
O3	-1621(7)	1811(4)	1840(3)	34.3(13)
O2	605(7)	-782(4)	1662(3)	29.6(13)
N1	-2989(8)	-2451(4)	1622(3)	20.6(13)
N2	-2795(9)	-3369(4)	-1448(4)	25.0(14)
C2	-1073(11)	-3368(6)	206(5)	26.8(16)
O1	-2673(8)	-5260(4)	-1872(3)	35.3(13)
C1	-1627(11)	-3398(5)	1239(5)	27.3(17)
C3	-3356(11)	-3435(5)	-460(5)	26.4(16)
C9	-3409(12)	-2486(6)	2654(4)	28.0(17)
C4	-4744(11)	-2458(6)	-50(5)	25.9(16)
C18	-1623(11)	803(6)	1645(4)	16.6(14)
C5	-5248(10)	-2510(6)	986(4)	22.8(16)
C17	690(11)	242(6)	1604(4)	16.3(14)
C7	-1722(13)	-4117(6)	-3046(5)	38.1(19)
C10	-4412(13)	-1434(6)	3188(5)	36.6(19)
C6	-2419(11)	-4299(6)	-2080(5)	24.0(16)
C11	-4740(9)	-1569(5)	4222(3)	39.1(18)
C12	-2894(8)	-1194(5)	4941(4)	62(3)
C13	-3168(9)	-1341(5)	5883(3)	76(3)
C14	-5288(10)	-1864(5)	6107(3)	59(2)
C15	-7134(8)	-2239(6)	5388(4)	120(5)
C16	-6860(8)	-2091(6)	4445(4)	114(5)
C8	-1320(80)	-5210(40)	-3670(30)	66(3)
C8A	680(20)	-4565(10)	-3240(8)	66(3)

Table 3 Anisotropic Displacement Parameters ($Å^2 \times 10^3$).
The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O4	7(2)	29(3)	48(3)	18(2)	4(2)	7.9(19)
O5	10(2)	29(3)	47(3)	13(2)	4(2)	2.6(19)
O3	20(3)	26(3)	57(3)	6(2)	6(2)	4(2)
O2	14(2)	23(2)	57(3)	15(2)	9(2)	7.7(19)
N1	9(3)	21(3)	34(3)	11(2)	-1(2)	5(2)
N2	18(3)	24(3)	36(3)	11(2)	7(2)	5(2)
C2	17(3)	24(4)	43(4)	12(3)	5(3)	11(3)
O1	37(3)	23(3)	48(3)	9(2)	5(2)	6(2)
C1	12(3)	26(4)	43(4)	6(3)	-1(3)	4(3)
C3	16(3)	27(4)	40(3)	13(3)	4(3)	4(3)
C9	17(4)	36(4)	33(3)	15(3)	0(3)	3(3)
C4	17(4)	29(4)	36(3)	11(3)	7(3)	7(3)
C18	9(2)	20(3)	22(3)	7(2)	3(2)	2(2)
C5	9(3)	26(4)	36(3)	13(3)	-1(3)	6(3)
C17	7(2)	23(3)	22(3)	9(2)	5(2)	4(2)
C7	47(4)	39(4)	35(4)	14(3)	7(3)	15(4)
C10	37(4)	41(4)	38(4)	14(3)	6(3)	18(4)
C6	13(4)	26(3)	36(3)	9(3)	0(3)	8(3)
C11	32(4)	49(5)	43(4)	16(3)	9(3)	20(3)
C12	60(5)	81(7)	43(4)	17(4)	-1(3)	-12(5)
C13	88(6)	98(7)	39(4)	13(4)	4(4)	2(5)
C14	66(5)	72(6)	50(4)	20(4)	19(3)	42(4)
C15	55(5)	238(14)	77(5)	77(6)	11(4)	-20(7)
C16	40(5)	238(14)	72(5)	78(7)	-6(4)	-29(6)
C8	72(7)	78(7)	59(6)	17(5)	26(5)	35(6)
C8A	72(7)	78(7)	59(6)	17(5)	26(5)	35(6)

Table 4 Bond Lengths

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O4	C17	1.250(7)	C9	C10	1.532(9)
O5	C18	1.295(7)	C4	C5	1.519(8)
O3	C18	1.194(7)	C18	C17	1.538(9)
O2	C17	1.245(7)	C7	C6	1.493(9)
N1	C1	1.482(7)	C7	C8	1.49(4)
N1	C9	1.499(8)	C7	C8A	1.542(13)
N1	C5	1.505(7)	C10	C11	1.519(7)
N2	C3	1.465(8)	C11	C12	1.3900
N2	C6	1.342(8)	C11	C16	1.3900
C2	C1	1.519(9)	C12	C13	1.3900
C2	C3	1.538(9)	C13	C14	1.3900
O1	C6	1.242(7)	C14	C15	1.3900
C3	C4	1.529(8)	C15	C16	1.3900

Table 5 Bond Angles

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C1	N1	C9	109.4(5)	O2	C17	O4	126.8(6)
C1	N1	C5	110.0(5)	O2	C17	C18	118.2(6)
C9	N1	C5	112.8(5)	C6	C7	C8A	110.5(6)
C6	N2	C3	121.5(6)	C8	C7	C6	111.4(17)
C1	C2	C3	111.0(5)	C11	C10	C9	109.3(5)
N1	C1	C2	111.3(5)	N2	C6	C7	116.7(6)
N2	C3	C2	110.4(5)	O1	C6	N2	121.3(6)
N2	C3	C4	110.9(5)	O1	C6	C7	122.0(6)
C4	C3	C2	108.4(5)	C12	C11	C10	119.9(4)
N1	C9	C10	114.4(5)	C12	C11	C16	120.0
C5	C4	C3	110.5(5)	C16	C11	C10	120.1(4)
O5	C18	C17	113.5(5)	C13	C12	C11	120.0
O3	C18	O5	124.7(6)	C14	C13	C12	120.0
O3	C18	C17	121.7(6)	C13	C14	C15	120.0
N1	C5	C4	111.1(5)	C16	C15	C14	120.0
O4	C17	C18	114.9(6)	C15	C16	C11	120.0

Table 6 Torsion Angles

A	B	C	D	Angle/°	A	B	C	D	Angle/°
O5	C18	C17	O4	164.8(5)	C9	C10	C11	C16	-89.5(6)
O5	C18	C17	O2	-18.1(8)	C5	N1	C1	C2	57.9(7)
O3	C18	C17	O4	-16.5(8)	C5	N1	C9	C10	-67.5(7)
O3	C18	C17	O2	160.6(6)	C10	C11	C12	C13	-178.4(5)
N1	C9	C10	C11	-179.6(5)	C10	C11	C16	C15	178.4(5)
N2	C3	C4	C5	-178.1(5)	C6	N2	C3	C2	85.2(7)
C2	C3	C4	C5	-56.7(7)	C6	N2	C3	C4	-154.6(6)
C1	N1	C9	C10	169.7(5)	C11	C12	C13	C14	0.0
C1	N1	C5	C4	-58.3(6)	C12	C11	C16	C15	0.0
C1	C2	C3	N2	178.2(5)	C12	C13	C14	C15	0.0
C1	C2	C3	C4	56.5(7)	C13	C14	C15	C16	0.0
C3	N2	C6	O1	4.4(9)	C14	C15	C16	C11	0.0
C3	N2	C6	C7	-176.5(6)	C16	C11	C12	C13	0.0
C3	C2	C1	N1	-58.2(7)	C8	C7	C6	N2	179(2)
C3	C4	C5	N1	58.5(7)	C8	C7	C6	O1	-2(2)
C9	N1	C1	C2	-177.7(5)	C8A	C7	C6	N2	122.0(8)
C9	N1	C5	C4	179.3(5)	C8A	C7	C6	O1	-58.9(10)
C9	C10	C11	C12	88.9(6)					

Table 7 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3)

Atom	*x*	*y*	*z*	U(eq)
H4	3698	545	1578	40
H5	-3360	-442	1785	42
H1	-2014	-1718	1607	25
H2	-2702	-2706	-1630	30
H2A	-188	-4016	-34	32
H2B	-59	-2656	191	32
H1A	-2553	-4133	1259	33
H1B	-131	-3342	1655	33
H3	-4332	-4175	-469	32
H9A	-1893	-2570	3007	34
H9B	-4520	-3167	2668	34
H4A	-3821	-1722	-67	31
H4B	-6255	-2508	-454	31
H5A	-6132	-1868	1242	27
H5B	-6247	-3228	997	27
H7AA	-2983	-3771	-3369	46
H7AB	-255	-3582	-2958	46
H7BC	-1605	-3295	-3067	46
H7BD	-2948	-4520	-3558	46
H10A	-3313	-744	3188	44
H10B	-5951	-1347	2854	44
H12	-1446	-837	4788	75
H13	-1907	-1085	6375	91
H14	-5475	-1965	6751	71
H15	-8583	-2596	5540	144
H16	-8121	-2347	3954	137
H8A	-734	-5723	-3269	100
H8B	-150	-5058	-4125	100
H8C	-2812	-5564	-4039	100
H8AA	1887	-4172	-2730	100
H8AB	1128	-4424	-3869	100
H8AC	543	-5385	-3244	100

Table 8 Atomic Occupancy

Atom	***Occupancy***	**Atom**	***Occupancy***	**Atom**	***Occupancy***
H7AA	0.215(12)	H7AB	0.215(12)	H7BC	0.785(12)
H7BD	0.785(12)	C8	0.215(12)	H8A	0.215(12)
H8B	0.215(12)	H8C	0.215(12)	C8A	0.785(12)
H8AA	0.785(12)	H8AB	0.785(12)	H8AC	0.785(12)

Experimental Details

Needle shaped crystals of $C_{18}H_{27}N_2O_5$ were submitted for structure determination. The crystals were small and were grown together. Even apparently single crystals were stacks of needles A crystal was selected and attached to a Micromount using paratone oil, then mounted on a Bruker Kappa APEX-II Duo diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2 [1], the structure was solved with the ShelXS [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.
3. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.

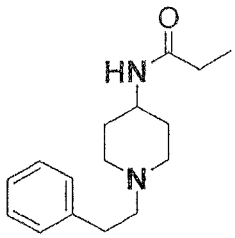
Crystal Data for $C_{18}H_{27}N_2O_5$ (M =351.41 g/mol): triclinic, space group P-1 (no. 2), a = 5.701(3) Å, b = 12.024(6) Å, c = 14.105(7) Å, α = 100.473(14)°, β = 93.997(14)°, γ = 95.571(15)°, V = 942.5(8) Å^3, Z = 2, T = 150.0 K, μ(MoKα) = 0.090 mm^{-1}, *Dcalc* = 1.238 g/cm^3, 7408 reflections measured (2.948° ≤ 2Θ ≤ 41.622°), 1965 unique (R_{int} = 0.0625, R_{sigma} = 0.0684) which were used in all calculations. The final R_1 was 0.0970 (I > 2σ(I)) and wR_2 was 0.2545 (all data).

Refinement model description
Number of restraints - 237, number of constraints -
Details:
1. Fixed Uiso
At 1.2 times of:
All C(H) groups, All C(H,H) groups, All C(H,H,H,H) groups, All N(H) groups
At 1.5 times of:
All C(H,H,H) groups, All O(H) groups
2. Restrained planarity
C11, C12, C13, C14, C15, C16, C10
with sigma of 0.1
3. Rigid bond restraints
O2, O5, C18, O4, C17, O3
with sigma for 1-2 distances of 0.01 and sigma for 1-3 distances of 0.01
C11, C16, C15, C14, C13, C12
with sigma for 1-2 distances of 0.01 and sigma for 1-3 distances of 0.01
4. Uiso/Uaniso restraints and constraints
Uanis(O3) ≈ Ueq, Uanis(C18) ≈ Ueq, Uanis(O5) ≈ Ueq, Uanis(O2) ≈ Ueq, Uanis(C17) ≈ Ueq, Uanis(O4) ≈ Ueq: with sigma of 0.005 and sigma for terminal atoms of 0.01
Uanis(C8) = Uanis(C8A)
5. Rigid body (RIGU) restrains
All non-hydrogen atoms
with sigma for 1-2 distances of 0.004 and sigma for 1-3 distances of 0.004
6. Others
Sof(H7BC)=Sof(H7BD)=Sof(C8A)=Sof(H8AA)=Sof(H8AB)=Sof(H8AC)=1-FVAR(1)
Sof(H7AA)=Sof(H7AB)=Sof(C8)=Sof(H8A)=Sof(H8B)=Sof(H8C)=FVAR(1)
7.a Ternary CH refined with riding coordinates:
N1(H1), C3(H3)
7.b Secondary CH2 refined with riding coordinates:
C2(H2A,H2B), C1(H1A,H1B), C9(H9A,H9B), C4(H4A,H4B), C5(H5A,H5B), C7(H7AA,

H7AB), C7(H7BC,H7BD), C10(H10A,H10B)
7.c Aromatic/amide H refined with riding coordinates:
N2(H2), C12(H12), C13(H13), C14(H14), C15(H15), C16(H16)
7.d Fitted hexagon refined as free rotating group:
C11(C12,C13,C14,C15,C16)
7.e Idealised Me refined as rotating group:
C8(H8A,H8B,H8C), C8A(H8AA,H8AB,H8AC)
7.f Idealised tetrahedral OH refined as rotating group:
O4(H4), O5(H5)
This report has been created with Olex2, compiled on 2014.07.22 svn.r2960 for OlexSys. Please let us know if there are any errors or if you would like to have additional features.

Appendix 3

Docket No. UA 15-023



μ-Opioid Antagonist/α-agonist (I)

Lipinski and Related Properties	Value	Condition	Note

Freely Rotatable Bonds 5(1)
H Acceptors 3(1)
H Donors 1(1)
H Donor/Acceptor Sum 4(1)
logP2.216 0.355Temp:25 C(1)
Molecular Weight260.37(1)

Lipinski's rule of five

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is a rule of thumb to evaluate druglikeness or determine if a chemical compoundwith a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules.[1][2]

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule.[3] Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market.[2][4]

Components of the rule

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen-hydrogen bonds)
- Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient[5] log *P* not greater than 5

Note that all numbers are multiples of five, which is the origin of the rule's name. As with many other rules of thumb, (such as Baldwin's rules for ring closure), there are many exceptions to Lipinski's Rule.

Appendix 4

Docket No. UA 15-023

1. STUDY REFERENCES

Study title	*In Vitro* Pharmacology Study of One Compound	
Study number	100023322	FINAL REPORT
Experimental period		

2. PERSONS INVOLVED IN THE STUDY

Investigator	**Eurofins Cerep** Le Bois l'Evêque B.P. 30001 86 600 Celle l'Evescault France Tel: +33 (0)5 49 89 30 00 Fax: +33 (0)5 49 43 21 70	**Thierry JOLAS,Ph.D.** Principal Scientist, Pharmacology Tel: +33 (0)5 49 89 39 89 Fax: +33 (0)5 49 43 21 70
Study sponsor	**TECH LAUNCH ARIZONA** University of Arizona 220 W 6th Street 4th Floor TUCSON, AZ 85701 U.S.A.	**Dr. Paul EYNOTT** Licensing Manager, The College of Science

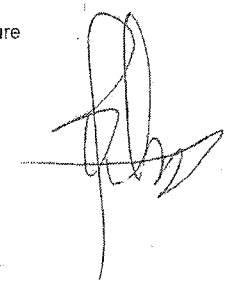
3. APPROVAL

Investigator	**Eurofins Cerep** Le Bois l'Evêque B.P. 30001 86 600 Celle l'Evescault France Tel: +33 (0)5 49 89 30 00 Fax: +33 (0)5 49 43 21 70	**Thierry JOLAS,Ph.D.** Principal Scientist, Pharmacology Tel: +33 (0)5 49 89 39 89 Fax: +33 (0)5 49 43 21 70

I certify that this report accurately reflects all relevant data collected in this study.

Date

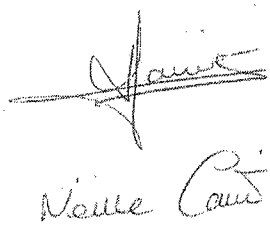
Signature



Quality assurance statement	Eurofins Cerep's Quality Unit certifies that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the raw data.

Date

Signature



4. TABLE OF CONTENTS

5. SUMMARY

The purpose of this study was to test HCV-3 in cellular and nuclear receptor functional assays.

5.1. Study Design

HCV-3 was tested at 1.0E-05 M.

5.2. Measurements

Cellular agonist effect was calculated as a % of control response to a known reference agonist for each target and cellular antagonist effect was calculated as a % inhibition of control reference agonist response for each target.

5.3. Results

Results showing an inhibition or stimulation higher than 50% are considered to represent significant effects of the test compounds.

Such effects were not observed at any of the receptors studied.

6. COMPOUNDS

6.1. Test Compounds

Manufacturer: TECH LAUNCH ARIZONA

Client Compound ID	Compound ID	Reference Number	Batch Number	FW	MW	Purity	Received Form	Stock solution	Flag
HCV-3	100023322-1	-	1	296.84	260.38	-	Powder	1.E-02 M H2O	-

FW: Formula Weight - MW: Molecular Weight

6.2. Reference Compounds

In each experiment and if applicable, the respective reference compound was tested concurrently with HCV-3, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins validation Standard Operating Procedure.

7. RESULTS

7.1. *In Vitro* Pharmacology: Cellular and Nuclear Receptor Functional Assays

7.1.1. Agonist Effect: Test Compound Results

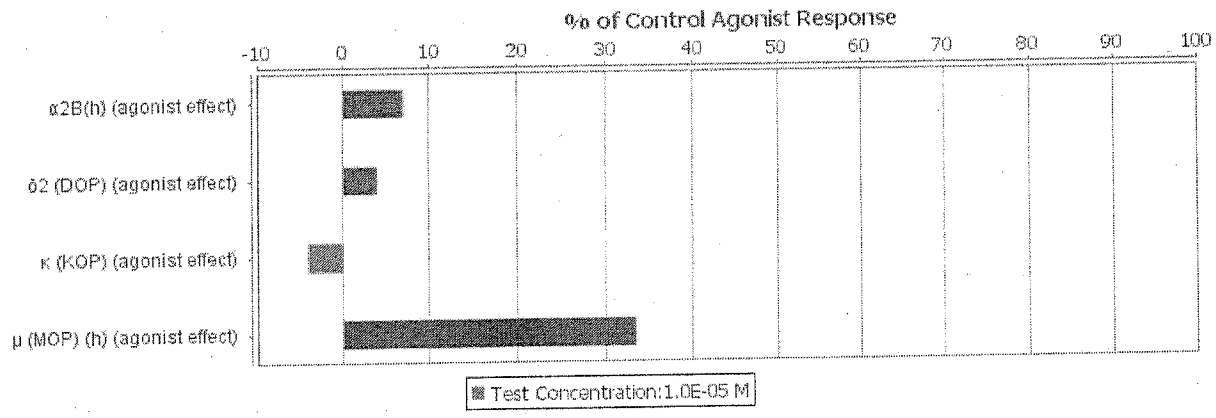


Figure 1. Histogram for HCV-3

Compound I.D.	Client Compound I.D.	Test Concentration	% of Control Agonist Response 1st	2nd	Mean
α_{2B} *(h)* (agonist effect)					
100023322-1	HCV-3	1.0E-05 M	9.2	5.1	7.1
δ_2 (DOP) (agonist effect)					
100023322-1	HCV-3	1.0E-05 M	4.0	4.0	4.0
κ (KOP) (agonist effect)					
100023322-1	HCV-3	1.0E-05 M	-10.3	1.8	-4.2
μ (MOP) *(h)* (agonist effect)					
100023322-1	HCV-3	1.0E-05 M	28.1	38.9	33.5

7.1.2. Reference Compound Results

Compound I.D.	EC_{50} (M)	nH
α_{2B} *(h)* (agonist effect)		
dexmedetomidine	1.3E-08 M	n/a
δ_2 (DOP) (agonist effect)		
DPDPE	1.5E-09 M	n/a
κ (KOP) (agonist effect)		
U 50488	1.6E-09 M	n/a
μ (MOP) *(h)* (agonist effect)		
DAMGO	4.2E-09 M	n/a

7.1.3. Antagonist Effect: Test Compound Results

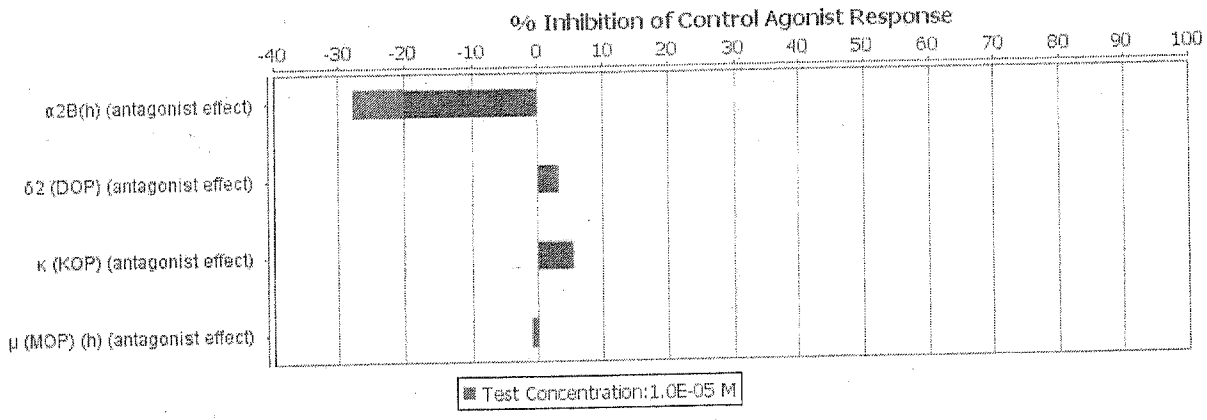


Figure 2. Histogram for HCV-3

Compound I.D.	Client Compound I.D.	Test Concentration	% Inhibition of Control Agonist Response 1st	2nd	Mean
α_{2B} *(h)* (antagonist effect)					
100023322-1	HCV-3	1.0E-05 M	-16.8	-38.7	-27.8
δ_2 (DOP) (antagonist effect)					
100023322-1	HCV-3	1.0E-05 M	0.8	5.9	3.3
κ (KOP) (antagonist effect)					
100023322-1	HCV-3	1.0E-05 M	-12.4	23.5	5.6
μ (MOP) *(h)* (antagonist effect)					
100023322-1	HCV-3	1.0E-05 M	-0.1	-1.8	-1.0

7.1.4. Reference Compound Results

Compound I.D.	IC_{50} (M)	K_B (M)	nH
α_{2B} *(h)* (antagonist effect)			
yohimbine	3.7E-07 M	4.8E-08 M	n/a
δ_2 (DOP) (antagonist effect)			
naltrindole	2.7E-10 M	4.6E-11 M	n/a
κ (KOP) (antagonist effect)			
nor-BNI	4.3E-10 M	7.2E-11 M	n/a
μ (MOP) *(h)* (antagonist effect)			
CTOP	2.1E-07 M	2.3E-08 M	n/a

8. RESULTS INTERPRETATION GUIDE

In Vitro Pharmacology

Results showing a stimulation or an inhibition higher than 50% are considered to represent significant effects of the test compounds. 50% is the most common cut-off value for further investigation (determination of EC_{50} or IC_{50} values from concentration-response curves).
Results showing a stimulation or an inhibition between 25% and 50% are indicative of weak to moderate effects (in some assays, they may be confirmed by further testing as they are within a range where more inter-experimental variability can occur).
Results showing a stimulation or an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level.

9. MATERIALS AND METHODS

9.1. Experimental Conditions

Minor variations to the experimental protocol described below may have occurred during the testing, they have no impact on the quality of the results obtained.

9.1.1. *In Vitro* Pharmacology: Cellular and Nuclear Receptor Functional Assays

Assay	Source	Stimulus	Incubation	Measured Component	Detection Method	Bibl.
Receptors						
α_{2B} *(h)* (agonist effect)	human recombinant (HEK-293 cells)	none (3 µM dexmedetomidine for control)	30 min 37°C	cAMP	HTRF	915
α_{2B} *(h)* (antagonist effect)	human recombinant (HEK-293 cells)	dexmedetomidine (100 nM)	30 min 37°C	cAMP	HTRF	915
δ_2 (DOP) (agonist effect)	NG-10815 cells (endogenous)	none (300 nM DPDPE for control)	37°C	impedance	Cellular dielectric spectroscopy	934
δ_2 (DOP) (antagonist effect)	NG-10815 cells (endogenous)	DPDPE (10 nM)	37°C	impedance	Cellular dielectric spectroscopy	934
κ (KOP) (agonist effect)	rat recombinant (CHO cells)	none (0.3 µM U 50488 for control)	10 min 37°C	cAMP	HTRF	819
κ (KOP) (antagonist effect)	rat recombinant (CHO cells)	U 50488 (3 nM)	10 min 37°C	cAMP	HTRF	819
µ (MOP) *(h)* (agonist effect)	human recombinant (CHO cells)	none (0.3 µM DAMGO for control)	10 min 37°C	cAMP	HTRF	260
µ (MOP) *(h)* (antagonist effect)	human recombinant (CHO cells)	DAMGO (20 nM)	10 min 37°C	cAMP	HTRF	260

9.2. Analysis and expression of results

9.2.1. *In Vitro* Pharmacology: Cellular and Nuclear Receptor Functional Assays

The results are expressed as a percent of control agonist response

$$\frac{\text{measured response}}{\text{control response}} * 100$$

and as a percent inhibition of control agonist response

$$100-(\frac{\text{measured response}}{\text{control response}} * 100)$$

obtained in the presence of HCV-3.

The EC_{50} values (concentration producing a half-maximal response) and IC_{50} values (concentration causing a half-maximal inhibition of the control agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting

$$Y=D+[\frac{A-D}{1+(C/C_{50})^{nH}}]$$

where Y = response, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, and C_{50}= EC_{50} or IC_{50}, and nH = slope factor.
This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).
For the antagonists, the apparent dissociation constants (K_B) were calculated using the modified Cheng Prusoff equation

$$K_B=\frac{IC_{50}}{1+(A/EC_{50A})}$$

where A = concentration of reference agonist in the assay, and EC_{50A} = EC_{50} value of the reference agonist.

10. BIBLIOGRAPHY

260. Wang, J.B. et al. (1994), *FEBS Lett.*, 338 : *217-222.*
819. Avidor-Reiss, T. et al. (1995), *FEBS Lett.*, 361 : *70-74.*
915. Eason, M.G. et al. (1992), *J. Biol. Chem.*, 267 : *15795-15801.*
934. Law, P.Y. and Loh, H.H. (1993), *Mol. Pharmacol.*, 43 : *684-693.*

Appendix 5

Docket No. UA 15-023

Table of Contents

AUTHENTICATION STATEMENT

I, the undersigned, hereby declare that the work described in this report was performed according to the study protocol and/or standard procedures, and to the best of my knowledge, this report provides a correct record of the results obtained.

Study Manager

Laboratory QC Review:

Barry Press, Ph.D.
Principal Scientist &
Quality Manager

1 PURPOSE

The purpose of this study was to evaluate the mutagenicity potential of a test article (HCV-3) in the Ames assay using the microplate fluctuation (MPF) method.

2 STUDY CONDITIONS

This study was performed under non-GLP conditions. All work was performed with appropriate local health regulations and ethical approval.

3 EXPERIMENTAL DESIGN

3.1 AMES MFP: Experimental Conditions

Test Article	Test Concentrations* (µg/mL)	Reference Compounds
HCV-3	31.3, 62.5, 125, 250, 500, 1000	2-aminoanthracene 2-nitrofluorene 4-nitroquinoline N-oxide

*The top concentration in the dose response reflects the highest soluble concentration of the test article in Ames assay conditions.

Experimental Procedure: Approximately ten million bacteria are exposed in triplicate to test agent (six concentrations), a negative control (vehicle) and a positive control for 90 minutes in medium containing a low concentration of histidine (sufficient for about 2 doublings.) The cultures are then diluted into indicator medium lacking histidine, and dispensed into 48 wells of a 384 well plate (micro-plate format, MPF). The plate is incubated for 48 hr at 37°C, and cells that have undergone a reversion will grow in a well, resulting in a color change in wells with growth. The number of wells showing growth are counted and compared to the vehicle control. An increase in the number of colonies of at least two-fold over baseline (mean + SD of the vehicle control) and a dose response indicates a positive response. An unpaired, one-sided Student's T-test is used to identify conditions that are significantly different from the vehicle control.

Where indicated, S9 fraction from the livers of Aroclor 1254-treated rats is included in the incubation at a final concentration of 4.5%. An NADPH-regenerating system is also included to ensure a steady supply of reducing equivalents.

Strains used in this study:
S. typhimurium TA98: hisD3052, rfa, uvrB / pKM101; detects frame-shift mutations.
S. typhimurium TA100: hisG45, rfa, uvrB / pKM101; detects base-pair substitutions.

4 RESULTS AND CONCLUSIONS

4.1 AMES MPF: Data Summary

Test Article	Test Strain	S9	AMES Result (Positive/Negative)	Highest Conc. Tested*	Comment
2-nitrofluorine + 4-nitroquinoline N-oxide	TA98	no	Positive	4 µg/ml + 2 µg/ml	positive control
2-nitrofluorine + 4-nitroquinoline N-oxide	TA100	no	Positive	4 µg/ml + 2 µg/ml	positive control
aminoanthracene	TA98	yes	Positive	5 µg/ml	positive control
aminoanthracene	TA100	yes	Positive	5 µg/ml	positive control
HCV-3	TA98	no	Negative	1,000 µg/mL	
	TA100	no	Negative	1,000 µg/mL	
	TA98	yes	Negative	1,000 µg/mL	
	TA100	yes	Negative	1,000 µg/mL	

*Highest soluble concentration of test article in Ames assay conditions.

Conclusion: Test article HCV-3 was assessed for its mutagenic potential in the Ames reverse mutation assay. This test was performed in the absence and presence of S9 metabolic activation. HCV-3 was found to be negative for genotoxicity against both strains used in this study (TA98 and TA100) up to a maximum tested concentration of 1 mg/ml. The positive controls behaved as expected.

Company Name: Tech Launch Arizona
Cyprotex Study Number: CYP1199-R1

4.2 AMES MPF: Individual Data

Significant fold increase over baseline values (≥2-fold) are indicated in **bold red**. Significant T-test probabilities ($P < 0.05$) appear red and ($P<0.01$) are indicated in **bold red**.

HCV-3
TA 98 -S9

Assay 8/10/2015

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	3	1.33		1.53	2.86		
31.3	3	1.33		1.53		0.47	0.5000
62.5	3	1.00		1.00		0.35	0.3838
125	3	1.00		1.00		0.35	0.3838
250	3	1.67		2.08		0.58	0.4170
500	3	0.67		0.58		0.23	0.2593
1000	3	0.67		0.58		0.23	0.2593
Pos. Control	3	26.00		2.65			

HCV-3
TA 98 +S9

Assay 8/10/2015

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	3	4.00		1.00	5.00		
31.3	3	5.67		2.52		1.13	0.1732
62.5	3	6.67		2.31		1.33	0.0702
125	3	6.00		3.61		1.20	0.2035
250	3	6.00		4.36		1.20	0.2409
500	3	3.67		2.08		0.73	0.4075
1000	3	5.00		4.00		1.00	0.3480
Pos. Control	3	34.33		8.33			

Company Name: Tech Launch Arizona
Cyprotex Study Number: CYP1199-R1

HCV-3
TA 100 -S9 Assay 8/10/2015

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	3	5.67		1.53	7.19		
31.3	3	2.33		0.58		0.32	0.0121
62.5	3	3.67		2.08		0.51	0.1254
125	3	3.67		2.52		0.51	0.1523
250	3	2.67		0.58		0.37	0.0167
500	3	2.67		0.58		0.37	0.0167
1000	3	2.33		0.58		0.32	0.0121
Pos. Control	3	47.67		0.58			

HCV-3
TA 100 +S9 Assay 8/10/2015

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	3	2.00		1.73	3.73		
31.3	3	2.67		0.58		0.71	0.2807
62.5	3	3.33		1.53		0.89	0.1870
125	3	3.33		2.52		0.89	0.2459
250	3	4.33		1.53		1.16	0.0775
500	3	4.00		1.00		1.07	0.0792
1000	3	4.33		1.15		1.16	0.0621
Pos. Control	3	25.33		12.10			

5 REFERENCE

Umbuzeiro, G. et al. (2010). "Comparison of the Salmonella/microsome micro-suspension assay with the new microplate fluctuation protocol for testing the mutagenicity of environmental samples". Environ. Mol. Mutagen. **51**(1):31-38.

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UA 15-023 PCT

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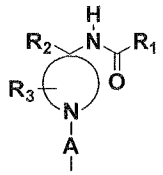
Incorporation by reference

Each document, patent, patent application or patent publication cited by or referred to in this disclosure is incorporated by reference in its entirety. However, no admission is made that any such reference constitutes prior art, and the right to challenge the accuracy and pertinence of the cited documents is reserved.

Claims:

1. A compound having the formula



Ar , or (HetAr), or $COOR_5$, or $CON(R_6)_2$ (III)

wherein

R_1 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or hetaryl;

R_2 is H, -CH_2O-C_{1-4} alkyl; COO-C_{1-4} alkyl; -$CONR_4$;

R_3 is H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or unsubstituted aryl or hetaryl;

A is substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl;

Ar or HetAr is substituted or unsubstituted monocyclic or polycyclic aromatic or heteroaromatic moiety;

$COOR_5$, or $CON(R_6)_2$, where R_5 and R_6 are H, substituted or unsubstituted C_1-C_{10} alkyl, alkylene, alkynyl, or substituted or unsubstituted aryl or hetaryl;

the central nitrogen-containing ring is a substituted or unsubstituted 5- to 7-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound.

2. The compound of claim 1, wherein the compound belongs to a series of N-(1-arylethylpiperidin-4-yl)acylamides.
3. The compound of claim 1, wherein the compound is N-(1-phenethylpiperidin-4-yl)propionamide.
4. The compound of claim 1, wherein the compound is N-(1-phenethylpiperidin-4-yl)propionamide oxalate.
5. A process for preparing a compound of claim 1, comprising the following steps:
   (a) reacting a cyclic ketone having a protecting group in a Grignard or Reformatsky reaction to obtain a first product;
   (b) reacting the product of step (a) in a Ritter reaction to obtain a second product; and
   (c) deprotecting the product of step (b) with acylation or alkylation to obtain a compound of claim 1.
6. A process for preparing a compound of claim 1, comprising the following steps:

(a) reacting a cyclic ketone having a protecting group in a Strecker reaction to obtain a first product;

(b) reacting the product of step (a) in a selective carbalkoxy group transformation to obtain a second product; and

(c) deprotecting the product of step (b) with acylation or alkylation to obtain a compound of claim 1.

7. A process for the preparation of N-(1-phenethylpiperidin-4-yl)propionamide, comprising the following steps:

(a) reacting phenethylpiperiding-4-one with hydroxylamine hydrochloride in ethanol in the presence of a base, to produce 1-phenethylpiperidin-4-one oxime;

(b) reducing the oxime obtained in step (a) with iso-amyl alcohol and sodium metal to produce 1-phenethylpiperidin-4-amine; and

(c) acylating the product of step (b) with propionic acid chloride in chloroform in the presence of triethylamine to produce N-(1-phenethylpiperidin-4-yl)propionamide.

8. The process of claim 7, wherein the N-(1-phenethylpiperidin-4-yl)propionamide is further treated with oxalic acid to produce N-(1-phenethylpiperidin-4-yl)propionamide oxalate.

Generic Display Report

Analysis Info

Analysis Name	D:\Data\June_15\15_0427_HVC-3_000001.d	Acquisition Date	
Method	April_good_method		
Sample Name	15_0427_HVC-3	Operator	
Comment	Saghar M.; ACN:H2O:FA	Instrument	apex-Qe

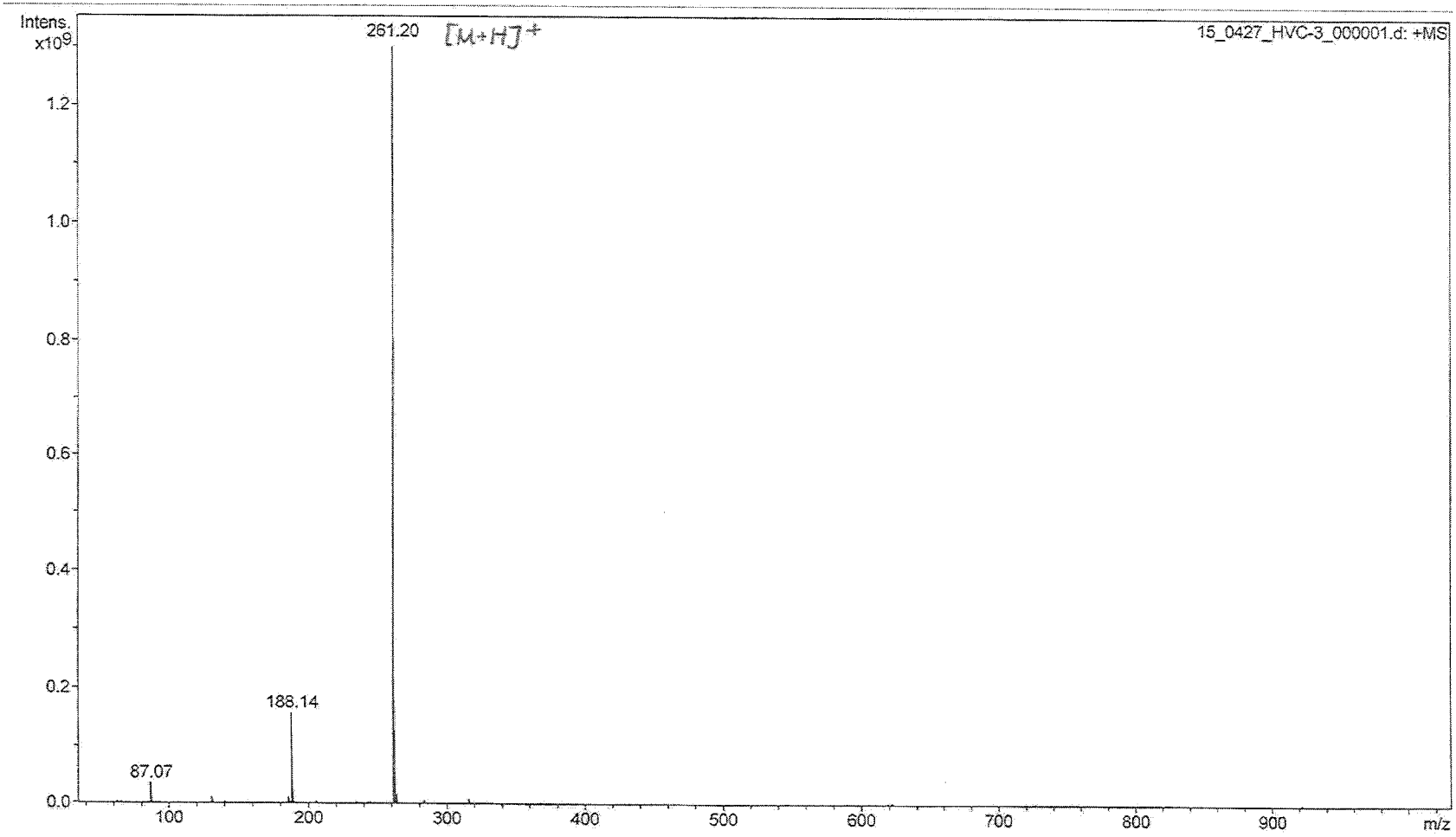


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/046585

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 207/04 (2015.01)
CPC - C07D 207/04 (2015.12)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07D 207/02, 207/04 (2015.01)
CPC - C07D 207/02, 207/04 (2015.12)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/183, 408, 426; IPC(8) - C07D 207/02, 207/04 (2015.01); CPC - C07D 207/02, 207/04 (2015.12) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, STN, Google Patents, Google Scholar, PubChem, SureChEMBL
Search terms used: pyrrolidin, formamide, formylaminopyrrolidin, formamidopyrrolidin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUBCHEM. Substance Record for SID 56006844. Deposit Date: 2008-10-08. [retrieved on 11 November 2015]. Retrieved from the Internet. <URL: http://pubchem.ncbi.nlm.nih.gov/substance/56006844#section=Top>. entire document	1
A	US 4,029,801 A (CAVALLA et al) 14 June 1977 (14.06.1977) entire document	1
A	US 4,649,144 A (MATSUMOTO et al) 10 March 1987 (10.03.1987) entire document	1
A	US 2004/0147503 A1 (ZIPFEIL) 29 July 2004 (29.07.2004) entire document	1
A	WO 2007/058482 A1 (LG LIFE SCIENCES LTD) 24 May 2007 (24.05.2007) entire document	1
A	PUBCHEM. Substance Record for SID 150462038. Deposit Date: 2012-10-23. [retrieved on 10 November 2015]. Retrieved from the Internet. <URL: https://pubchem.ncbi.nlm.nih.gov/substance/150462038/version/1>. entire document	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2015	11 JAN 2016

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Blaine Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/046585

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Claim 1 has been analyzed subject to the restriction that the claim reads on a compound having the instant formula as described (See first Extra Sheet). The claim is restricted to compound having the instant formula wherein R1 is H, R2 is H, R3 is H, A is substituted C1 alkyl, Ar is substituted monocyclic aromatic, the central nitrogen-containing ring is a substituted 5-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound.

(Continued on first Extra Sheet)

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2015/046585

<Continued from Box No. III>

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: Claims 1-4 are drawn to a compound having the formula (III).

Group II: Claims 5-8 are drawn to a process for preparing a compound.

The first invention of Group I+ is restricted to a compound having the formula (III), wherein R1 is H, R2 is H, R3 is H, A is substituted C1 alkyl, Ar is substituted monocyclic aromatic, the central nitrogen-containing ring is a substituted 5-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound. It is believed that claim 1 reads on this first named invention and thus this claim will be searched without fee to the extent that it reads on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional method to be searched in a specific combination for each formula by paying additional fees for each election. An exemplary election would be a compound having the formula (III), wherein R1 is substituted C1 alkyl, R2 is H, R3 is H, A is substituted C1 alkyl, Ar is substituted monocyclic aromatic, the central nitrogen-containing ring is a substituted 5-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound. Additional inventions will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I+, a compound having the formula (III), are not present in Group II; and the special technical features of Group II, a process for preparing a compound thereof, are not present in Group I+.

The Group I+ compounds do not share a significant structural element, requiring the selection of alternatives for the compound variables R1, R2, R3, A, Ar, HetAr, COOR5, CON(R6)2, and the central nitrogen-containing ring.

The Groups I+ and II share the technical features of a compound having the formula (III) and pharmaceutically acceptable salts of said compound. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 4,029,801 A to Cavalla et al. teach a compound having the formula (III), wherein R1 is unsubstituted aryl, R2 is H, R3 is H, A is unsubstituted C2 alkyl, Ar is unsubstituted aryl, and the central nitrogen-containing ring is an unsubstituted 6-membered heterocyclic ring; and pharmaceutically acceptable salts of said compound (Col. 24, Lns. 25-39, 4-Benzamido-1-phenethylpiperidine; Col. 37, Lns. 55-68, 4-Benzamido-1-phenethylpiperidine).

The inventions listed in Groups I+ and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

<End of Box No. III>