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Discovery of MK-0952, a selective PDE4 inhibitor for the treatment of long-term memory loss and mild cognitive impairment

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ABSTRACT

The structure–activity relationship of a novel series of 8-biarylnaphthyridinones acting as type 4 phosphodiesterase (PDE4) inhibitors for the treatment of long-term memory loss and mild cognitive impairment is described herein. The manuscript describes a new paradigm for the development of PDE4 inhibitor targeting CNS indications. This effort led to the discovery of the clinical candidate **MK-0952**, an intrinsically potent inhibitor ($IC_{50} = 0.6$ nM) displaying limited whole blood activity ($IC_{50} = 555$ nM). Supporting in vivo results in two preclinical efficacy tests and one test assessing adverse effects are also reported. The comparative profiles of **MK-0952** and two other Merck compounds are described to validate the proposed hypothesis.

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There are three distinct steps involved in the generation of memory. The first step, acquisition, requires a certain level of attention and alertness. The second step, called short-term memory is thought to reside within the hippocampus and acts as a switchboard to determine which memory should be stored longterm and what type of information is required for retrieval. The third step, termed the consolidation step, generates long-term memories and is a process that requires new protein synthesis. Biochemically, short-term memory (or early phase long-term potentiation) is primarily driven by calcium as the second messenger. Repeated stimulation of calcium-mediated events leads to the activation of cAMP which mediates the late phase long-term potentiation (L-LTP) cascade, which is thought to be associated with long-term memory.¹ Within the L-LTP cascade, stimulation of cAMP results in the activation of protein kinase A, which results in the subsequent phosphorylation of the transcription factor cAMP response element binding protein (CREB). Multiple target genes are rapidly transcribed following CREB activation² (some of which are involved in L-LTP) resulting in new protein synthesis, strengthening of existing synaptic connections, and de novo establishment of synaptic connections. Physiologically, hydrolysis of cAMP is catalyzed by a multigene super-family of cyclic nucleotide phosphodiesterases (PDEs). To date, 11 individual families of PDEs

have been described; among them is the type 4 cAMP-specific phosphodiesterase (PDE4) which is particularly important for the control of intracellular cAMP.³ Consequently, it is hypothesized that inhibition of PDE4 will lead to the accumulation of cAMP which will facilitate the L-LTP cascade and cognition enhancement.⁴ The most convincing evidence to date that supports the involvement of PDE4 in cognition comes from animal tests. Rolipram, a first-generation selective PDE4 inhibitor has been shown to exert a reversal of the L-LTP deficit in aged mice,⁵ and to reverse recognition memory impairment in rats.⁶

Adverse effects (AEs) associated with PDE4 inhibitors: First generation PDE4 inhibitors are known to produce dose-limiting AEs⁷ including emesis, nausea, colitis and vasculitis rendering their clinical development challenging. AEs linked to PDE4 inhibition can be triggered both centrally and/or peripherally based on known tissue distribution. Numerous strategies to identify PDE4 inhibitors displaying acceptable therapeutic window of efficacy over AEs have been proposed. For example, efforts have been made to identify isozyme-selective inhibitors,⁸ to pinpoint the molecular target of emesis and efficacy by using highly emetic photoaffinity probes⁹ or finally to focus on peripherally restricted inhibitors.¹⁰ However, to target a centrally-mediated disease such as cognitive impairment, a new paradigm is needed to establish the necessary therapeutic window.

Hypothesis: Inhibition of the centrally located PDE4 enzyme, due to low protein content in CSF, should correlate best with the

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intrinsic activity of the inhibitor rather than its potency in whole blood. On the other hand, AEs may be restricted predominately to its peripheral whole blood activity. Therefore, a brain penetrating PDE4 inhibitor exhibiting a high plasma protein shift (i.e., low whole blood potency) but retaining intrinsic potency on the enzyme could demonstrate an improved therapeutic window. Moreover, a compound with high oral bioavailability and rapid absorption would minimize gastrointestinal (GI) related AEs by reducing local GI exposure. The Condition Taste Aversion¹¹ (CTA) test (vide infra) was employed to evaluate malaise in rats while in vivo efficacy was measured using the Novel Object Recognition¹² (NOR) and the Water Maze Delayed Matching to Position (WM) tests.¹³

PDE4 enzymatic activities: Inhibition of PDE4 leads to an increase in intracellular cAMP which in turns inhibits the release of inflammatory mediators such as cytokines (TNF- α , IL-2, IL-12, LTB4, IFN- γ) as well as the recruitment and activation of inflammatory cells.¹⁴ This observation can be quantified in vitro by measuring the ability of PDE4 inhibitors to block the LPS-stimulated TNF- α release in human (HWB) or rat (RWB) whole blood thereby allowing a direct measurement of their relative potency.¹⁵ Intrinsic activity of inhibitors against the four PDE4 isozymes A, B, C, and D was assessed on their catalytic domain using a PDE4-GST constructs, expressed in SF9 cells via the baculovirus expression system.¹⁶ PDE4 inhibitors described in this manuscript displayed little or no isozyme specificity. Consequently, the PDE4 inhibitory enzyme potencies described in this manuscript are averages of the IC₅₀'s of the relevant isozymes A, B, and D. These three isozymes are widely distributed in the human brain, with the latter two being more abundant. In contrast, PDE4C exhibit a more restricted distribution in the human brain and is found only in the cortex, some thalamic nuclei and the cerebellum). In the rat brain, PDE4C is found exclusively in olfactory bulb.¹⁷

Our initial approach consisted in combining previously optimized scaffolds of PDE4 inhibitors discovered in our laboratories exhibiting in vitro activities in-line with our working hypothesis (Table 1). Designed for the treatment of respiratory diseases, clinical candidates **MK-0359**^{18,19} and **MK-0873**^{20,21} were optimized for whole blood potency and exhibited desirable low nanomolar intrinsic activity on the PDE4 enzyme. Additionally, second generation inhibitors, as exemplified by **MK-0873**, were found to address the issue of undesirable CYP2C9 competitive inhibition primarily by the introduction of the naphthyridinone pharmacophore. Finally,

Table 1

PDE4 inhibitors developed at Merck



^a IC₅₀ determinations are averages of at least two experiments.

^b PDE4^{QT} is the average of the IC₅₀'s on isozymes PDE4A, B and D.

 $^{\rm c}\,$ Human whole blood activity as measured by inhibition of TNF- α release. $^{\rm d}\,$ Competitive CYP2C9 inhibition.

third generation inhibitors,²² in which a carboxylic acid moiety was introduced (Compounds **1** and **2**), displayed a significant reduction of the whole blood potency but maintained intrinsic activity on the PDE4 enzyme. This high plasma protein shift is believed to be mediated by their high affinity for serum albumin, that is, commonly observed for lipophilic carboxylic acids.²³

SAR studies were initiated by combining the naphthyridinone core with the carboxylic acid biaryl pharmacophore. Oral bioavailability in rat was also measured for most compounds to validate our initial hypothesis regarding reduction in GI occupancy. The position of the cyclopropanecarboxylic acid residue, relative to the biphenyl tether, was evaluated first (Table 2). Unsubstituted phenyl propionic acids were not explored as they were previously found to display low oral bioavailability in rats due to extensive glucuronidation and β-oxidative mediated degradation.²² Meta and *para*-substituted analogs **3** and **4** displayed superior intrinsic enzyme activities over their *ortho* analog **5**. Moreover, the *para* analog 3 exhibited the highest high whole blood to intrinsic activity ratio. The gem-dimethyl analogs 6 and 7, which were designed to lower glucuronidation rate (steric hindrance) and alleviate β-oxidation (removal of benzylic hydrogen), respectively, displayed lower oral bioavailability but superior half-life compared to the cyclopropane analog **3**. Neither the benzylic dimethyl **7** nor the *cis*-cyclopropane analog **8** exhibited the preferred whole blood potency. Finally, the tetrazole 9, a carboxylic acid isostere of **3**, presented the optimum in vitro profile, but unfortunately did not meet our bioavailability criteria. Consequently, the parasubstituted cyclopropane carboxylic acid 3 was determined to possess the best overall profile for further optimization. Although the amide moiety present in compound 3 has previously been optimized²¹ with regard to both metabolic stability and enzyme activity, we suspected that the introduction of the acidic moiety may have rendered earlier SAR studies obsolete. Consequently, this area of the naphthyridinone scaffold was optimized next.

Although numerous amide analogs were prepared and profiled, none were found to be superior to the cyclopropyl amide **3** (Table 3). Noteworthy was the loss of inhibitory of activity upon increasing the size of the nitrogen substituent, exemplified by **11**, or by the introduction of polarity, as shown by the tertiary alcohol **12**. Equipotent analogs (**13**, **14**, and **16**) were identified but they displayed lower oral bioavailability. The cyclopropylmethyl **15** offered no real advantages over **3**.

Table 2

SAR studies on the carboxylic acid residue



Entry	IC ₅₀ ^a		Ratio Rat PK ^b		t PK ^b
	PDE4 ^{QT} (nM)	HWB (µM)	HWB/PDE4 ^{QT}	F (%)	$t_{1/2}$ (h)
3	0.57	0.30	526	78	1.7
4	0.54	0.11	203	100	1
5	4.6	1.3	282	35	4.7
6	0.88	0.61	693	30	6.1
7	0.85	0.075	88	62	5.1
8	1.6	0.16	100	30	3.9
9	0.55	>5	>9090	5	2.4

^a IC₅₀ determinations are averages of at least two experiments.

^b Rat PK studies were conducted in Sprague–Dawley rats at 10 mg/kg PO in 0.5% methocel (n = 2) and 5 mg/kg iv in PEG200 60% (n = 2).

Table 3SAR studies on the amide residue



Entry	IC ₅₀		Katlo	Kat PK	
	PDE4 ^{QT} (nM)	HWB (µM)	HWB/PDE4 ^{QT}	F (%)	$t_{1/2}(h)$
3	0.57	0.30	526	78	1.7
11	32	>40	>1142		
12	17	6.4	376		
13	0.58	0.28	482	58	4.1
14	0.51	0.51	1085	25	3.2
15	0.35	0.20	571		
16	0.52	0.22	432	20	2.4

^a IC₅₀ determinations are averages of at least two experiments.

^b Rat PK studies were conducted in Sprague–Dawley rats at 10 mg/kg PO in 0.5% methocel (n = 2) and 5 mg/kg iv in PEG200 60% (n = 2).

Having completed SAR studies on both the amide and acid portion of the naphthyridinone scaffold, we next focused on the resolution of compound **3**. Initially, the pair of enantiomers **17** and **18** (Table 4) were separated using chiral chromatography and their respective absolute configuration was established by X-ray crystallography. Despite the fact that the pair of enantiomers displayed similar intrinsic potency on the PDE4 enzymes, they differed significantly in whole blood potency, with the R,R enantiomer 17 displaying a three-fold greater whole blood shift. Unfortunately, while compound 17 did meet our selection criteria of intrinsic versus whole blood activity, it was found to elicit an unacceptable increase in rat liver protein covalent binding as a function of time (Fig. 1). Based on previous SAR of naphthyridinone containing analogs, we speculated that the bioactivation event leading to this phenomenon resulted from the formation of a short lived and highly reactive metabolite derived from the cyclopropane carboxylic acid. A follow-up SAR study demonstrated that the introduc-

Table 4

Discovery of MK-0952



Entry		IC ₅₀ ^a	Ratio	
	PDE4 ^{QT} (nM)	$HWB^{b}\left(\mu M ight)$	HWB/PDE4 ^{QT}	
17	1.0	$1.0 \pm 0.26 \ (n = 12)$	1000	
18	0.66	$0.23 \pm 0.049 \ (n = 21)$	350	
MK-0952	0.53	$0.55 \pm 0.097 \ (n = 17)$	1040	
20	0.46	$0.15 \pm 0.041 \ (n = 11)$	330	

^a IC₅₀ determinations are averages of at least two experiments.

^b Standard error (mean/sq. root of the *n* value).

tion of a fluorine atom at the *ortho* position in relation to the cyclopropane carboxylic acid addressed this problem and ultimately led to the discovery of the clinical candidate **MK-0952**.^{24,25}

The whole blood activity of **MK-0952** in rats and rhesus monkey was found to be similar to that in human with $IC_{50} = 450 \pm 80$ nM (n = 9) and 940 ± 270 nM (n = 4), respectively. Surprisingly, the squirrel monkey whole blood potency ($IC_{50} = 52 \pm 11$ nM; n = 6) was significantly higher. Pharmacokinetic studies (Table 5) in preclinical species and tissue distribution studies in rats (i.e., brain penetration, Table 6) were performed to complete the characterization of **MK-0952**. Overall, **MK-0952** is orally bioavailable in all preclinical species examined, and exhibits moderate clearance and half-life. **MK-0952** was not found to be highly brain penetrant in rats (2–3%), but reaches brain concentrations 20-fold over its intrinsic enzyme activity (0.65 nM) at a dose of 0.1 mg/kg. At this dose, the corresponding plasma level is equivalent to its whole blood potency (0.55 μ M).

The metabolic profile of **MK-0952** was determined in vitro by performing hepatocyte and microsomal incubations and in vivo using bile duct cannulated rats. A low extent of metabolism was observed in hepatocytes: 2-10% in human (n = 3); 5-8% in male rat (n = 2); 20% in female rat (n = 1); 10% in rhesus monkey (n = 1); and 30% in dog (n = 1) and all species exhibited the same major metabolites. The metabolic fate of **MK-0952** is summarized in Figure 2. The major metabolites were **21**, the acyl glucuronide and **22** the mono hydroxylated analog. Metabolite structures were confirmed with synthetic standards.



Figure 1. Rat liver covalent binding following oral dosing.

 Table 5

 Pharmacokinetic profile of MK-0952 in preclinical species

Table 6

Brain versus plasma exposure of MK-0952 as a function of dose

Dose ^a (mg/kg)	Brain ^b (µM)	Plasma ^c (μ M)	Brain/plasma (%)
0.1	0.012	0.59	2.1
0.5	0.047	1.34	3.5
1	0.11	3.41	3.2
5	0.29	8.19	3.5
10	0.59	18.84	3.1

^a **MK-0952** was dosed orally as a sodium salt in 0.5% methocel.

^b Whole brain. Contamination by residual blood <0.5%. LOQ = 1 nM.

^c Plasma and brain level measured 90 min post-dosing at C_{max} .



Figure 2. Structures of MK-0952's metabolites.

In human hepatocytes, 21 was the major metabolite. Incubations with rat uridine 5'-diphospho-glucuronosyl transferase (UGT's) showed that UGT 1A1, 1A3, 1A6, 1A8, and 1A9 contributed to the formation of **21**. Incubations with rat CYP's indicated that formation of 22 was mediated by CYP 1A, 3A and 2C19. Analysis of bile from permanently bile-cannulated male rats dosed iv with 3 H-L-001037116 (3 mg/kg, 100 μ Ci/kg) showed that the major component excreted in bile was the parent, accounting for >35% of total radioactivity (0–8 h). The metabolites 21 and 22 and two additional unknown metabolites (possibly a taurine conjugate) were also observed. Analysis of plasma samples showed mainly parent, with minor levels of 21 and 22. MK-0952 was neither a CYP inhibitor (IC₅₀ >100 µM on CYP2C9, 3A, 2D6 and 1A2) nor an inducer (23% increase CYP3A4 protein levels was observed at 10 µM in human primary cultured hepatocytes relative to rifampicin at 10 μ M) using Western blot). Based on the overall metabolic and CYP profile of MK-0952, we believe that the risk for this compound to be either the victim or perpetrator of drug-drug interactions is low.

In general, the PDE4 inhibitors described herein were prepared according to the synthetic sequence described in Scheme 1. The core naphthyridinone **III** was obtained in four steps starting from 2-chloronicotinoyl chloride. The latter was condensed with triethyl

orthoformate in the presence of acetic anhydride to afford 2-chloronicotinoyl acrylate. Upon addition of 3-bromo aniline in CH₂Cl₂ at room temperature the amino acrylate I was formed. Cyclization of the latter to the 1-bromophenyl-1,4-dihydro[1,8]naphthyridin-4-one II was achieved by treatment of I with NaH at room temperature. The naphthyridinone ester II was subsequently converted in one step to the corresponding amide III by condensation with an appropriately substituted and commercially available amine following dropwise addition of AlMe₃. Two parallel strategies were used to couple the naphthyridinone core to the acidic moiety. In one the bromoaryl III was first converted to the pinacole boronate IV by a palladium catalyzed coupling with pinacolato diboron.

Subsequent Suzuki coupling of the resulting boronate ester **IV** with an appropriately substituted aryl bromide **V** afforded the desired PDE4 inhibitor **VI**. Alternatively, the bromo aryl **III** was coupled by Suzuki coupling with the boronate ester **VII**, prepared as previously described. The chiral cyclopropane esters **X** were prepared from the corresponding styrenes **IX** using an enantiose-lective cyclopropanation described by Evans and co-workers.²⁶ The required styrenes were synthesized via Wittig olefination reaction of the corresponding benzaldehydes **VIII**. Racemic *trans* cyclopropane were prepared via Horner–Wadsworth–Emmons reaction followed by Simmons–Smith cyclopropanation. Finally,



Scheme 1. Synthesis of Naphthyridinone type PDE4 inhibitors. Reagents and conditions: (a) (i) Triethyl orthoformate/Ac₂O/130 °C; (ii) 3-bromo aniline/CH₂Cl₂/rt; (b) NaH/ THF/0 °C-rt; (c) HNR¹R²/1,2-dichloroethane/then AlMe₃ dropwise, or (i) LiOH/MeOH/THF; (ii) NEt₃/isobutyl chloroformate/CHCl₃/rt; (iii) HNR¹R²; (c) 5% Pd(OAc)₂/PPh₃ (1/3), Na₂CO₃, *n*-propanol, 80 °C; (d) 5% PdCl₂(dppf)₂, KOAc, pinacolato diboron, DMF, 70 °C; (e) 5% Pd(OAc)₂/PPh₃ (1/3), Na₂CO₃, *n*-propanol, 80 °C; (f) (i) (Ph)₃PCH₃Br/THF/-78 °C/ add *n*-BuLi (ii) **IX**/-78 °C-rt; (g) (i) Cu(OTf)/(+ or -) 2,2'-isopropylidene-bis(4-*tert*-butyl-2-oxazoline)/CHCl₃/rt; (ii) add **IX** then N₂CHCOOEt dropwise.

the β -phenylisovaleric ester required for compound **7** was prepared according to the procedure described by Corse and Rohrmann.²⁷ A detailed description of all the experimental conditions can be found in referenced patents

In vivo efficacy of **MK-0952**: In the Novel Object Recognition test, rats were initially presented with two identical objects (sample phase). After 24 h, they were presented with a triplicate copy of the original object and a novel object (choice phase). The time spent exploring the familiar object versus the novel object was recorded. If a rat recognizes the original object, it will spend more time with the novel object. When rats were pretreated with **MK-0952** prior to the sample phase, they spent more time exploring

the novel object at both doses of 0.01 and 0.1 mg/kg compared to vehicle-treated controls (Fig. 3). These results reflect an improvement in long-term recognition memory. In these studies, **MK-0359** was included as a positive control, and showed similar results to **MK-0952** at a dose of 0.1 mg/kg.

In the Water Maze Delayed Matching to Position test, rats were placed into the water maze at one of four randomly determined start locations and were given four trials per day (maximum length 60 s, with an inter trial interval (ITI) 30 s) for eight days to find a hidden platform submerged below the water surface using extra maze cues (training phase). The platform was placed in a different location each day, and the mean escape latency and swim speed



Figure 3. Assessment of efficacy of **MK-0952** and **MK-0359** in the Novel Object Recognition test. The data shown are the mean \pm standard error of mean (SEM) of the d2 score (time spent exploring the novel object-time spent exploring the familiar object/total exploration time) and the percentage score. **MK-0952** and **MK-0359** were dosed orally each day (0.5% methocel) 90 min and 30 min, respectively, before the sample trial corresponding to their individual C_{max} . The statistically significant differences between groups (n = 21-24) were determined using Newman Keuls tests. Any animal that lay two SDs away from the group mean on two or more performance measures were excluded from the analysis: p < 0.05 versus vehicle-treated animals. See Ref. 28 for full experimental details.



Figure 4. Assessment of efficacy of **MK-0952** and **MK-0359**^{19a} in the Water Maze Delayed Matching to Position test. The data shown are the mean \pm SEM savings score. **MK-0952** and **MK-0359** were dosed orally each day (0.5% methocel) 90 min and 30 min, respectively, before the sample trial. The statistically significant differences between groups (*n* = 10) were determined using Newman Keuls tests: *p* <0.05 versus vehicle-treated animals, *p* <0.01 versus vehicle-treated animals. See Ref. 29 for full experimental details.



Figure 5. Assessment of AEs induced by **MK-0952** in the Conditioned Test Aversion test. The data shown are the mean \pm SEM percentage change in liquid consumption from baseline scores. **MK-0952** was administered orally each day in 0.5% methocel immediately following the conditioning sessions. The statistically significant differences between groups (n = 6-8) were determined using Dunnett's tests: * p < 0.01 versus vehicle-treated animals. See Ref. 30 for full experimental details.

Table 7

Comparative in vivo profile of three distinct PDE4 inhibitors

	Water maze Delayed Matching to Position test						
	PDE4 ^{QT} (nM)	MED ^a (mg/kg)	Plasma ^b (nM) ^b	Brain ^b (nM) ^b	B/P (%)	B/PDE4 ^c	
MK-0359	1.0 ± 0.3	0.3	27	5.1	19	5.1	
MK-0873	5.1 ± 0.9	0.3	258	15	5.8	2.9	
MK-0952	0.5 ± 0.1	0.1	390	9.2	2.4	15	
		Conditioned Taste Aversion test					
	RWB (nM)	MED (mg/kg)	Plasma	(nM)	Brain (nM)	P/RWB ^d	
MK-0359	23 ± 5	1.0	113		21	4.9	
MK-0873	64 ± 24	1.0	828		48	13	
MK-0952	450 ± 80	10	12,050		290	27	

^a Minimum efficacious dose.

^b Plasma and brain exposure were measured on the tested animal as previously describe in Table 6.

^c Whole brain concentration to inhibitory enzyme potency ratio.

^d Plasma concentration to rat whole blood activity ratio.

measures were recorded on each trial. The ability of the test compound to improve cognition is evaluated over a five days testing phase. Rats may receive vehicle or the test compound. After a 4 h delay between trials one and two, the difference in escape latencies across these trials (termed the savings score) was recorded. The ability of **MK-0952** to improve cognition in this test was evaluated over a series of four experiments so that a comprehensive dose range, namely 0.01–3.0 mg/kg can be tested (Fig. 4). Previously disclosed results for **MK-0359**^{19a} were included as a comparator. In all experiments, **MK-0952** was shown to improve performance in the water maze test with a minimum efficacious dose (MED) of approximately 0.1 mg/kg.

In vivo assessment of **MK-0952** induced AEs: The potential of **MK-0952** to induce malaise (emesis) in rats was assessed in the Conditioned Taste Aversion test in two experiments. For two conditioning days, rats were given 20 min access to a solution of 0.1% saccharin and the amount consumed was recorded. Immediately after, rats received vehicle, **MK-0952**, or the positive control LiCl. On test day, rats were presented with the saccharin solution again and the amount of liquid consumed was recorded. The premise was that if the compound administered induced malaise in the rat, this ill-feeling would be associated with the novel saccharin solution, and would be reflected by reduced consumption on the test day. As a result, **MK-0952** was shown to induce malaise in the rat at a MED of 10 mg/kg (Fig. 5).

Comparative profile of **MK-0952**, **MK-0359** and **MK-0873**: All three compounds were evaluated in the WM and CTA tests and the overall data are summarized in Table 7. Despite its low brain penetration, **MK-0952** compares favorably to **MK-0359** and **MK-0873** in the WM test as a result of its superior intrinsic enzymatic activity over its brain concentration. In general, efficacy in the WM

test was observed at whole brain concentrations slightly above the intrinsic enzymatic activity. These results do not take into account the possibility that distribution within different brain section might vary among the inhibitor tested. Nevertheless, the ratio of whole brain inhibitor concentration to intrinsic enzymatic activity is a good tool to assess and compare different series of PDE4 inhibitors. In the CTA test, MK-0952 was better tolerated than MK-0359 and MK-0873 despite its significantly higher brain and plasma exposure over its counterparts. The ratio of plasma level to whole blood activity seems to be a useful measurement of tolerance towards AEs induced by the inhibition of the PDE4 enzymes as exemplified by the comparative ratios of the three studied compounds. The therapeutic widow of MK-0952, determined by the WM MED to CTA MED ratio, is 100. It is significantly superior to the therapeutic widow (i.e., three) of the two other compounds studied. In conclusion, the results presented in this manuscript validate our initial hypothesis. Following successful safety studies in rats and rhesus monkeys, MK-0952 completed Phase I clinical trials to assess its human pharmacokinetic profile. Results from these efforts will be disclosed in a separate manuscript.

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- 28 Habituation: male hooded Lister rats (Harlan, UK) were habituated over two days (2 \times 5 min) to the empty box (1 m \times 1 m). Sample phase: two identical objects are placed in the far corners of the box. Rat was placed in the middle of the box and the total time spent exploring the two objects was determined. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Turning around or sitting on the object was not considered exploratory behavior. The sample trial lasts for a maximum of 3 min, but was terminated once the rat had scored 25 s of combined exploration on the sample objects. Choice phase: rat was reintroduce in the box after 30 min delay and was presented with the same object as before (cleaned between trial to remove olfactory cues) and a new object. The total time spent exploring the two objects was determined. The same sequence of events was repeated 72 h later and the objects were switched (first object introduce correspond to second one in the first experiment).
- 29. Male hooded Lister rats (Harlan, UK) were trained over eight days to find a submerged platform (10 cm diameter) in a 2 m diameter pool filled with opaque water and surrounded by visual cues. The platform position remained constant during the day but was changed from day to day, and the movements of the animals were tracked using an HVS image and software system (HVS Image Ltd, UK). Each animal received four trials per day with each trial lasting 60 s. If an animal failed to find the platform within this time, it was guided to the platform by the experimenter. The animal spent 30 s on the platform before being removed prior to its next trial.
- 30. Singly-housed male hooded Lister rats (Harlan, UK) were deprived of water for 22.5 h in each 24 h period for two days prior to two conditioning days. On each conditioning day, all rats were given 20 min access to a solution of 0.1% saccharin that has a distinct and novel flavor. The amount of this liquid consumed was recorded. Rats received either vehicle, test compound or the positive control lithium chloride (30.0 mg/kg, ip) in a volume of 1 ml/kg. All compounds were dosed immediately after consumption of the novel flavor (*n* = 8 per group). Subsequently, on the test day the rats were presented with the flavor again and the amount of liquid consumed was recorded.