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Development of a novel therapeutic suppressor of brain proinflammatory cytokine up-regulation that attenuates synaptic dysfunction and behavioral deficits

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Abstract—We report the development of a novel, aqueous-soluble, safe, small molecule, experimental therapeutic that suppresses injury-induced, proinflammatory cytokine increases in the brain, with resultant attenuation of synaptic protein biomarker loss and improvement in hippocampus-dependent behavioral deficits. A GMP production scheme for the active pharmaceutical ingredient, compound **17**, is presented. The development and large-scale availability of this novel compound allow exploration of new, potentially disease-modifying, therapeutic approaches to CNS disorders.

Over-production of proinflammatory cytokines contributes to the progression of pathology in a diverse array of diseases,[1–5] and the recent approval of protein therapies[5–8] that seek to control the level or activity of proinflammatory cytokines provides a proof-of-concept for targeting proinflammatory cytokine levels in drug discovery. However, the macromolecular therapeutic approach is not as amenable to clinical use in some diseases, such as central nervous system (CNS) disorders, creating an unmet need for small molecule, brain-penetrant compounds as new classes of disease-modifying drugs. An accumulating body of evidence from animal model studies and clinical observations supports the concept[1,3,4,9,10] that development of such therapies for CNS disorders could have potential impact on both acute and chronic disorders as diverse as Alzheimer's disease (AD), stroke, traumatic brain injury, neuropathic pain, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, age-related macular degeneration, and non-AD dementias. The prevailing hypothesis in this regard is that targeting the up-regulation of proinflammatory cytokine production by activated glia, the main cellular source of cytokines in the CNS, will alter disease progression through attenuation of the subsequent neuronal synaptic dysfunction, the cellular basis of clinical symptoms and behavioral alterations. However, there are no such current therapies available and there are no consensus molecular targets for such discovery efforts. The current state of the art requires, therefore, the use of the more classical and unbiased functional approach to drug discovery.[11]

Recently, we described[12,13] a contemporary version of the functional approach for CNS drug discovery that resulted in a novel lead compound with the desired in vivo functions in animal models of disease. The discovery platform used de novo lead compound generation that started with an inactive phenylpyridazine fragment to generate a focused synthetic chemical library.[12,14,15] The proprietary library was screened for the ability to suppress, in a concentration-dependent manner, the increased production of proinflammatory cytokines by activated glia in cell culture.[12,13] Cell-based screening hits were then tested in hierarchal, in vivo screens to eliminate highly toxic compounds and to identify compounds with good in vivo efficacy in animal models of disease. We identified[13] a novel, safe, orally active, lead compound (MW01-5-188WH; compound **1** in Table 1), that was efficacious in animal models of AD-relevant pathophysiology.

Keywords: Alzheimer's disease; Pyridazine; Process chemistry; Proinflammatory cytokine; Neurodegeneration.
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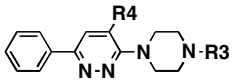
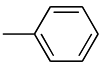
Table 1. Identification of lead compound



Compound	R4	R3	M_W[a]	IL-1β[b]
1			394.47	2.5 ± 0.7
2			408.50	5.3 ± 0.3
3			395.46	25.8 ± 3.1
4			374.48	6.1 ± 1.2
5	CH_3		332.40	8.3 ± 2.1
6	Cl		352.82	9.5 ± 2.3
7	CH_3		332.40	46.1 ± 11.6
8	CH_3		331.41	17.7 ± 3.6
9	CH_3		330.43	15.5 ± 5.2
10	CH_3		336.47	31.4 ± 3.5
11	CH_3	CH_3	268.36	48.9 ± 12.8

[a] M_W = molecular weight.
[b] Concentration (μM) for 50% inhibition; means ± SEM; IL-1β = interleukin-1β.

Compound **1** has attractive efficacy and safety screening profiles,[13] but its aqueous solubility is not ideal for clinical development. Extensive analyses indicate the significant influence of a compound's molecular properties on pharmacokinetics, toxicity, and efficacy, with success generally associated with low M_W and good aqueous solubility, especially drugs that fall into the top class of the biopharmaceutics classification scheme.[16–20] However, increasing the aqueous solubility of a compound while decreasing M_W and retaining in vivo biological function is a non-trivial goal often considered paradoxical, especially for CNS lead compounds. We summarize in this report the discovery of a novel lead compound, compound **5** (Table 1), that has improved molecular properties but retains in vivo efficacy. In addition we report the development of a synthetic scheme for good manufacturing protocol (GMP) production of compound **17**, a highly soluble and stable dihydrochloride hydrate of compound **5**. Compound **17** is the active pharmaceutical ingredient (API) currently in clinical development.[21]

The rationale for efforts to improve molecular properties while retaining activity was to replace the R4 phenyl ring of compound **1** (Table 1). Compound **5** fulfilled these criteria. Compounds **2**–**6** (Table 1) all have a M_W similar to, or less than, compound **1**, but have better computed log$S$ values (respectively, −5.4, −5.3, −4.4, −4.9, −4.1, and −4.6 for compounds **1**–**6**). Compounds **2**, **4**, **5**, and **6** retain activity in the glia cell-based screen for concentration-dependent suppression of IL-1β production, but there was some loss of activity with compound **3** (Table 1). Previous screening results showed[15] that there is no activity when R4 = H. Therefore, compound **5** was the most promising due to its potential improved aqueous solubility, retention of activity, and a decreased M_W (332.40 compared to 394.47). Experimental determination of aqueous solubility confirmed that compound **5** (0.016 mg/ml) is much more soluble in water than compound **1** (<0.002 mg/ml).

Interestingly, an analog of compound **5** has been previously described in the literature.[22] The CNS drug min-

aprine is also a 3-amino-4-methyl-6-phenylpyridazine. However, minaprine is not active as a suppressor of pro-inflammatory cytokine production by activated glia, suggesting that the details of R3 may be critical for this difference in function. In order to gain insight into key features of compound **5** that distinguish it from minaprine, we examined the effects of diversification at R3 in the context of compound **5** (synthesis of compounds **7**–**11**) on retention of activity. Compounds **7**–**11** (Table 1) are analogs of compound **5** that differ at R3. Diversification included alternatives to the pyrimidinyl ring, including compounds with a pyrazinyl (compound **7**), pyridinyl (compound **8**), phenyl (compound **9**), or cyclohexyl (compound **10**) ring. Preference for an aromatic group at R3 for full activity in the context of a methyl at R4 is suggested by the loss of activity with a cyclohexyl (compound **10**) or methyl (compound **11**) at R3, and the partial retention of activity with a pyridinyl or phenyl (compounds **8** and **9**). However, this hypothesis is contradicted by compound **7**, which has a pyrazinyl at R3 yet has a lower activity comparable to the cyclohexyl (compound **10**) or methyl (compound **11**) analogs. The results indicate that only certain types of aromatic rings can substitute for the pyrimidine ring in the context of 3-amino-6-phenylpyridazines represented by compounds **1** and **5**. Although compound **5** is distinct from minaprine in its structural requirements at R3, it is similar to minaprine in its retention of partial activity with the presence of a benzyl at R6 instead of a phenyl (compound **12**) or loss of activity with the presence of a methyl (compound **13**).[21] However, further pursuit of structure–activity relationships was not done as part of this investigation because the discovery of compound **5** provided attainment of our discovery goal, and the diversification at R3 revealed a strict preference for an arylpiperazine, a feature distinguishing compound **5** from minaprine.

A seminal discovery for the studies summarized here is that compound **5** retains the in vivo functions of compound **1** (Figs. 1, 2, and S1[21]). Specifically, compound **5** mimics compound **1** in oral bioavailability, safety, and CNS disease model efficacy. Compound **5** produces no histological liver injury after chronic oral administration at a potential therapeutic dose, or after acute, escalating-dose oral administration at doses up to 40 times a potential therapeutic dose (Fig. 1). It also shows cardiovascular safety, as measured by lack of prolongation of cardiac QTc interval in guinea pigs following FDA recommended protocols. Compound **5** demonstrates brain penetration (LogBB=0.45; Fig.S1) **21** comparable to other CNS drugs and compound **1**.[13] Compound **5** is efficacious in an AD-relevant mouse model of human amyloid-beta (Aβ)-induced injury (Fig. 2). A single, daily oral administration of compound **5** at a low dose (2.5 mg/kg) for 2 weeks, beginning 21 days after initiation of the human Aβ injury, effectively suppressed the up-regulation of the proinflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor alpha (TNFα), and S100B measured at day 60 after the start of injury. The number of activated GFAP-positive astrocytes and F4/80-positive microglia was also reduced. The loss of the synaptic marker proteins synaptophysin and PSD-95 was attenuated. Deficits in the Y-maze, a hippocampus-dependent behavioral task, were ameliorated. These data document that compound **5** retains the desired in vivo functions of the original lead compound **1**.

The discovery of compound **5** as an analog of compound **1** that retains full in vivo functions of compound **1**, but has a lower M_W and better aqueous solubility, provided the starting point for development and characterization of an API to allow clinical investigations. Standard diversification chemistries used for the synthesis of compounds such as those in Table 1 are not attractive for chemical processes amenable to large-scale GMP production. For example, a Pd-catalyzed reaction from a common 4-chloropyridazine precursor (compound **6**) was used initially to synthesize compound **5** and other analogs, summarized in Scheme S1,[21] as part of the in-parallel synthetic approach to diversification.[12] However, Pd-catalyzed reaction protocols raise specific concerns for GMP production and are not readily scalable. Therefore, we developed an efficient production protocol (Scheme 1) to facilitate manufacture for clinical studies. In addition, the protocol offers the potential for use of water as an alternative solvent in the penultimate amination step. We also added as a last

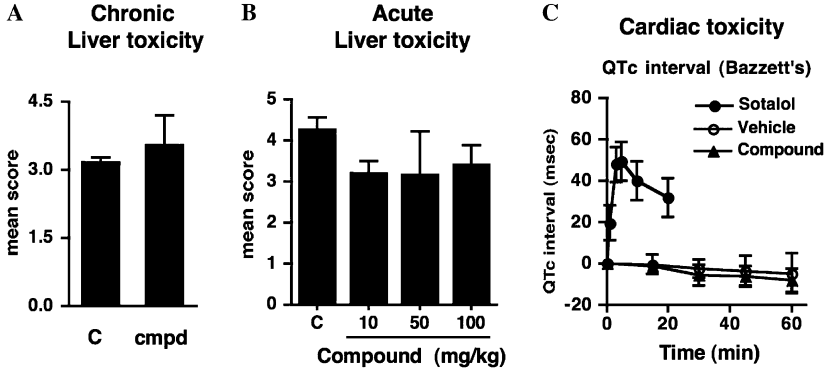


Figure 1. Oral administration of compound **5** does not reveal in vivo liver or cardiac toxicity. (A) Chronic therapeutic dose test of liver injury: there is no histological evidence of liver injury after oral administration of compound **5** (2.5 mg/kg) to mice once daily for 2 weeks. (B) High dose test of liver injury: no histological evidence of liver injury is seen after oral administration of increasing doses up to 100 mg/kg to mice once daily for 3 days. (C) Physiological test for cardiotoxicity at high dose: no evidence of prolongation of QTc interval after oral administration (15 mg/kg) to guinea pigs; sotalol is a positive control compound.

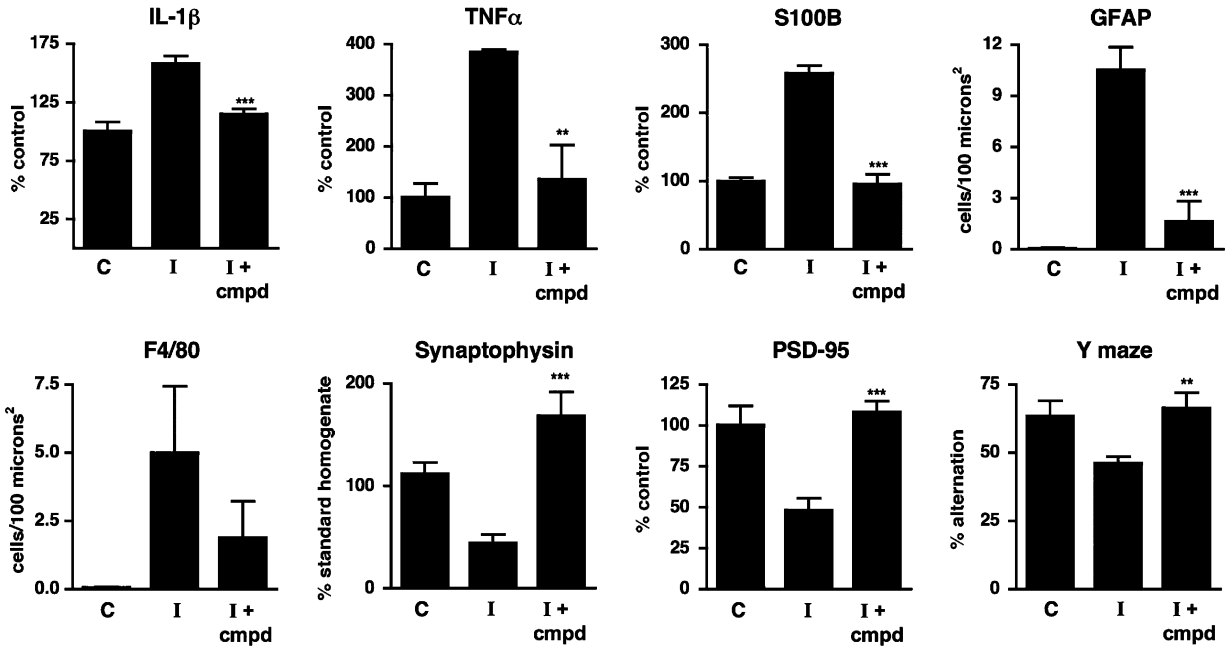
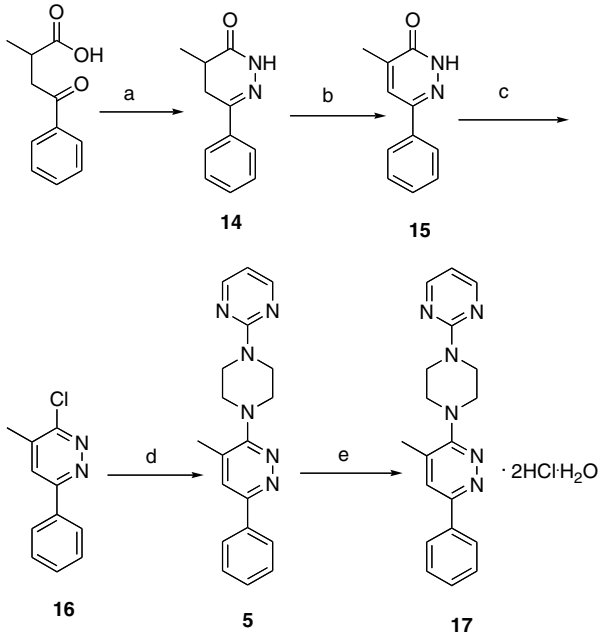


Figure 2. Compound **5** is efficacious in an Alzheimer's disease mouse model. Compound **5** suppresses up-regulation of the proinflammatory cytokines IL-1β, TNFα, and S100B; decreases astrocyte (GFAP) and microglia (F4/80) activation; prevents loss of the synaptic proteins synaptophysin and PSD-95; and attenuates hippocampal-dependent behavioral deficits in the Y-maze. C, control; I, Aβ-injured; I + compd, Aβ-injured + oral administration (2.5 mg/kg once daily for two weeks beginning three weeks after start of injury). Data are means ± SEM of $n = 5$–10 mice per group. Significantly different from Aβ-injured: $^{**}p < 0.01$, $^{***}p < 0.001$.

step the production of the dihydrochloride hydrate, compound **17**, which is the API,[21] as the final product. Hydrochloride hydrates are a common salt form used in API production for diverse drugs,[23] including the drug minaprine. As summarized in Scheme 1, the commercially available 2-methyl-4-oxo-4-phenyl butanoic acid was cyclized with hydrazine to obtain compound **14**, which was then dehydrogenated to 4-methyl-6-phenylpyridazinone compound **15**. Chlorination with phosphorus oxychloride provided the 3-chloropyridazine compound **16**. Compound **5** was then obtained by amination. The corresponding dihydrochloride hydrate, compound **17**, was synthesized by treatment with HCl in isopropanol. As detailed in supporting data,[21] this scheme and its variations have been used for generation of the API at the multi-gram level in the laboratory and at the kg level under GMP conditions.



Scheme 1. Reagents and conditions: (a) N_2H_4, EtOH, reflux; (b) $CuCl_2$, CH_3CN, reflux; (c) $POCl_3$, CH_3CN, reflux; (d) 1-(2-pyrimidyl)piperazine, water, reflux; (e) HCl, isopropanol.

Compound **17** is water-soluble and stable. Compound **17** has improved solubility at 37 °C compared to the lead base compound **5**, and compared to compound **18**,[21] the analogous dihydrochloride hydrate of compound **1**. The experimental values for compound **17** and compound **18** are, respectively, 322 mg/ml and 0.04 mg/ml. In contrast to differences in experimentally determined aqueous solubility, these compounds possess similar experimentally determined log *P* values (2.3 and 2.7), as measured by octanol/water partition coefficient. Compound **17** has a melting point >215 °C, a temperature at which it decomposes, compared to a melting point of 115.7–116.4 °C for the base compound **5**. When compound **17** was tested for chemical stability in water, dilute acid, or dilute base at 37 °C over 5 days, there was <5% loss of material in water and acid. Compound **17** was less stable in basic conditions, with only 53% remaining after 5 days. Compound **17** also showed <10% loss of material when treated with 3% (v/v) H_2O_2 at ambient temperature (24 °C) for up to 48 h. These data provide experimental evidence that the

molecular properties were improved as a result of refinement, and demonstrate that the API is a water-soluble, stable product.

In summary, we have described the discovery and development of a novel, water-soluble, safe, experimental therapeutic with potential to be a new type of disease-modifying drug. Currently, IND-enabling preclinical toxicology and pharmacokinetic analyses are validating and extending the findings of the initial screens reported here, and GMP manufacture of the API for clinical studies has been accomplished.[21]

Acknowledgment

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Supplementary data

The contents of supporting information include the following: details of synthetic procedures, analytical chemistry methods, physical property determinations, NMR spectra, and biological procedures, including screening data for oral bioavailability. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.028.

References and notes

1. Craft, J. M.; Watterson, D. M.; Van Eldik, L. J. *Expert Opin. Ther. Targets* **2005**, *9*, 887.
2. Mrak, R. E.; Griffin, W. S. T. *Neurobiol. Aging* **2005**, *26*, 349.
3. Wyss-Coray, T. *Nat. Med.* **2006**, *12*, 1005.
4. Watkins, L. R.; Maier, S. F. *Nat. Rev. Drug Disc.* **2003**, *2*, 973.
5. Maini, R. N.; Taylor, P. C. *Annu. Rev. Med.* **2000**, *51*, 207.
6. Scott, D. L.; Kingsley, M. B. *N. Engl. J. Med.* **2006**, *335*, 704.
7. Tobinick, E.; Gross, H.; Weinberger, A.; Cohen, H. *Med Gen. Med.* **2006**, *8*, 25.
8. Mpofu, S.; Fatima, F.; Moots, R. J. *Rheumatology* **2005**, *44*, 271.
9. Guo, Z.; Cupples, L. A.; Kurz, A.; Auerbach, S. H.; Volicer, L.; Chui, H.; Green, R. C.; Sadovnick, A. D.; Duara, R.; DeCarli, C.; Johnson, K.; Go, R. C.; Growdon, J. H.; Haines, J. L.; Kukull, W. A.; Farrer, L. A. *Neurology* **2000**, *54*, 1316.
10. D'Ambrosio, R.; Perucca, E. *Curr. Opin. Neurol.* **2004**, *17*, 731.
11. Sams-Dodd, F. *Drug Discovery Today* **2006**, *11*, 465.
12. Wing, L. K.; Behanna, H. A.; Van Eldik, L. J.; Watterson, D. M.; Ralay Ranaivo, H. *Curr. Alzheimer. Res.* **2006**, *3*, 205.
13. Ralay Ranaivo, H.; Craft, J. M.; Hu, W.; Guo, L.; Wing, L. K.; Van Eldik, L. J.; Watterson, D. M. *J. Neurosci.* **2006**, *26*, 662.
14. Velentza, A. V.; Wainwright, M. S.; Zasadzki, M.; Mirzoeva, S.; Schumacher, A. M.; Haiech, J.; Focia, P.; Egli, M.; Watterson, D. M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3465.
15. Hu, W.; Ralay Ranaivo, H.; Craft, J. M.; Van Eldik, L. J.; Watterson, D. M. *Curr. Alz. Res.* **2005**, *2*, 197.
16. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
17. van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem.* **2001**, *44*, 1313.
18. Wenlock, M. C.; Austin, R. P.; Barton, P.; Davis, A. M.; Leeson, P. D. *J. Med. Chem.* **2003**, *46*, 1250.
19. Vieth, M.; Siegel, M. G.; Higgs, R. E.; Watson, I. A.; Robertson, D. H.; Savin, K. A.; Durst, G. L.; Hipskind, P. A. *J. Med. Chem.* **2004**, *47*, 224.
20. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
21. Details of synthetic procedures, analytical chemistry methods, physical property determinations, NMR spectra, and biological procedures, including screening data for oral bioavailability and brain uptake, can be found in Supporting Information. Information about the clinical development of compound **17** under the name of Minozac can be accessed on the website of NeuroMedix (NMX) Inc., http://www.neuromedix.com.
22. Wermuth, C. G.; Schlewer, G.; Bourguignon, J.-J.; Maghioros, G.; Bouchet, M.-J.; Moire, C.; Kan, J.-P.; Worms, P.; Biziere, K. *J. Med. Chem.* **1989**, *32*, 528.
23. Wermuth, C. G.; Stahl, P. H. In *Handbook of Pharmaceutical Salts*; Stahl, P. H., Wermuth, C. G., Eds.; Wiley-VCH, 2002; p 249.